RESEARCH ARTICLE

Filamentous sulfur bacteria, *Beggiatoa* spp., in arctic marine sediments (Svalbard, 79°N)

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Abstract

Fjord sediments on the west coast of the arctic archipelago Svalbard were surveyed to understand whether large filamentous sulfur bacteria of the genus *Beggiatoa* thrive at seawater temperatures permanently near freezing. Two sediments had abundant populations of *Beggiatoa*, while at six sites, only sporadic occurrences were observed. We conclude that *Beggiatoa*, although previously unnoticed, are widespread in these arctic fjord sediments. *Beggiatoa* ranged in diameter from 2 to 52 μm and, by those tested, stored nitrate in vacuoles at up to 260 mM. The 16S rRNA gene sequence of a 20-μm-wide filament is closely associated with other large, marine, nitrate-storing *Beggiatoa*. The *Beggiatoa* mostly occurred in the upper 2–5 cm of oxidized surface sediment between oxygen and the deeper sulfidic zone. In spite of a very low or an undetectable sulfide concentration, sulfate reduction provided abundant H_2S in this zone. The total living biomass of *Beggiatoa* filaments at one study site varied over 3 years between 1.13 and 3.36 g m^{-2}. Because of their large size, *Beggiatoa* accounted for up to 15% of the total prokaryotic biomass, even though the filament counts at this site were rather low, comprising $< 1/10\,000$ of the bacterial numbers on a cell basis.

Introduction

Filamentous, sulfide-oxidizing bacteria of the genus *Beggiatoa* are widespread in marine surface sediments that have a sufficiently high production of sulfide from bacterial sulfate reduction. Such sediments occur in the eutrophic coastal zone and in highly productive upwelling systems along continental margins, often associated with the low oxygen concentration in the bottom water. *Beggiatoa* spp. also occur as benthic mats in many other marine environments where sulfide is introduced by advective flow, for example at cold seeps or at hydrothermal vents (Jørgensen & Boetius, 2007).

Beggiatoa spp. are most often noticed when growing as white mats on the seafloor, and yet the most widespread occurrence is probably as scattered filaments hidden within the uppermost few centimetres of the sediment. This more diffuse distribution requires a designated approach to find and quantify the organisms, although they are conspicuous as they have the size of micro- or meiofauna. It is a property unique for these large, multicellular bacteria that they can be identified and counted by simple light microscopy. Using this approach, it has been demonstrated that *Beggiatoa* generally occur in the 'suboxic zone' between the few millimetres thick oxic zone at the sediment surface and the diffusion front of sulfide starting several centimetres below (Jørgensen, 1977; Mußmann *et al.*, 2003; Jørgensen & Nelson, 2004; Preisler *et al.*, 2007).

Beggiatoa display tactic responses that enable them to avoid both increasing oxygen and increasing sulfide concentrations (Møller *et al.*, 1985; Preisler *et al.*, 2007). The microaerophilic organisms only accumulate at the sediment surface when the population density is sufficiently high to create a steep oxygen gradient in the diffusive boundary layer, thereby ensuring a low oxygen concentration at the exposed mat surface (Jørgensen & Revsbech, 1983). It was proposed that a prerequisite for the subsurface occurrence of *Beggiatoa* in sediments is the presence of a distinct sulfide zone that prevents filaments from becoming lost at depth without a chemotactic clue (Preisler *et al.*, 2007).

The predominant *Beggiatoa* populations in marine sediments are filaments with diameters of several micrometres to several tens of micrometres. Filaments of these size groups were found to accumulate nitrate in internal

vacuoles, often up to concentrations of several hundred millimoles (McHatton *et al.*, 1996; Ahmad *et al.*, 1999; Mußmann *et al.*, 2003; Preisler *et al.*, 2007). *Beggiatoa* use nitrate as an alternative electron acceptor when living in the suboxic zone. Where studied, nitrate is not denitrified, but is rather reduced to ammonium (McHatton, 1998). Internal nitrate accumulation and reduction to ammonium is a property shared with other large, marine sulfur bacteria of the genera *Thioploca* with whom *Beggiatoa* form a monophyletic group (Otte *et al.*, 1999; Jørgensen & Nelson, 2004; Jørgensen *et al.*, 2005b).

In coastal environments, *Beggiatoa* have so far mostly been observed under temperate conditions where the organic sedimentation is high and where experimental measurements of sulfate reduction demonstrate high sulfide production rates. In these moderately warm sediments, *Beggiatoa* have gliding motility and express highly developed phobic responses to oxygen and sulfide. At temperatures > 5–$10\,^{\circ}C$, the filaments glide at a velocity of 2–4 $\mu m\,s^{-1}$ and effectively migrate within their preferred environmental zone (Dunker *et al.*, 2010). Coastal sediments of the Arctic and Antarctic have temperatures permanently near $0\,^{\circ}C$, which may require a psychrophilic adaptation of the indigenous *Beggiatoa* communities, not only of their metabolic rate but also of their gliding motility, in order to metabolize and orient effectively. Such a psychrophilic adaptation has been demonstrated for arctic communities of sulfate-reducing bacteria (Knoblauch & Jørgensen, 1999; Knoblauch *et al.*, 1999), but has not yet been described for large, sulfide-oxidizing bacteria such as *Beggiatoa*. The present study therefore aimed to explore the occurrence of *Beggiatoa* spp. in arctic sediments with respect to their distribution, size spectrum and biomass and to understand whether these large bacteria are equally well adapted to permanently cold environments as they are to temperate environments. Concurrent studies of the temperature regulation of their metabolic rate, expressed through their gliding velocity, will be published elsewhere (Dunker *et al.*, 2010).

Materials and methods

Field sampling and sediment properties

Sediment was collected from different fjords on the west coast of Spitsbergen, the main island of the archipelago Svalbard bounding the North Atlantic and the Arctic Ocean. The location and relevant characteristics of these sites are given in Fig. 1 and Table 1. All sites were located between 78° and 80° northern latitude and, apart from a small lagoon, had temperatures permanently near $0\,^{\circ}C$. In several of the 70–200 m deep Spitsbergen fjords, cores of 15 cm diameter and 20–30 cm depth were collected using a HAPS corer (Kanneworff & Nicolaisen, 1973) during cruises with MS FARM (Longyearbyen). Subcores were taken immediately on board using 26 or 36 mm inner diameter acrylic tubes. The cores were kept submerged in seawater at *c.* $0\,^{\circ}C$ during the following 1–3 days on the ship. Back in the laboratory, cores were transferred to an incubator at $0.5 \pm 0.5\,^{\circ}C$ until further subsampling within 1–2 days.

Porosity, density and ignition loss of the sediments were determined at 2 cm depth intervals. Core segments of defined volume were transferred to preweighed crucibles and the sediment weight was determined before and after drying at $105\,^{\circ}C$ overnight. Porosity was determined from the water loss per volume of sediment upon drying. Porosity data were used for the calculation of sulfate reduction rates (SRR).

Biomass determination

Sediment subcores were sectioned into 0.5 cm depth increments. Subsamples of 20–30 mg were taken using a clean mini-spatula, transferred to a tarred microscope slide and weighed to ± 0.2 mg accuracy. The sediment was immediately wetted with drops of seawater, mechanically suspended and smeared over the surface of the slide and finally covered with a large cover slip. *Beggiatoa* filaments were quantified using direct light microscopy according to Jørgensen (1977) by scanning the entire slide systematically using the $\times 10$ objective. The length ($\times 10$) and diameter ($\times 40$) of all detected filaments were measured using a calibrated ocular micrometer. Only motile, colorless, multicellular filaments with distinct sulfur inclusions were counted as *Beggiatoa*. Some *Beggiatoa*, in particular those of smaller diameters, may have been missed and the counts thus represent minimum values.

Beggiatoa filaments were also quantified using a modified approach whereby a weighed sediment sample of *c.* 0.5 g was added to 10 mL seawater and suspended. A 0.35 g subsample was then smeared on a glass slide and the filaments were counted. This procedure improved the statistical representation of the bacterial density in case these had a patchy distribution. Determination of length and diameter was also performed by digital photography using imaging software (IMAGETOOL, The University of Texas Health Science Center, San Antonio, TX).

For quantification, diameters were grouped in 2–3 μm increments. Based on the mean dimensions of each diameter group, *Beggiatoa* biomass was calculated using the cylinder volumes of the filaments and assuming a density of $1\,g\,cm^{-3}$. All population data were recalculated from gram wet weight to volume (cm^3) of sediment based on the sediment densities determined. Adding up the data over the entire depth interval revealed the population size as fresh biomass per sediment surface area ($g\,m^{-2}$).

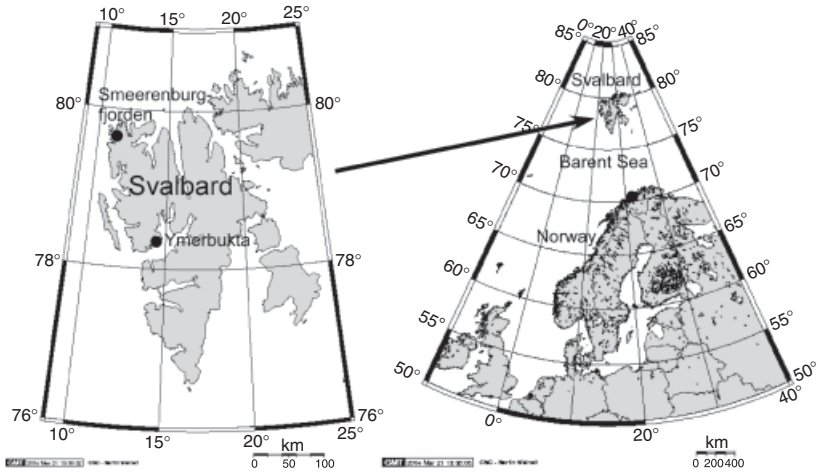

Fig. 1. Map of Svalbard with sampling sites.

Phylogenetic analyses

Single filaments intended for phylogenetic analyses were stored separately at −20 °C, preserved in 1 × TE buffer (Promega Corporation, Madison, WI). Before PCR amplification, each filament was separated from the TE buffer by centrifugation for 3 min at 5000 ***g*** and dissolving in 5 μL of PCR-grade water (Sigma-Aldrich Biochemie GmbH, Hamburg, Germany). The complete volume of 5 μL was then used as a template in the following amplification reaction. Universal bacterial primers GM3F (5′-AGAGTTTGATCMTGGC-3′; Muyzer *et al.*, 1995) and GM4R (5′-TACCTTGTTAC GACTT-3′; Muyzer *et al.*, 1995) were used for amplification of nearly full-length 16S rRNA gene sequences. Amplification reactions were set up as follows: 1 × MasterTaq buffer with 1.5 mM Mg^{2+}, 0.3 mg mL^{-1} bovine serum albumin (Sigma-Aldrich Biochemie GmbH), 250 μM of each dNTP (Roche, Mannheim, Germany), 0.5 μM of each primer (Biomers, Ulm, Germany) and 0.025 U μL^{-1} MasterTaq (5 Prime, Hamburg, Germany) in a total volume of 50 μL. After an initial denaturation for 15 min at 95 °C, 30 cycles of 95 °C for 1 min, 42 °C for 1 min and 72 °C for 3 min were performed before a final elongation step at 72 °C for 10 min.

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), ligated into pGEM-T Easy vector (Promega Corporation) and transformed into One Shot TOP10 Chemically Competent *Escherichia coli* (Invitrogen Corporation, Karlsruhe, Germany) according to the manufacturer's instructions. Three representative clones were selected for plasmid preparation (Montage Plasmid $Miniprep_{HTS}$ Kit; Millipore GmbH, Schwalbach, Germany). Purified plasmids were subjected to *Taq* cycle sequencing using an ABI Prism 3130x Genetic Analyzer (Applied Biosystems, Foster City, CA).

Partial sequences were assembled using SEQUENCHER 4.6 software (Gene Codes Corporation, Ann Arbor, MI) and checked manually. Examination for chimeric signals was performed using the PINTAIL program (Ashelford *et al.*, 2005), with nearest neighbors obtained using the SILVA-based Webaligner (http://www.arb-silva.de/aligner/; Pruesse *et al.*, 2007). No genuine chimeric signals were detected. All three sequences were nearly identical (similarity values of 99.5–99.7%). Finally, clone S3678 was chosen for further phylogenetic analyses.

A phylogenetic tree was constructed using the neighbor-joining and maximum-likelihood (RAxML) algorithm, as included in the ARB software package (Ludwig *et al.*, 2004). Initial calculations were conducted with nearly full-length sequences (≥1200 bp) and by applying different filters. Partial sequences L41043, L40999 and AF129012 were subsequently inserted by applying parsimony criteria and without allowing changes in the overall tree topology. Deltaproteobacterial sequences were used as an outgroup. A consensus tree based on the different reconstruction approaches was built, wherein unstable branching orders were visualized by multifurcation.

The sequence of the uncultured *Beggiatoa* spp. clone S3678 from Smeerenburgfjorden (Station J) has been submitted to the EMBL database under accession no. FN561862.

Chemical measurements

Sediment subcores were sectioned in 1 cm depth increments and pore water was obtained by squeezing through 0.45-μm pore-size membrane filters under N_2 according to Reeburgh (1967). Sulfate was measured by nonsuppressed ion chromatography (Waters IC with a conductivity detector). A subsample was diluted 30–50-fold in double-distilled water and membrane-filtered just before analysis.

Profiles of O_2, H_2S and pH were measured using microsensors in cores mounted in a mini-flume, which provided a constant temperature of 0 °C and a water flow of 1–2 cm s^{-1} at 1 cm above the sediment surface (Jørgensen *et al.*, 2005a). O_2 and H_2S were measured using Clark-type

Table 1. Sampling sites visited during field campaigns in 2003, 2005 and 2008

Station	Location	Coordinates		Depth (m)	Temperature (°C)	SRR (mmol m^{-2} day^{-1})	Description
J	Smeerenburgfjorden	79°42′815N	011°05′189E	214	0.4	2.71	Light gray until 2–4 cm, dark gray below, silt and very fine sand, rich in macrofauna Many *Beggiatoa* of different diameters
DA	Ymerbukta	78°16′61N	014°02′69E	0.3	6.5	2.04*	Light brown until 2–4 cm, silt with fine sand of 30–80 μm, gray to black fine sand below, patches of *Beggiatoa* or cyanobacteria overlying black sediment Many *Beggiatoa* of mostly 5 and 12 μm
A	Adventfjorden	78°15′44N	015°30′90E	69	0.4	4.48	Gray-brown until 5–6 cm, mottled gray below; silt and fine sand of 30–100 μm diameter, not very sulfidic No *Beggiatoa*
O	Borebukta	78°19′557N	014°27′760E	94	1.4	2.50/1.59	Gray-brown until 3 cm, gray below, silt and very fine sand of < 30 μm One *Beggiatoa* filament of 6 μm on top
BN	Ymerbukta S	78°15′352N	013°58′417E	104	0.3	1.99	Gray-brown until 4 cm, gradually into gray below, silt-clay, no sand, pelletized Three *Beggiatoa* of 15–20 μm in top 2 cm
E	St Jonsfjorden W	78°32′599N	012°17′909E	168	1.6	1.87	0–3 cm gray-brown, gray below, silt and fine sand One *Beggiatoa* filament of 4 μm at 2 cm
F	Kongsfjorden	78°55′234N	012°13′912E	114	1.8	1.33/1.11	Silty sediment colored red from hematite Few scattered *Beggiatoa* of 7 and 20 μm
S	Blomstrandhalvoya N	78°59′929N	011°59′213E	77	0.0	2.40	Red-gray, with depth dark gray mottled in red, silt and fine sand of 50–150 μm Few *Beggiatoa* of 4 and 7 μm in top 2 cm
R	Blomstrandhalvoya S	78°57′424N	012°09′873E	90	0.0	1.32	Red, with depth gray mottled in red, silt and very fine sand of < 30 μm No *Beggiatoa*
K	Raudfjorden	79°46′144N	012°04′433E	154	−0.5	2.09	Gray-brown to 6 cm, mottled gray below, silt No *Beggiatoa*
I	Magdalenefjorden	79°34′052N	011°03′597E	124	−1.0	1.87	Gray-brown to 5 cm, mottled gray below, silt Three *Beggiatoa* of 18–20 μm

*Data from Sawicka *et al.* (in press) measured in 2007.

A general description of the sediment and an indication for the occurrence of *Beggiatoa* are provided. Stations are all located in fjords on the west and north coast of the island Spitsbergen. Water depths and sediment temperature at the time of sampling are indicated. Areal rates of sulfate reduction (SRR) were integrated over the top 0–15 cm of the sediment and represent the mean of three cores. Two numbers are given when measurements were conducted during different years.

microelectrodes. The O_2 electrode had an internal reference and a guard cathode (Revsbech, 1989). The tip size of the O_2 sensors was 10–20 μm, the stirring sensitivity $< 1\%$ and the 90% response time was ~1 s. A two-point calibration was made by positioning the sensor in air-saturated seawater and in anoxic sediment. The H_2S electrode (Jeroschewski *et al.*, 1996) had a tip diameter of 20–50 μm, stirring sensitivity $< 2\%$ and a 90% response time of ~3 s. The H_2S electrode was calibrated in anoxic, stirred 0.2 M phosphate buffer at pH 7.5, to which appropriate volumes of 100 mM sodium sulfide solution were added. The electrode current was read after each addition and a subsample of the calibration solution was fixed in 2% Zn-acetate for later photometric analysis (Cline, 1969). Total sulfide was calculated from the H_2S concentrations using the sediment pH and a pK_1 of 6.87 as calculated from the seawater salinity and temperature of the incubated cores (Millero *et al.*, 1988). The pH was measured with freshly filled liquid ion exchange microsensors (de Beer *et al.*, 1997), calibrated with standard pH buffers.

With the help of a dissection microscope, all sensors were positioned vertically above the sediment surface. Data acquisition started above the sediment and the electrodes were moved downwards in 100 μm steps using a computer-controlled motor (Faulhaber, Germany). The O_2 and H_2S electrode currents were read using a pA-meter and the pH potential was determined using an mV-meter. Data were transferred to an A-D converter (National Instruments) and stored on a laptop computer.

For the determination of nitrate concentrations in *Beggiatoa* vacuoles, 50–100 filaments were picked for each analysis with a clean glass needle into 1 mL of a NaCl solution isotonic to seawater. After gentle centrifugation, 0.9 mL of the supernatant water was collected separately. Water and *Beggiatoa* samples were acidified at pH 1 with 6 M HCl and stored frozen. Three freeze–thaw cycles between liquid N_2 and 90 °C warm water ensured breakage of the vacuoles and release of the nitrate. Nitrate was analyzed using a Chemoluminescence NO/NOx Analyser (Eco Physics, Germany) with the isotonic NaCl solution as the control. The supernatant samples were also analyzed to check for nitrate leakage before freezing. The mean biovolume of *Beggiatoa* filaments was determined by photographing at random individual filaments ($n = 73$) from a bulk sample of the mat. The length and width of each photographed filament was determined using calibrated image analysis software (IMAGETOOL). The mean biovolume was used to calculate the internal nitrate concentration.

SRR

SRR were measured by whole core injection using ^{35}S-labelled sulfate (Jørgensen, 1978). At each 1 cm depth interval, 2 μL carrier-free $^{35}SO_4^{2-}$ tracer (~100 kBq) was injected and the core was incubated for 8–12 h at *in situ* temperature. Sulfate reduction was stopped by mixing 1 or 2 cm depth sections with 10 mL cold Zn-acetate (20% w/v) and freezing. The samples were later treated with cold chromium distillation according to Kallmeyer *et al.* (2004). SRR were calculated according to Jørgensen (1978):

$$\mathrm{SRR} = [\mathrm{sulfate}] \times (^{35}\mathrm{S\text{-}CRS}/^{35}\mathrm{S\text{-}sulfate}) \times (1.06/t)\ \mathrm{nmol}\ SO_4^{2-}\ \mathrm{cm}^{-3}\ \mathrm{day}^{-1} \quad (1)$$

where [sulfate] is the sulfate concentration in nmol cm^{-3} of wet sediment, ^{35}S-CRS is the radioactivity of total reduced sulfur at the end of the incubation, ^{35}S-sulfate is the initial radioactivity of sulfate added to the experiment, 1.06 is a correction factor for the expected isotope discrimination against ^{35}S-sulfate vs. the bulk ^{32}S-sulfate by the sulfate-reducing bacteria and t is the incubation time measured in days.

Results

Filamentous bacteria observed and quantified microscopically in sediments from Svalbard fjords were recorded as belonging to the genus *Beggiatoa* based on the following criteria (Strohl, 2005): (1) the filaments were freely motile and were not surrounded by a visible sheath common to several filaments. (2) The filaments ranged in diameter from 2 to 50 μm, were multicellular and had rounded, never tapered, terminal cells. (3) The cells were always rich in light-refracting, spherical sulfur globules. In the wider filaments, with diameters > 5 μm, sulfur globules were distributed in the periphery of the individual cells, typical of the morphology of *Beggiatoa* spp. containing nitrate vacuoles. Filaments of similar appearance, but devoid of sulfur globules, were not counted.

Occurrence of *Beggiatoa* around the arctic archipelago Svalbard

We conducted a survey of *Beggiatoa* in fjords on the west and north coast of the main Svalbard island, Spitsbergen. Sediment cores were sampled, generally in the deeper and central part of the fjords, and the cores were screened for *Beggiatoa* using the described approach. For each site, a total of 5–10 samples of *c.* 30 mg wet sediment each were screened from the oxidized surface zone. Thus, a total of 150–300 mg sediment was completely screened per station. The results are summarized in Table 1.

The main conclusion from this survey was that only two out of 11 investigated sites, i.e. Smeerenburgfjorden and Ymerbukta, had abundant *Beggiatoa* during all three sampling years. At six sites, only one or a few *Beggiatoa* were found, with diameters mostly ranging from 4 to 20 μm. At

the remaining three sites, no *Beggiatoa* were found. Given the volume of sediment screened, those sites had statistically < 3–6 filaments cm^{-3}, if any.

Filament frequency and dimensions

During the entire study, close to 1000 *Beggiatoa* filaments were counted and measured. In Fig. 2, the frequency distribution and mean dimensions are shown after an arbitrary classification of all filaments into different diameter groups. When compiling data from all locations and years, there was an almost continuous variation in the diameters, but a strong variation in their frequency (Fig. 2a). The two narrowest size classes, 2–3 and 4–5 μm, were the most abundant and comprised 77% of all *Beggiatoa* filaments counted. Filaments narrower that 2 μm were not found, although they were particularly searched for in several of the sediment samples. There were notably many *Beggiatoa* with diameters of 11–22 μm. A small number of very wide filaments of > 23 μm diameter were also found. The widest filament encountered was 52 μm in diameter. The mean filament length of each diameter class increased with the filament width (Fig. 2b). The narrowest filaments of 2–5 μm had a mean length of 0.25 mm, while the widest were about 1.5 mm long. The longest individual filaments were > 3 mm in length.

The biomass (living wet weight) of individual filaments was calculated based on their measured volume, assuming a density of 1 g cm^{-3}. It should be noted that a large part of this biomass is not active cytoplasm, but is comprised of vacuoles inside the cells. As shown in Fig. 2c, the mean biomass of whole filaments increased > 1000-fold from 1.3 ng of the 2–3 μm class to 1500 ng of the 30–50 μm class. Because of the large difference in biomass per filament between narrow and wide diameters (Fig. 2c), the widest filaments often dominated the biomass in spite of their low numbers. Typically, the 5% widest filaments comprised 50% of the total *Beggiatoa* biomass. It is striking that, in spite of the small mean individual filament lengths ranging from 0.25 to 1.5 mm, the accumulated length of all filaments reached 50 cm cm^{-3} of sediment and was typically a few tens of cm cm^{-3}.

There was a rather constant ratio between filament (i.e. cell) diameter and cell length (i.e. the height of the cylinder-shaped individual cells) for the different size classes. The narrow filaments had longer cylindrical cells than the wide filaments, which had flatter disk-shaped cells. Thus, among the 2–5-μm-wide filaments, the mean cell diameter was 3.7 ± 1.1 μm and the mean cell length was 5.4 ± 0.4 μm. Among the 17–22-μm-wide filaments, the mean cell diameter was 19.2 ± 0.5 μm and the mean cell length was 9.0 ± 1.2 μm. The mean volume of the two size classes of cells was 18 and 830 μm^3, respectively, i.e. 100- and 5000-fold larger than the mean size of other sediment bacteria with a mean volume of 0.2 μm^3. The mean number of cells in each filament was 50 cells for the 2–5 μm size class and 160 cells for the 17–22 μm size class. The latter, multicellular filaments were thereby close to a million-fold larger than the mean size of other sediment bacteria.

Smeerenburgfjorden *Beggiatoa* community

Station J was situated in central Smeerenburgfjorden at 214 m water depth (Table 1) and was particularly rich in *Beggiatoa*. The station was sampled during three different summers in 2003, 2005 and 2008. The Smeerenburgfjorden is a channel on the north-west coast of Spitsbergen and connects to the ocean both towards the west and towards the north. The sediment was a silty mud mixed with fine sand and rich in burrowing macrofauna, particularly in tube-building polychaetes. The upper 2–4 cm of sediment was oxidized and light gray to brown in color, while the sediment below was light to dark gray and black due to iron sulfides, with a mottled appearance due to the heterogeneity caused by ventilation and mixing by the fauna.

During sampling in 2003 and 2005, *Beggiatoa* occurred until 2.5 cm depth in the oxidized surface sediments (Fig. 3). *Beggiatoa* filaments with a narrow size range of 2–2.5 μm width dominated in numbers in 2005 and made up 70% of all *Beggiatoa* counted there. In the more sulfidic (based on coloration) of two sediment cores analyzed in 2005, nearly all of these narrow filaments were found in the top 0–5 mm, while the wider filaments of > 5 μm mostly occurred at the subsurface at 0.5–2.5 cm depth (Fig. 3a). Filament numbers

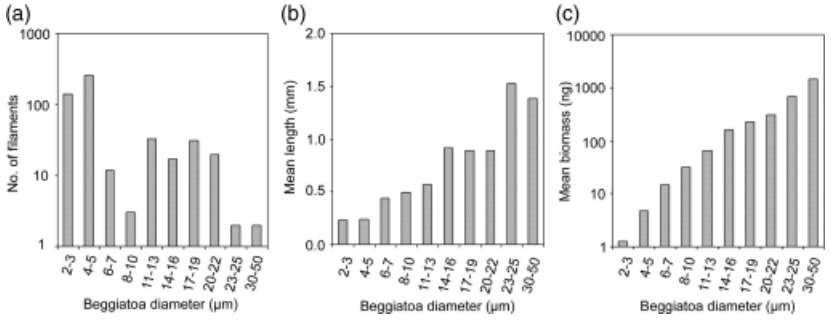

Fig. 2. Size frequency and dimensions of all *Beggiatoa* measured. (a) Frequency distribution of filament diameters. (b) Mean filament lengths of different size groups. (c) Mean fresh biomass of different size groups. Notice log scales in (a) and (c).

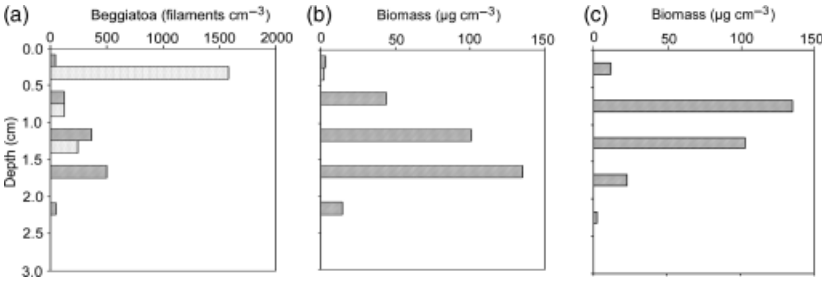

Fig. 3. Depth distribution of *Beggiatoa* in sediment of Station J, Smeerenburgfjorden, during two sampling seasons. (a) Numbers of filaments in 2005. (b) Biomass of filaments in 2005. In (a) and (b) dotted bars show 2–2.5-µm-wide filaments while hatched bars show > 5-µm-wide filaments. (c) Biomass of filaments in 2003.

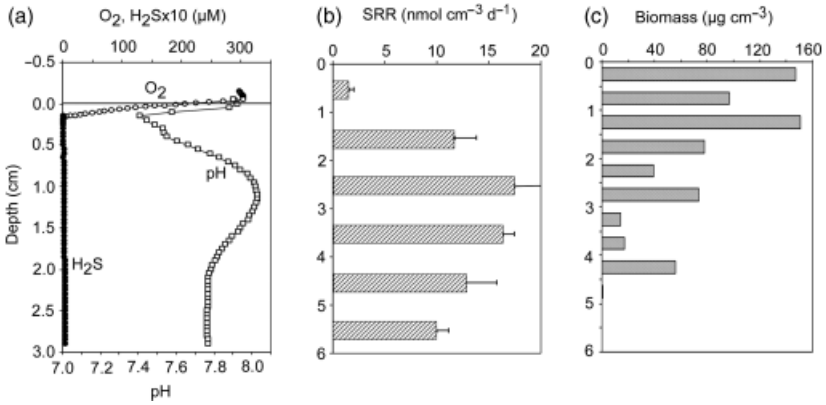

Fig. 4. Station J, Smeerenburgfjorden, in 2008. (a) Microsensor measurements of O_2, total H_2S and pH. (b) SRR measured by the ^{35}S-tracer technique (depth resolution 1 cm). (c) Depth distribution of *Beggiatoa* wet biomass. Note the difference in depth scales.

reached 1600 cm^{-3} for the 2–2.5 µm group and 500 cm^{-3} for the > 5-µm-wide group. Because of the large size difference, however, the > 5 µm *Beggiatoa* completely dominated the biomass (Fig. 3b). Comparison with the 2003 data shows a high reproducibility in biomass distribution between these 2 years (Fig. 3c).

In order to understand the parameters controlling *Beggiatoa* distribution, high-resolution microprofiles of oxygen, sulfide and pH were recorded in 2008 in sediment cores retrieved from Station J. The oxidized zone was deeper that year, 5 cm, judging from sediment color. The primary oxygen front penetrated only 1–2 mm into the sediment (Fig. 4a). Free sulfide (total H_2S) was detected from 2 mm depth and downwards at a very low concentration, reaching 3 µM at 2–3 cm depth. The pH was 7.9 in the overlying seawater and showed a sharp minimum of 7.4 at the oxic–anoxic interface at 1.5 mm. A broad pH maximum of 8.0 occurred in the middle of the oxidized zone. Although free H_2S was hardly detectable, sulfate reduction took place throughout the sediment, reaching maximum values of up to 17 nmol cm^{-3} day^{-1} in 2–3 cm depth (Fig. 4b). *Beggiatoa* were observed throughout the oxidized zone with high abundances and biomass down to 4.5 cm (Fig. 4c).

The maximum biomass of *Beggiatoa* varied between the 3 years from 70 to 150 µg cm^{-3} (Figs 3 and 4). The total biomass of *Beggiatoa* (in g wet biomass m^{-2}) was 1.38 g m^{-2} in 2003, 1.13 g m^{-2} in 2005 (mean of two cores with 0.76 and 1.49 g m^{-2}, respectively) and 3.36 g m^{-2} in 2008.

Ymerbukta *Beggiatoa* community

The other site visited during all 3 years was a protected lagoon in Ymerbukta on the north coast of Isfjorden (Station DA, Table 1). Because of the shallow water depth, 20–30 cm, and the 24 h of daylight during summer, the sediment surface was relatively warm, 6–7 °C, at the time of sampling. The sediment was mixed silt and fine sand of mostly 20–80 µm grain size and was light brown and oxidized to 4 cm depth. The sediment below was gray to black fine sand.

Beggiatoa occurred throughout the 4 cm deep oxidized zone (Fig. 5) and two distinct size classes prevailed. As in Smeerenburgfjorden, there was a distinct difference in the depth distributions of narrow and wide *Beggiatoa*. There was a predominance of *Beggiatoa* filaments with a narrow size range of 5 ± 0.5 µm width, which comprised 90% of all filaments counted at this site. Most of the wider *Beggiatoa* were 12 µm in diameter. The narrow 5 µm *Beggiatoa* had the highest density at 1–2 cm depth, while the wider 12 µm *Beggiatoa* had maximum at 2–3 cm depth (Fig. 5a). Because of their much larger individual filament size, the biomass of the 12 µm filaments was overall the highest (Fig. 5b). The

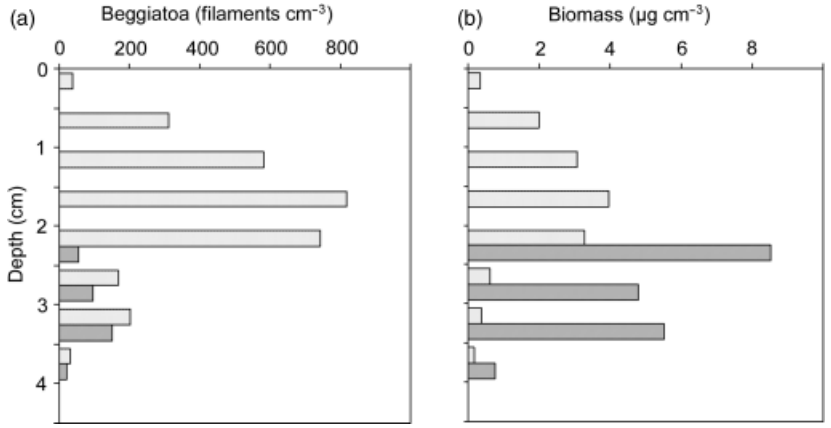

Fig. 5. Depth distributions of *Beggiatoa* in sediment at Station DA, Ymerbukta lagoon, 2005. (a) Numbers of filaments per cm^3 sediment. (b) Biomass of filaments per cm^3 sediment. Dotted bars, 5 µm filaments; hatched bars, > 5 µm filaments. Mean of two cores.

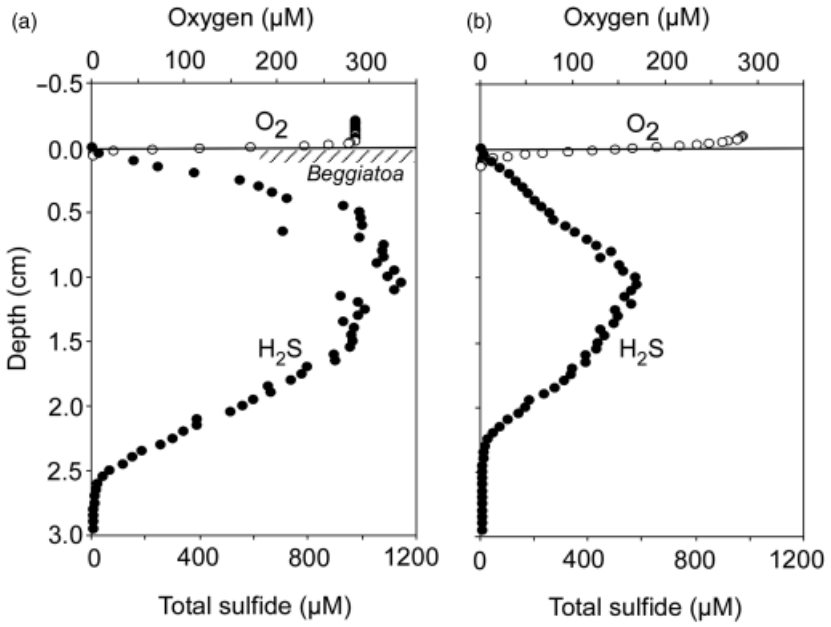

Fig. 6. Oxygen and sulfide (total H_2S) profiles in sediment from a shallow lagoon in Ymerbukta (Station DA). (a) Patch of white *Beggiatoa* mat. (b) Sediment just outside of the visible *Beggiatoa* mat.

mean areal biomass was 0.167 g m^{-2}, of which the 5-µm-wide filaments accounted for 40%.

On the sediment surface patches of white mats of *Beggiatoa* were also scattered, typically a few hundred cm^2 in size and dominated by 2- and 8–10-µm-wide filaments. Some patches were green to blue-green and were dominated by *Oscillatoria*-like cyanobacteria with filaments of mostly 18 or 25 µm diameter. Interestingly, these filamentous cyanobacteria often occurred down to > 4 cm depth in the sediment. The cyanobacterial patches also contained many *Spirulina*-like cyanobacteria, some *Beggiatoa* and different pennate diatoms. The sediment beneath the white and green patches was gray to black and highly sulfidic.

In a studied patch with a *Beggiatoa* mat on the surface, oxygen penetrated only to 0.4 mm depth (Fig. 6a), while the penetration depth just outside the visible *Beggiatoa* mat was 0.8 mm (Fig. 6b). In both cases, sulfide overlapped a few hundred µm with oxygen, but did not reach the sediment surface. The *Beggiatoa* mat was only about 1 mm thick and covered the O_2–H_2S interface and the uppermost front of the sulfide zone. The maximum sulfide concentration was found at 1 cm depth and was twice as high below the *Beggiatoa* mat (1100 µM, Fig. 6a) as just outside the mat (600 µM, Fig. 6b). These near-surface peaks of sulfide were extremely high and indicated intensive sulfate reduction driven by a high pool of organic matter in the sediment. Accordingly, we found abundant remains of decomposing macroalgae buried just under the sediment surface, probably brought into the lagoon during storms and covered by fine-grained sediment during calm weather. The steep decrease in sulfide below the peak indicates that a large pool of reactive Fe(III) was also mixed into the sediment and was

precipitating different iron–sulfide minerals and thus causing the black color of the sediment.

Nitrate-accumulating arctic *Beggiatoa*

Beggiatoa filaments of different diameters were used to either measure the internal nitrate concentration or to determine their phylogenetic affiliation. Two parallel batches of 16–20-μm-wide filaments from Station J in Smeerenburgfjorden contained 86 and 134 mM nitrate, respectively, while a batch of 13–15-μm-wide filaments contained 260 mM nitrate. The mean nitrate concentration for these large *Beggiatoa* was thus *c.* 130 μM. In contrast, *Beggiatoa* of 8–10 μm diameter from a mat in Ymerbukta contained only 2.7 ± 0.2 mM nitrate.

Phylogenetic analysis of 20 μm wide *Beggiatoa* from Smeerenburgfjorden (clone S3678; Fig. 7) revealed that the closest relatives include nitrate-storing *Beggiatoa* spp. from the brackish Limfjorden, Denmark (AF532775), and from an intertidal mud flat at Dangast, German Wadden Sea (AF532769). All these *Beggiatoa* are relatively large, with diameters between 9 and 17 μm, and accumulate nitrate in intracellular vacuoles (Mußmann *et al.*, 2003). Information on possible nitrate storage in the closest relative, a marine, uncultured *Beggiatoa* from Tokyo Bay (AB108786), was not provided by the original investigators, but seems likely due to the presence of an internal vacuole (Kojima & Fukui, 2003).

Discussion

The fjords of Svalbard provide some of the most extreme arctic, coastal sediments, given the latitudes ranging between 78° and 80°N, continuous daylight in summer for 3–4 months and corresponding dark night in winter, and seawater temperatures always near the freezing point. Our study demonstrates for the first time the widespread, yet scattered occurrence of large filamentous sulfur bacteria, *Beggiatoa* spp., in such permanently cold sediments. An earlier study of a conspicuous white bacterial mat of unidentified *Gammaproteobacteria* in Young Sound on the north-east coast of Greenland also reported the presence of *Beggiatoa*, but in very low numbers (Glud *et al.*, 2003).

The occurrence of *Beggiatoa* is best known from sites where sulfide reaches the sediment surface and where thin, visible mats are formed at the narrow oxygen–sulfide interface (Jørgensen & Revsbech, 1983). Previously, white mats of sulfur bacteria have been observed in the cold deep sea and also in the Arctic. These mats are, however, often associated with seep systems where sulfidic pore fluid emerges at the sediment surface. A remarkable example is the Håkon Mosby mud volcano in the deep North Atlantic west of Spitsbergen, where dense *Beggiatoa* mats have been observed at 1250 m water depth thriving at a temperature of -0.5 °C (de Beer *et al.*, 2006; Niemann *et al.*, 2006). It is therefore not a new finding that marine *Beggiatoa* can live at near-zero temperatures, provided that sufficient sulfide is available. In this study, *Beggiatoa* mats on the sediment surface were only observed in the shallow lagoon of Ymerbukta where microbial sulfate reduction coupled to decomposing macroalgae just beneath the sediment surface provided a high sulfide flux from below. At most other stations, *Beggiatoa* were found to thrive within the sediment at depths down to 2–5 cm.

Controls on *Beggiatoa* distribution

There is no indication that low temperature is directly inhibitory to the growth and distribution of *Beggiatoa*. The arctic communities of *Beggiatoa* are apparently cold adapted, and at some sites, large communities are able to thrive at temperatures permanently near the freezing point. Recent measurements of the gliding speed of 8–10 μm wide

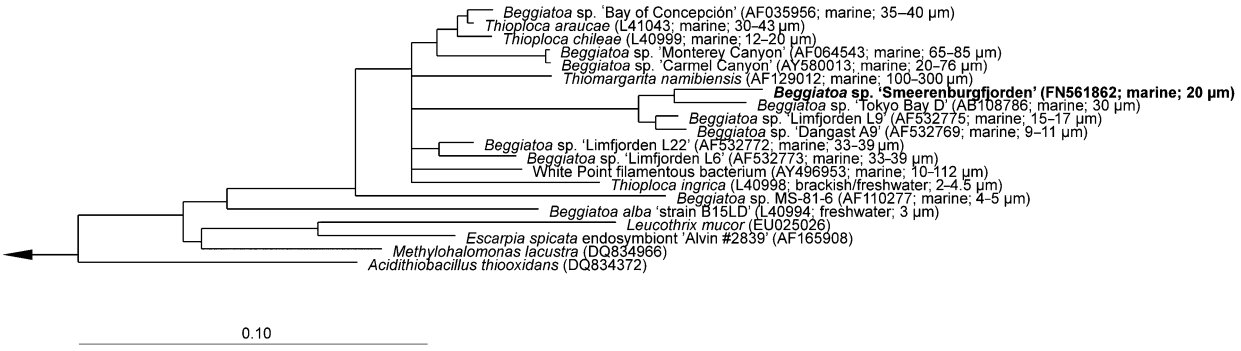

Fig. 7. Phylogenetic 16S rRNA gene-based tree showing the affiliation of the uncultured *Beggiatoa* spp. clone S3678 from Smeerenburgfjorden in Svalbard (FN561862, indicated by bold type) to selected reference sequences within the *Gammaproteobacteria*. Following each accession number, information on the general habitat (marine/freshwater) and cell diameter is given in parentheses. The bar indicates 10% estimated phylogenetic divergence.

Svalbard *Beggiatoa* showed that these were moderately psychrophilic, with an optimum at 17 °C. They continued their gliding motility even down to −5 °C and immediately recovered, without motility loss, from a transient freezing at that temperature (Dunker *et al.*, 2010). Because the upper part of the lagoonal sediment in Ymerbukta freezes solid in winter, it is an important observation that both *Beggiatoa* and sulfate-reducing bacteria from this environment survive freezing without or with only a slight detrimental effect on their metabolic rates (Dunker *et al.*, 2010; Sawicka *et al.*, in press; Mountfort *et al.*, 2003). By comparison, *Beggiatoa* from a sediment of the temperate North Sea were immobilized, and probably killed, by transient freezing, but survived freezing, provided that the population had been cold adapted over a few months (Dunker *et al.*, 2010).

The availability of H_2S is critical for *Beggiatoa* to establish a community in marine sediments. The SRR measured in the permanently cold Svalbard sediments, 1.3–4.5 mmol m^{-2} day^{-1} (Table 1), tend to be of a similar magnitude as the rates measured in temperate sediments (Canfield *et al.*, 2005; Jørgensen & Kasten, 2006). The key control on these rates is provided by the phytoplankton productivity in the water column and thus the deposition and burial of degradable organic matter in the sediment. The fjords of Svalbard are relatively deep, typically 70–200 m, with steep rocky coasts and hardly any terrestrial organic material coming from the barren and extensively ice- and snow-covered land. Yet, the primary productivity during the ice-free season is relatively high, and the rates of microbial metabolism (oxygen uptake, metal reduction and sulfate reduction) in the sediments are correspondingly high (Glud *et al.*, 1998; Arnosti & Jørgensen, 2006; Vandieken *et al.*, 2006).

The most common, yet mostly unnoticed occurrence of marine *Beggiatoa* is within the top several cm of slightly oxidized sediment (Jørgensen, 1977). This zone is characterized by the abundance of oxidized iron minerals, which provide the sediment with a light gray to brown color. The zone generally has no detectable sulfide, and yet often a high rate of sulfate reduction. The sulfide produced is thus turned over immediately, mostly by reaction with oxidized iron minerals, but also by *Beggiatoa*. It is a prerequisite for the occurrence of *Beggiatoa* in this zone that the filaments are able to glide through the sediment. It has been noticed in temperate sediments that a mixture of silt and very fine sand may physically exclude *Beggiatoa* because gliding motility is not possible (Jørgensen, 1977).

The occurrence of visible *Beggiatoa* mats on the sediment surface was associated with buried macroalgae, which were the source of intense sulfide production. The oxygen flux into the *Beggiatoa* mat was more than twice the oxygen flux just outside the *Beggiatoa* patch (61 and 24 mmol m^{-2} day^{-1}, respectively, Fig. 6). The corresponding sulfide fluxes were 34 and 13 mmol m^{-2} day^{-1}, respectively, yielding flux ratios of 2.1 : 1 (O_2 : H_2S) in the mat and 1.8 : 1 outside the mat. This corresponds in both cases to a complete oxidation of sulfide to sulfate with oxygen, which would imply a 2 : 1 stoichiometry:

$$2O_2 + H_2S \rightarrow SO_4^{2-} + 2H^+ \quad (2)$$

A flux ratio of 2 : 1 or slightly higher was also found by microsensor studies of a temperate *Beggiatoa* mat (Jørgensen & Revsbech, 1983). This stoichiometry is characteristic of a community in a steady state where the *Beggiatoa* biomass or their elemental sulfur content is not increasing (Nelson *et al.*, 1986). The complete sulfide oxidation to sulfate outside of the *Beggiatoa* mat could be due to other, nonconspicuous sulfide-oxidizing bacteria, for example of the *Thiobacillus* or *Thiomicrospira* group. A purely chemical sulfide oxidation would expectedly not lead to a quantitative conversion to sulfate, but rather to sulfur compounds of an intermediate oxidation state, such as elemental sulfur or thiosulfate.

In the sediments where the oxygen and sulfide were separated by an intermediate oxidized zone inhabited by *Beggiatoa* (Fig. 4), the pH profile showed a sharp minimum near the oxygen front. Because there was no significant gradient of free sulfide, the pH minimum may be due to the oxidation of elemental sulfur to sulfate in *Beggiatoa* as proposed by Sayama *et al.* (2005). It may also be due to the reoxidation of free Fe^{2+} produced by iron reduction in the intermediate zone. Preisler *et al.* (2007) concluded from a similar pH minimum in a *Beggiatoa*-inhabited Baltic Sea sediment that the oxidant for Fe^{2+} was MnO_2 rather than O_2, both of which would produce excess H^+ and thus a pH minimum. On the contrary, other oxidation processes with MnO_2 involving pyrite, iron sulfide or organic matter consume H^+ and may generate the broad pH maximum in the oxidized zone (Preisler *et al.*, 2007).

In Table 1, we compare the occurrence of *Beggiatoa* to the SRR, i.e. to the rate of sulfide formation and, thus, presumably to the availability of sulfide for these sulfide-oxidizing bacteria. Station A in Adventfjorden had the highest areal rates of sulfate reduction, and yet *Beggiatoa* were not found. Perhaps this sediment was physically not accessible to *Beggiatoa* due to the lack of pore space for gliding motility. Smeerenburgfjorden sediment with abundant *Beggiatoa* was also in the higher end of the SRR range, but overall, there was no clear correlation between the rates of sulfide production and the occurrence of *Beggiatoa* (Table 1).

Ecology of arctic *Beggiatoa*

The ability to store nitrate enables the large marine *Beggiatoa* of Svalbard to thrive in the oxidized, but anoxic zone within the upper 2–4 cm of the sediment. Near the sediment

surface, they may take up nitrate, which, in the bottom seawater in fjords on the west coast of Svalbard, is mostly present at 1–10 μM concentration (Eilertsen *et al.*, 1989; Wang *et al.*, 2009). In the sediment below, they may effectively utilize the sulfide produced from bacterial sulfate reduction, which proceeds throughout this zone, although sulfide is near or below detection. Thus, in Smeerenburgfjorden, SRR reached 17 nmol cm^{-3} day^{-1} at 2–3 cm depth, where *Beggiatoa* were abundant, but where free sulfide concentrations did not exceed 3 μM (Fig. 4). With a measured porosity of 0.72 at that depth, this rate corresponds to 24 μM SO_4^{2-} reduced per day or 2 μM SO_4^{2-} reduced per hour. The turnover time of total sulfide in the *Beggiatoa* zone is therefore in the order of 1 h. The experimentally determined SRR data show that *Beggiatoa* do indeed have sulfide available, which they can oxidize with intracellularly stored nitrate. The low concentration and relatively fast turnover of free sulfide shows that the sulfide was oxidized at the depth where it was produced and did not diffuse away.

Even at the highest density of *Beggiatoa* found in Smeerenburgfjorden, they probably did not play an important role in the overall sulfide oxidation, as the following calculations show. The cell-specific rate of nitrate reduction in marine *Beggiatoa* of 24–30 μm diameter from a temperate sediment at 15 °C was found to be 13 mM NO_3^- day^{-1} (Preisler *et al.*, 2007). Arctic *Beggiatoa* of this size class living at 0 °C may have *c.* 5-fold lower metabolic rate, estimated from the difference in gliding speed (Dunker *et al.*, 2010), i.e. about 3 mM NO_3^- day^{-1}. Because the dominant biomass of *Beggiatoa* in Smeerenburgfjorden belonged to the wider size range (Fig. 3b) that stored on the order of 130 mM NO_3^-, this rate of nitrate reduction could keep the filaments supplied with electron acceptors for (130/3 =) *c.* 40 days without a refill. If the filaments carry out dissimilatory nitrate reduction to ammonium and oxidize sulfide completely to sulfate, then the stoichiometry of nitrate reduction to sulfide oxidation is 1 : 1 and the cell-specific rate of sulfide oxidation would also be about 3 mM sulfide day^{-1}:

$$H_2S + NO_3^- + H_2O \rightarrow SO_4^{2-} + NH_4^+ \quad (3)$$

This rate can be compared with the rate of sulfide production from the sulfate reduction measured. At 0–1 cm depth in the Smeerenburgfjorden sediment, the biomass of *Beggiatoa* was 150 μg cm^{-3}. This is equal to 0.15 mm^3 of *Beggiatoa* biovolume cm^{-3} of sediment and these *Beggiatoa* could oxidize ($0.15 \times 3 \times 10^{-6}$ =) 0.5 nmol sulfide cm^{-3} day^{-1}. The measured SRR at 0–1 cm was equal to 2 nmol H_2S cm^{-3} day^{-1} produced, i.e. fourfold higher. The same calculation for the 2–3 cm depth interval yielded 60 μg cm^{-3} of *Beggiatoa* biomass, which could oxidize 0.2 nmol sulfide cm^{-3} day^{-1}. The measured SRR at 2–3 cm was equal to 17 nmol H_2S cm^{-3} day^{-1} or nearly 100-fold higher. The conclusion is that, even at their highest biomass density in Smeerenburgfjorden, the *Beggiatoa* did not contribute significantly to the overall rate of sulfide oxidation. This is the same conclusion as that reached for a rather similar *Beggiatoa* population in a Baltic Sea sediment (Preisler *et al.*, 2007).

Mat-forming *Beggiatoa* of 8–10 μm diameter in Ymerbukta stored only 2.7 mM nitrate. It is possible that their chemoautotrophic life at the narrow interface of overlapping O_2 and H_2S (Fig. 6) is not as selective for a large internal nitrate storage as the subsurface life in Smeerenburgfjorden sediment with many hours or days of gliding away from the oxic surface zone.

Beggiatoa – members of the 'rare biosphere'?

During the microscopic search for *Beggiatoa* in Svalbard sediments, we also looked for other cells containing light-refractive globules that could indicate sulfur bacteria with elemental sulfur inclusions. This search for morphologically conspicuous sulfur bacteria was generally without positive results. In one core from Smeerenburgfjorden, however, we found a distinct sheath that harbored five filaments of 23 μm diameter. The sheath was 3.5 mm long and 70 μm wide and the individual filaments were 0.5–1.1 mm in length and rich in light-refractive inclusions. The terminal cells were rounded and it was thus not possible to distinguish the filaments morphologically from free-living *Beggiatoa*. The presence of a common sheath for a bundle of filaments, however, is diagnostic of the genus *Thioploca* and the diameter of 23 μm classified the filaments taxonomically into the species *Thioploca chileae* (Jørgensen *et al.*, 2005b). In contrast to the observed filaments, *Thioploca* spp. most often have tapered terminal cells.

The scattered occurrence of *Beggiatoa* of highly variable diameters in many sediments indicates that these bacteria are widely present in the Arctic, but in low numbers. A different approach than that used here would be required to scan a larger sediment volume ($\gg$ 0.1 g) in the search for *Beggiatoa*, for example by extracting DNA and searching for *Beggiatoa*-related 16S rRNA genes, provided that appropriate primers for amplification could be established. Such an environmental genomic approach, however, misses the unique advantage provided by the distinct morphology of *Beggiatoa*. *Beggiatoa* are of the size of micro- and meiofauna, rather than of the size of normal bacteria, and the organisms can therefore be quantified with similar sample volumes and techniques. Because of their extraordinary size, *Beggiatoa* are rarely recorded in marine sediments, although they are widely distributed. The main reason is that they are not detected by normal direct bacterial counts using fluorescent stains such as

acridine orange or DAPI. This becomes clear from the following example.

The densities of *Beggiatoa* filaments in Svalbard varied from < 10 to 1000 filaments cm^{-3} sediment. By direct fluorescence counts of bacteria, about 1 μg of sediment may be scanned under the light microscope at $\times 1000$ magnification. As the cell density of bacteria in normal fjord sediments is $> 10^9$ cells cm^{-3}, this would suffice to count > 1000 bacteria. However, in 1 μg of sediment, the likelihood of finding just one *Beggiatoa* at the above densities is $< 10^{-5}$–10^{-3}, i.e. highly unlikely. By the counting of *Beggiatoa*, we routinely counted all filaments in 30 mg of sediment, i.e. in a 10^5-fold larger sediment volume than is needed to count all other bacteria.

Even at their highest density, *Beggiatoa* filaments comprise only a millionth of all bacteria in the sediment. Yet, due to the very large biomass of each filament, 1–1000 ng, *Beggiatoa* may comprise a significant fraction of the total prokaryotic biomass. In Smeerenburgfjorden sediment, the mean total bacterial numbers were 3.4×10^9 cells cm^{-3} sediment in the top 0–2 cm (Ravenschlag *et al.*, 2000). With a typical biovolume of 0.2 μm^3 of sediment bacteria (Kuwae & Hosokawa, 1999), the living biomass per bacterial cell is 0.2 pg. The total prokaryotic biomass (excluding *Beggiatoa*) is thus 700 μg cm^{-3}. The total biomass of *Beggiatoa* at 0.5 cm depth was 50–100 μg cm^{-3}, i.e. 7–15% of the total prokaryotic biomass of other bacteria. This shows that, although a normal direct count of total bacteria in the sediment would not have detected any *Beggiatoa*, they may still account for a significant fraction of the total bacterial biomass. With respect to biomass and metabolic activity, they may thus be an important component of the microbial community. With respect to numbers, they are less than a millionth (on a per cell basis $< 1/10\,000$) and belong to the 'rare biosphere' (Sogin *et al.*, 2006), even at their highest densities.

Conclusions

Our results show that large, nitrate-accumulating *Beggiatoa* occur widespread in permanently cold sediments of the high Arctic, similar to temperate sediments (Mußmann *et al.*, 2003; Preisler *et al.*, 2007). The bacteria are not inhibited in their metabolic rate or in their motility and chemotactic behavior by temperatures near freezing, but are well adapted to the cold (Dunker *et al.*, 2010). Only in two out of 11 investigated fjord sediments were *Beggiatoa* abundant and arctic *Beggiatoa* were not found to contribute significantly to sulfide oxidation in the sediments studied. Nevertheless, our findings provide a new and interesting perspective on the biogeography of *Beggiatoa* by showing that these giant, conspicuous sulfur bacteria occur frequently as members of the rare biosphere in marine sediments.

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