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Quantitative Molecular Analysis of the Microbial Community in Marine Arctic Sediments (Svalbard)

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Fluorescence in situ hybridization (FISH) and rRNA slot blot hybridization with 16S rRNA-targeted oligonucleotide probes were used to investigate the phylogenetic composition of a marine Arctic sediment (Svalbard). FISH resulted in the detection of a large fraction of microbes living in the top 5 cm of the sediment. Up to 65.4% ± 7.5% of total DAPI (4′,6′-diamidino-2-phenylindole) cell counts hybridized to the bacterial probe EUB338, and up to 4.9% ± 1.5% hybridized to the archaeal probe ARCH915. Besides δ-proteobacterial sulfate-reducing bacteria (up to 16% 52) members of the *Cytophaga-Flavobacterium* cluster were the most abundant group detected in this sediment, accounting for up to 12.8% of total DAPI cell counts and up to 6.1% of prokaryotic rRNA. Furthermore, members of the order *Planctomycetales* accounted for up to 3.9% of total cell counts. In accordance with previous studies, these findings support the hypothesis that these bacterial groups are not simply settling with organic matter from the pelagic zone but are indigenous to the anoxic zones of marine sediments. Members of the γ-proteobacteria also constituted a significant fraction in this sediment (6.1% ± 2.5% of total cell counts, 14.4% ± 3.6% of prokaryotic rRNA). A new probe (GAM660) specific for sequences affiliated with free-living or endosymbiotic sulfur-oxidizing bacteria was developed. A significant number of cells was detected by this probe (2.1% ± 0.7% of total DAPI cell counts, 13.2% ± 4.6% of prokaryotic rRNA), showing no clear zonation along the vertical profile. Gram-positive bacteria and the β-proteobacteria were near the detection limit in all sediments.

Knowledge of the microbial diversity of marine pelagic and benthic communities has been greatly extended recently by molecular studies based on the analysis of 16S rDNA sequences (see, for example, references 9, 10, 20, 43, 44, 48, 50, 52, and 67). Numerous new 16S rDNA sequences have been retrieved both from marine sediments and from the water column, indicating that the vast majority of species has not been cultivated yet. Several studies using the cultivation-independent approach of 16S rDNA cloning have helped to elucidate common features within the microbial communities of specific habitats such as marine benthic environments (10, 35, 36, 52, 67). Furthermore, they have provided additional sequence information for the design and evaluation of nucleic acid probes for the identification and quantification of distinct bacterial populations.

While microbial diversity can be readily studied by PCR-based 16S rDNA cloning, community structure cannot be deduced from cloning studies (3) due to potential biases introduced during DNA retrieval and amplification (17, 53, 65). For reliable characterization of community structure, quantitative methods such as fluorescence in situ hybridization (FISH) or rRNA slot blot hybridization are more suitable (3). To date, a number of studies have been performed using either of these two methods to quantify different groups in marine sediments (15, 37, 38, 51, 57–59, 68). Most of these studies, however, focused on specific microbial groups such as sulfate-reducing bacteria (51, 58, 59) or *Archaea* (38, 68).

We describe here the community composition of a marine Arctic sediment (Smeerenburgfjorden, Svalbard) using both FISH and rRNA slot blot hybridization for quantification. The sulfate-reducing community of Smeerenburgfjorden sediment has recently been described in detail (51); sulfate reducers accounted for up to 16% of the total cell numbers and up to 29% of the prokaryotic rRNA. In the present study, we report the contribution of other major phylogenetic groups, such as the β- and γ-proteobacteria, the *Cytophaga-Flavobacterium* cluster, the *Planctomycetales*, and gram-positive bacteria, to the total microbial community along vertical gradients.

To the best of our knowledge, there is only a single previous study which has described the abundance of the different classes of proteobacteria, the *Cytophaga-Flavobacterium* cluster, the *Planctomycetales*, and gram-positive bacteria in marine sediments (37). Llobet-Brossa et al. used FISH for quantification. The present study, however, reports the first rRNA profiles of these major phylogenetic groups in marine sediments.

In addition to the quantification of these major phylogenetic groups, a new probe specific for a cluster of 16S rDNA clone sequences affiliated with free-living and endosymbiotic sulfur-oxidizing bacteria of invertebrates was developed and applied. Sequences of this group were abundant in a Svalbard sediment clone library (52) and also dominant in clone libraries from other marine sediments, e.g., different deep-sea sediments off Japan (35, 36), coastal sediments off Japan (67), and seagrass-colonized sediments from the Bassin d'Archachon (7). The potential ecological importance of this group is discussed with regard to its abundance, the stratification of its distribution, and the possible occurrence of symbiotic and free-living forms.

MATERIALS AND METHODS

Study site and sampling. Sediment samples were collected on 28 July 1998 from Smeerenburgfjorden, Svalbard, Arctic Ocean (79°42′815″N, 11°05′189″E,

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TABLE 1. Oligonucleotide probes used in this study

Probe	Specificity	Sequence (5′-3′)	Target RNA	Position[a] (nucleotide range)	FISH (FA)[b]	Slot blot T_d (°C)[c]	Source or reference
UNI1390	Universal—all organisms	GACGGGCGGTGTGTACAA	16S, 18S	1390–1407	NU	44*	72
ARCH915	*Archaea*	GTGCTCCCCCGCCAATTCCT	16S	915–935	35	56*	2
EUK1379	*Eucarya*	TACAAAGGGCAGGGAC	18S	1379–1394	NU	42*	28
EUB338	*Bacteria*	GCTGCCTCCCGTAGGAGT	16S	338–355	10	54*	1
NON338	Negative control	ACTCCTACGGGAGGCAGC	16S	338–355	10	NU	69
GP1199	Most gram-positive bacteria	AAGGGGCATGATG	16S	1199–1211		34*	B. J. MacGregor et al.[d]
GAM42a	γ-Proteobacteria	GCCTTCCCACATCGTTT	23S	1027–1043	35	60	40
BET42a	β-Proteobacteria	GCCTTCCCACTTCGTTT	23S	1027–1043	NU	58	40
PLA886	*Planctomycetales*, some *Eucarya*	GCCTTGCGACCATACTCCC	16S	886–904	35	62	46
CF319a	*Cytophaga-Flavobacterium* cluster	TGGTCCGTGTCTCAGTAC	16S	319–336	35	56	39
GAM660	16S rDNA clone sequences affiliated with endosymbionts and some other species in the γ-proteobacteria	TCCACTTCCCTCTAC	16S	660–674	35–40	52	This study

[a] Position in the 16S or 23S rRNA of *E. coli*.
[b] FA, formamide concentrations in the hybridization buffer calculated as the percent (vol/vol). NU, not used.
[c] *, Dissociation temperatures (T_d) were determined with the washing buffer containing 1× SSC and 1% SDS.
[d] B. J. MacGregor, S. Toze, E. W. Alm, R. Sharp, C. J. Ziemer, and D. A. Stahl, submitted for publication.

"station J"). The sediment temperature was 0°C, the surface water temperature was 5°C, and the water depth was 218 m. Sediment was sampled with a Haps-corer, subsampled, and kept at in situ temperature during transport (72 h). The sediment was characterized by a soft brown silty oxidized surface (upper 2 cm) overlaying a transition zone of darker, black-streaked clayey mud. Below the transition zone (2 to 6 cm) a black sulfidic zone followed. Worm tubes as well as small shells (2 to 3 mm) were present in the sediment to a depth below 10 cm. Two parallel cores subsampled from the same Haps-corer (distance between the two core samples, ca. 10 cm) were sliced: one-half of each slice was frozen in liquid nitrogen for RNA extraction (stored at −80°C); the other half was fixed for 2 to 3 h at a final concentration of 3% formaldehyde, washed twice with 1× phosphate-buffered saline (PBS; 10 mM sodium phosphate [pH 7.2], 130 mM NaCl), and was finally stored in 1× PBS-ethanol (1:1) at −20°C.

RNA extraction and slot blot hybridization. RNA was extracted from 1.5 ml of wet sediment (per layer) by bead beating, phenol extraction, and isopropanol precipitation as described previously (57). The quality of the RNA was checked by polyacrylamide gel electrophoresis. Approximately 50 ng of RNA was blotted onto nylon membranes (Magna Charge; Micron Separations, Westborough, Mass.) in triplicate and hybridized with radioactively labeled oligonucleotide probes as described by Stahl et al. (63). Membranes were washed at different temperatures depending on the dissociation temperature (T_d) of the probe. The probes used and dissociation temperatures are given in Table 1. The dissociation temperatures of the probes were determined as described by Raskin et al. (49) with slight modifications. For T_d determinations and hybridizations (probes BET42a, GAM42a, GAM660, CF319a, and PLA886), washing buffer with a lower sodium dodecyl sulfate (SDS) concentration was used (1× SSC [150 mM NaCl, 15 mM sodium citrate; pH 7.0]; 0.1% SDS). However, for hybridizations with probes Uni1390, EUB338, EUK1379, and ARCH915 a washing buffer with 1% SDS was used.

Quantification. Hybridization signal intensity was measured with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.) and quantified as described previously (59). Reference rRNA isolated from pure cultures of *Cytophaga lytica* (DSM 7489), *Pirellula marina* strain 1, *Methanolobus tindarius* (DSM 2278), *Arthrobacter* strain KT1113.15, *Zoogloea* strain Cadagno, *Halothiobacillus kellyi* (DSM 13162), and sulfur-oxidizing bacterium strain OAII 2, as well as the rRNA of *Saccharomyces cerevisiae* and *Escherichia coli* (purchased from Roche, Mannheim, Germany) served as standards for hybridization with the probes given in Table 1.

FISH. PBS-ethanol-stored samples were diluted and treated by mild sonication with an MS73 probe (Sonopuls HD70; Bandelin, Berlin, Germany) at a setting of 20 s, with an amplitude of 42 μm, and <10 W. An aliquot of 10 μl of a 1:40 dilution was filtered on 0.2-μm-pore-size GTTP polycarbonate filters (Millipore, Eschborn, Germany). Hybridization and microscopy counts of hybridized and 4′,6′-diamidino-2-phenylindole (DAPI)-stained cells were performed as described previously (62). Means were calculated from 10 to 20 randomly chosen fields on each filter section, corresponding to 800 to 1,000 DAPI-stained cells. Counting results were always corrected by subtracting signals observed with the probe NON338. Formamide concentrations are given in Table 1.

Oligonucleotides. Oligonucleotides were purchased from Interactiva (Ulm, Germany). For FISH, oligonucleotide probes were synthesized with the fluorescent dye Cy3 at the 5′ end.

RESULTS

Total cell counts and domain-specific probing. Total cell numbers were determined by DAPI staining. They were in the range of (2.1 to 4.7) × 10^9 ml of wet sediment^{-1} and showed little variation among two parallel sediment cores. There was no significant decrease of total cell numbers with increasing sediment depth (Table 2), even to a 19-cm depth.

Bacteria and *Archaea* were quantified by both FISH and rRNA slot blot hybridization, with domain-specific probes. FISH resulted in the detection of a large fraction of microbes living in the top 5 cm of the sediment. Up to 65.4% ± 7.5% of total DAPI cell counts hybridized to the eubacterial probe EUB338, and up to 4.9% ± 1.5% of the DAPI cell counts hybridized to the archaeal probe ARCH915 (mean of two cores along the vertical profile). The EUB338 detection rate strongly decreased by factors of 2.6 (core A) and 4.6 (core B) along a vertical profile from the sediment surface to a 10-cm depth. At a >10-cm depth, the detection rate was too low for further FISH analysis (<20% of total DAPI cell counts). Depth profiles of bacterial rRNA were in good accordance with profiles of FISH-detected cells. Recovered rRNA was highest near the surface (up to 13.6 μg ml of sediment^{-1}) and decreased with depth to 2.3 μg ml^{-1}.

Archaea mainly occurred only in numbers near the detection limit, set at 1% of DAPI-stained cells (Table 2). Only in the uppermost layer were *Archaea* found in higher numbers, with up to 6.4% of DAPI cell counts and 1.9 × 10^8 cells ml^{-1}. Below the surface layer, the relative contribution of *Archaea* remained relatively constant at approximately 1.0 to 1.5% of total DAPI cell counts along a vertical profile. No increase of *Archaea* cell numbers was detected in sediment layers at depths of 11 to 15 cm. Quantification of *Archaea* by slot blot hybrid-

TABLE 2. Quantification of bacteria by FISH and rRNA slot blot hybridization in Smeerenburgfjorden sediments (core A/core B)

Depth (cm)	Absolute prokaryotic cell nos (10^9 ml^{-1})	Total RNA, probe Uni1390 (ng of RNA ml^{-1})	*Bacteria* (probe EUB338)			*Archaea* (probe ARCH915)			*Eucarya* (probe EUK1379)		*Cytophaga-Flavobacterium* (probe CF319a)		
			% Total DAPI cell counts	% Universal RNA	ng of RNA ml^{-1}	% Total DAPI cell counts	% Universal RNA	ng of RNA ml^{-1}	% Universal RNA	ng of RNA ml^{-1}	% Total DAPI cell counts	% Prokaryotic RNA	ng of RNA ml^{-1}
0.25	2.9/2.1	15,658/18,429	57.9/72.9	61.3/47.8	9,601/9,845	6.4/3.3	1.7/0.9	264/169	19.2/26.3	3,010/4,841	9.6/12.8	6.1/5.4	606/481
0.75	3.5/3.3	14,750/22,860	55.9/73.6	66.7/59.3	9,845/13,564	1.8/1.8	1.3/1.2	191/282	12.4/15.9	1,829/3,629	10.3/10.4	5.5/5.8	554/799
1.25	3.1/3.4	13,943/20,448	56.1/65.4	51.5/50.8	7,179/10,391	2.9/1.8	1.0/1.0	142/206	25.1/30.4	3,500/6,225	7.4/6.8	4.1/4.1	299/433
1.75	4.2/4.2	11,449/13,035	53.1/57.7	58.9/62.8	6,744/8,183	2.1/1.5	1.2/1.1	135/142	23.9/15.2	2,734/1,983	7.2/4.8	4.3/3.8	297/315
2.25	4.1/3.5	11,276/18,647	57.5/48.4	62.3/50.5	7,028/9,417	1.8/1.5	1.2/1.0	133/188	18.0/20.8	2,026/3,883	7.6/3.0	4.2/5.4	299/522
2.75	3.7/3.3	7,795/8,318	47.9/51.2	63.4/69.6	4,944/5,791	1.4/0.8	1.2/0.9	93/75	21.4/12.0	1,666/995	8.5/7.1	4.3/3.6	217/213
3.25	2.7/3.2	8,266/9,157	43.7/46.6	56.1/69.3	4,638/6,344	1.4/1.7	1.0/0.8	86/73	25.8/12.0	2,129/1,258	7.6/3.9	4.1/3.3	193/215
3.75	3.1/3.7	7,896/9,342	42.8/50.0	64.9/70.8	5,125/6,613	1.4/1.7	1.1/0.6	87/58	12.1/13.7	954/1,181	8.2/3.3	3.7/3.4	192/229
4.25	3.5/3.4	5,964/6,494	43.5/47.3	59.2/77.3	3,531/5,019	1.4/0.4	1.4/0.8	83/52	18.3/12.6	1,093/895	4.3/2.8	4.9/3.1	177/159
4.75	3.4/3.6	5,559/5,952	28.4/42.2	63.4/73.5	3,526/4,373	1.4/1.1	1.4/0.9	79/54	15.3/13.8	853/811	4.4/1.8	4.2/3.2	152/140
5.5	3.5/4.6	3,306/4,956	26.0/36.0	77.7/80.6	2,570/3,997	ND[a]	1.4/1.0	46/49	8.3/15.5	274/769	1.8/2.0	4.0/3.4	106/137
6.5	3.7/4.7	3,345/4,897	24.1/27.4	87.6/90.8	2,929/4,448	ND	1.6/0.9	55/46	10.1/13.1	339/643	1.0/1.7	3.7/2.7	112/122
7.5	4.7/3.9	3,283/3,972	23.6/23.6	80.1/87.5	2,631/3,474	ND	0.9/1.2	31/46	12.4/13.5	408/536	0/0	3.5/3.1	93/110
8.5	2.9/3.1	3,599/4,331	28.9/22.6	78.5/73.9	2,824/3,200	1.0/1.7	1.3/1.1	45/46	11.7/12.9	421/559	0/0.5	3.4/3.5	97/112
9.5	3.7/3.9	3,199/3,589	22/15.7	71.1/79.7	2,273/2,861	1.0/0.9	1.1/1.0	35/38	12.6/14.9	402/534	0/0	3.5/3.1	81/90
11	3.9/3.4					1.7/0.7							
13	2.7/3.4					0.6/0.8							
15	2.5/3.4					0.6/1.2							
17	3.3/2.7												
19	2.7/2.1												

[a] ND, not determined.

TABLE 3. Further quantification of bacteria by FISH and rRNA slot blot hybridization in Smeerenburgfjorden sediments (core A/core B)

Depth (cm)	β-Proteobacteria, probe BET42a		γ-Proteobacteria (probe GAM42a)			Clone sequences and symbionts affiliated with the γ-Proteobacteria (probe GAM660)			*Planctomycetales* (probe PLA886)			Gram-positive bacteria (probe GP 1199)	
	% Prokaryotic RNA	ng of RNA ml^{-1}	% Total DAPI cell counts	% Prokaryotic RNA	ng of RNA ml^{-1}	% Total DAPI cell counts	% Prokaryotic RNA	ng of RNA ml^{-1}	% Total DAPI cell counts	% Universal RNA	ng of RNA ml^{-1}	% Prokaryotic RNA	ng of RNA ml^{-1}
0.25	1.6/1.7	161/155	12.1/9.0	17.6/19.4	1,739/1,744	2.9/2.9	11.7/6.9	1,153/621	2.2/1.9	30.7/17.0	4,815/3,131	0/0.2	0/17
0.75	1.8/1.7	180/229	11.6/9.2	20.7/19.4	2,075/2,813	1.8/2.7	9.5/10.5	950/1,449	3.0/2.8	23.5/16.1	3,464/3,684	0/0.4	0/52
1.25	1.8/1.5	132/159	8.9/9.5	22.2/17.8	1,626/1,888	2.5/2.8	11.6/10.8	851/1,142	3.6/3.5	53.6/19.7	7,468/4,037	0/0.5	0/49
1.75	1.4/1.6	99/134	8.0/6.9	20.2/16.8	1,388/1,402	0.8/2.0	17.0/8.2	1,170/682	2.8/2.7	26.0/20.6	2,975/2,683	0.1/1.4	4/116
2.25	1.3/1.6	96/158	7.3/6.2	17.5/16.2	1,254/1,558	1.6/3.1	10.8/11.4	772/1,094	3.7/3.8	19.1/17.2	2,154/3,204	0.5/1.3	35/129
2.75	1.1/1.2	58/72	5.5/6.1	14.5/13.3	733/780	1.2/2.1	18.1/6.1	910/359	2.8/3.9	15.8/13.1	1,229/1,091	0.7/2.2	36/129
3.25	1.3/1.2	60/75	4.7/4.7	14.5/12.5	686/804	2.6/2.7	18.4/6.7	871/431	3.5/3.3	19.1/14.1	1,578/1,287	0.3/2.6	16/167
3.75	1.3/1.1	70/77	8.7/6.0	14.7/12.6	764/841	2.6/2.8	20.1/9.0	1,050/599	3.1/3.6	19.3/13.5	1,524/1,257	0.2/2.1	13/137
4.25	1.1/1.1	40/57	4.2/4.2	13.7/11.8	495/596	2.2/2.5	17.6/7.0	635/353	1.7/1.9	16.2/15.2	964/984	0/1.2	0/63
4.75	1.3/1.1	47/45	5.1/3.6	13.4/11.5	483/509	2.2/2.7	20.4/9.5	734/423	1.8/1.8	26.5/14.0	1,475/831	0/0.7	0/31
5.5	1.2/1.1	31/45	4.4/5.0	11.9/12.0	311/486	2.8/2.4	14.2/14.0	3,730/565	2.9/1.5	21.8/16.0	720/793	0/0.3	0/13
6.5	1.2/1.2	36/56	5.8/4.0	11.2/11.6	336/520	1.8/2.1	16.6/14.2	496/638	2.5/3.1	27.8/14.3	931/701	0/0.1	0/6
7.5	1.2/1.1	31/38	6.4/3.0	9.3/11.8	246/415	2.4/1.3	18.3/11.6	4,878/408	1.7/2.8	31.0/14.5	1,019/575	0/0	0/0
8.5	1.2/0.9	26/30	4.2/2.9	9.3/11.4	268/369	0.6/1.9	19.4/12.0	557/391	1.3/2.0	31.8/15.7	1,143/681	0/0.2	0/6
9.5	0.9/1.0	20/28	4.0/3.0	10.0/11.7	231/338	1.8/0.4	21.3/12.4	493/359	1.9/1.2	38.7/19.0	1,238/681	0/1.4	0/40

ization were in the same range (0.6 to 1.7% of total rRNA) as determined by FISH.

Eukaryotic rRNA was quantified using probe EUK1379. The highest percentages were detected in the upper layers (0- to 3.25-cm depth). The mean in this region was 20.8% ± 5.8% of total rRNA, as compared to 13.2% ± 2.3% in the layers between depths of 3.75 and 9.5 cm. rRNA detected by the bacterial, archaeal, and eukaryotic probes were 80 to 100% of total rRNA as quantified using universal probe UNI1390. Especially in the upper layers, only about 80% of total rRNA was detected with the domain-specific probes.

The two parallel cores were quite similar in total cell numbers, FISH detection rates, and recovered rRNA (Tables 2 and 3). Therefore, in the following sections the mean values of the two cores are discussed.

Quantification of different bacterial groups. (i) *Cytophaga-Flavobacterium* cluster. A large fraction of the microbial community could be affiliated with the *Cytophaga-Flavobacterium* cluster (Tables 2 and 3). Their relative abundance ranged from 11% (3.5×10^8 cells ml^{-1}) in the uppermost layers to 3% (2.0×10^8 cells ml^{-1}) at a 5-cm depth (Fig. 1). Below 5 cm, CF319a-target cells were near or below the detection limit. *Cytophaga-Flavobacterium* rRNA detection was also highest at the sediment surface (5.7% of prokaryotic rRNA) and decreased slightly to 3.3% at 9.5 cm. CF319a target cells were morphologically highly diverse and included long and short rods (0.5 to 1.5 μm in length), filaments (up to 10 μm in length) and cocci. About three-quarters of the detected cells were very small (≤0.5 μm). Several very thin filaments could barely be detected by DAPI staining. Some of the *Cytophaga-Flavobacterium* cells (>30%) were found attached to sediment particles or other organic matrices (Fig. 2). These cells were difficult to remove from the particles by sonication.

(ii) *Planctomycetales*. Probe PLA886 is specific for *Pirellula* spp., *Planctomyces* spp., *Isophaera* spp., and several clone sequences within the order *Planctomycetales*. Furthermore, the probe also binds to a wide variety of eukaryotic 18S rRNAs. For FISH analysis, this lack of specificity is not relevant because, in general, a visual differentiation of *Eucarya* and *Bacteria* is possible based on the smaller cell size of the latter. Members of the *Planctomycetales* made up a quantitatively important fraction of the microbial community in the Smeerenburgfjorden sediments and ranged between 1.5 and 3.7% of the total prokaryotic cell counts. There was no clear maximum visible at any specific depth. The highest detection corresponded to 1.4×10^8 cells ml of $sediment^{-1}$. The cells were usually large cocci, approximately 1 μm in diameter (Fig. 2), occurring as single or rosette-forming cells or in disordered clusters of about 10 cells. All target cells showed a bright fluorescence signal. In the slot blot hybridization, the problem of hybridization of PLA886 to eukaryotic rRNA became relevant. Very high values (13.1 to 38.7% of total rRNA) were detected. A comparison of slot blot profiles for probes PLA886 and EUK1379 showed similar maxima.

(iii) γ-Proteobacteria. γ-Proteobacteria, as detected by probe GAM42a, comprised a dominant group in the Smeerenburgfjorden sediments (Tables 2 and 3). In the upper layers, this group accounted for up to 10.5% of the total DAPI cell counts. Detection by FISH decreased slightly with increasing depth and was lowest at a 10-cm depth, with 3.5% of the total DAPI cell counts. The morphology of the GAM42a target cells was quite diverse (Fig. 2). The cell size varied, but a large fraction of detected cells was very small (size, ≤0.5 μm). The majority of target cells had a very bright FISH signal, indicating a high cellular rRNA content. The γ-proteobacterial rRNA also made up a quantitatively important fraction of the microbial-community rRNA, with up to 20.0% of prokaryotic rRNA hybridizing to GAM42a. The relative contribution to the prokaryotic rRNA decreased by a factor of approximately 2 from the surface to the 10-cm depth.

Potential sulfur-oxidizing bacteria within the γ-proteobacteria. Probe GAM660 was designed to be specific for clone sequences affiliated with free-living and endosymbiotic sulfur-oxidizing bacteria which were abundant in a Svalbard sediment clone library (52). Because of their phylogenetic affiliation, these sequences could potentially originate from sulfur-oxidizing bacteria. In addition to our clone sequences, probe GAM660 also targets closely related (up to 97.9%) γ-proteobacterial sequences which were retrieved from other marine sediments (7, 35, 36), endosymbionts of *Riftia pachyptila*, other vestimentiferan tubeworms and of several bivalves (11–14, 18, 31), *Thiobacillus ferrooxidans*, *H. kellyi* and *Coxiella burnetii* (Table 4). A clear discrimination between target and nontarget organisms was possible with FISH as well as with rRNA slot blot hybridization. Probe GAM660 hybridized to free-living bacteria in Smeerenburgfjorden sediment samples. Up to 2.9% of the total DAPI cell counts (9.4×10^7 cells ml^{-1}) were detected in the surface layer. In deeper layers, the detection rate remained relatively constant and varied between 0.4 and 3.1% of the total DAPI cell counts. In general, targeted cells were cocci that very often occurred as diplococci (Fig. 2). Due to their small size, it was impossible to investigate targeted cells for the presence of sulfur inclusion bodies. The FISH-detected fraction was relatively small compared with the fraction (13.2% ± 4.2% of prokaryotic RNA) detected by slot blot hybridization.

Other probes used. Members of the β-proteobacteria and of the gram-positive bacteria were only quantified by slot blot hybridization. For probe Bet42a, maximum values of 1.7% of the total DAPI cell counts were detected at the surface, decreasing to 1% in deeper layers. The rRNA of gram-positive bacteria was barely detected in the upper and lower layers and reached a maximal mean of 1.4% prokaryotic rRNA at 3 cm.

DISCUSSION

Total cell counts in the Smeerenburgfjorden sediments were relatively constant along a vertical profile from the sediment surface to a 20-cm depth. The average abundance of (3.4 ± 0.6) $\times 10^9$ ml^{-1} was comparable with previous reports for other marine sediments (see, for example, references 37, 57, and 70), although in contrast to our results, all other studies, including one of four other sampling sites off the coast of Svalbard (57), reported decreasing cell numbers with depth. In Svalbard sediments, Sahm et al. reported cell numbers decreased with depth by factors of 3, 7, and 9 within the first 28 cm (57). In Wadden Sea sediments, the total cell numbers decreased by a factor of 2.4 within the first 5 cm of the sediment (37). Wellsbury et al. (70) reported constant cell numbers in the uppermost layers (up to an 8-cm depth) of an estuarine

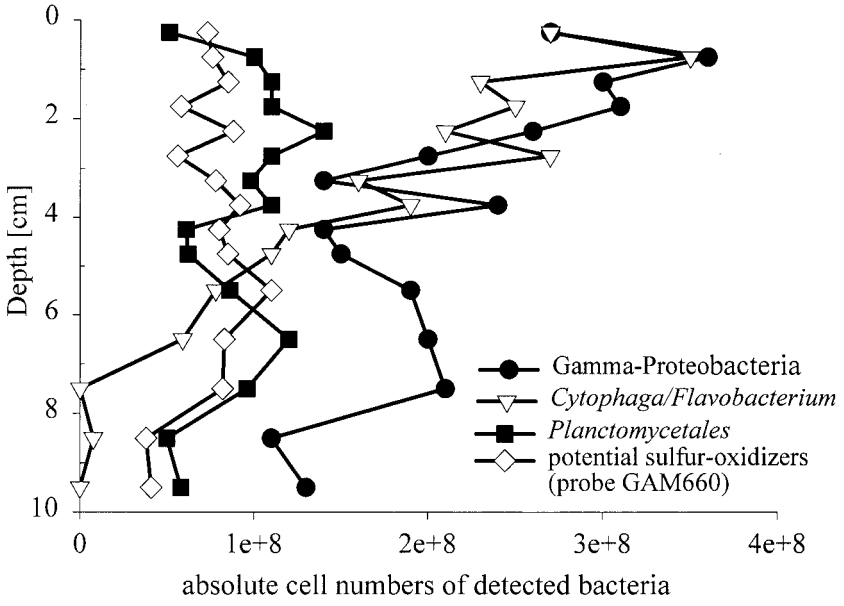

FIG. 1. Vertical profiles of absolute numbers of bacteria detected. The mean of two parallel cores is shown.

sediment. They explained this rather unusual depth profile by a high tidal influence and high sediment porosity. In our case, tidal influence can be excluded. The sediment, however, was characterized by a relative high water content in the first 2 to 3 cm. Since most sediment bacteria can be found attached to particles, a higher pore water content leads to lower cell numbers per milliliter and could be one cause of the constant cell numbers throughout the profile. In contrast, the total level of RNA detectable by universal or domain-specific probes decreased markedly over the same depth. The most likely reason for the decrease in total rRNA recovered with depth is a lower cellular ribosome content with depth according to the available organic substrates. This is supported by a lower mean FISH signal of the cells in the deeper layers of the sediment (51).

As in other sediments (57, 59, 61) the recovered rRNA was mainly of bacterial and eukaryotic origin. *Archaea* made up only a minor part of the microbial community, with about 1 to 3% of total cells and of prokaryotic rRNA. Although a relatively large number of cells were not detected by the domain-specific probes in FISH, the lack of detection of significant amounts of archaeal rRNA in the slot blot hybridization suggests that *Archaea* are not a major component of this arctic sediment. To date, probe ARCH915 includes more than 95% of currently available archaeal sequences in the databases. Low

TABLE 4. List of organisms or clone sequences targeted by GAM660

Target	Accession no.	Target	Accession no.
Solemya terraeregina gill symbiont	U62131	Uncultured γ-proteobacterium TK99	AB022639
Solemya pusilla gill symbiont	U62130	Unidentified γ-proteobacterium JTB256	AB015255
Thyasira flexuosa gill symbiont	L01575	Unidentified γ-proteobacterium JTB255	AB015254
Riftia pachyptila trophosome symbiont	M99451	Unidentified γ-proteobacterium JTB23	AB015248
Riftia pachyptila endosymbiont	U77478	Unidentified γ-proteobacterium BD5-16	AB015571
Lucinoma aequizonata gill symbiont	M99448	Unidentified γ-proteobacterium BD3-6	AB015548
Anodontia phillipiana gill symbiont	L25711	Unidentified γ-proteobacterium BD3-1	AB015547
Ridgeia piscesae endosymbiont	U77480	Unidentified marine eubacterium	L10949
Lamellibrachia columna endosymbiont	U77481	Uncultured γ-proteobacterium n7d	AF194195
Lucina pectinata gill symbiont	X84980	Uncultured γ-proteobacterium B2M61	AF223300
Escarpia spicata endosymbiont	U77482	Uncultured γ-proteobacterium B2M60	AF223299
Vestimentiferan endosymbiont Shinkai 6500	AF65907	Uncultured γ-proteobacterium B2M54	AF22329
Unidentified bacterium HNSS31	Z88573	Uncultured γ-proteobacterium B2M28	AF223297
Unidentified marine eubacterium	U84621	Uncultured γ-proteobacterium B2M23	AF223296
Uncultured γ-proteobacterium Sva1046	AJ240991	Uncultured marine eubacterium HstpL26	AF159683
Uncultured γ-proteobacterium Sva0864	AJ240988	Uncultured bacterium clone Car16fa	AF224800
Uncultured γ-proteobacterium Sva0071	AJ240986	Uncultured bacterium BURTON-10	AF142830
Uncultured γ-proteobacterium Sva0115	AJ240974	*Thiomicrospira* sp. strain Tms-MPN/Milos-CIVI	AJ247759
Uncultured γ-proteobacterium Sva0304	AJ240971	*Halothiobacillus kellyi*	AF170419
Uncultured γ-proteobacterium Sva0857	AJ240969	*Beggiatoa* sp. strain 1401-13	L40997
Uncultured γ-proteobacterium Sva0862	AJ240968	*Coxiella burnetii*	D89799
Uncultured γ-proteobacterium Sva0120	AJ240993	*Thiobacillus ferrooxidans*	M79416
Uncultured proteobacterium clone 1605-59	AJ007658	*Dechloromarinus chlorophilus*	AF170359
Uncultured γ-proteobacterium SA28	AB022641	*Alkalispirillum mobilis*	AF114783

Archaea counts are in accordance with previous studies from other marine sediments (37, 57).

The *Cytophaga-Flavobacterium* cluster and the order *Planctomycetales* typically contain aerobic species. *Cytophaga-Flavobacterium* have been shown to be abundant in the marine water column (16, 19). Recently, Llobet-Brossa et al. found significant cell numbers of both groups in Wadden Sea sediments, even in anoxic zones (37). Data from clone libraries derived from several marine sediments (20, 35, 36, 52) and a freshwater sediment (42) supported this finding. Input of complex organic substrates to anaerobic sediments resulted in a strong increase among members of the *Cytophaga-Flavobacterium* cluster (56). These findings indicate a potential ecological relevance of these bacteria as hydrolytic fermentative organisms in a mainly anaerobic habitat. In our study, the *Cytophaga-Flavobacterium* cluster, along with the γ-proteobacteria and sulfate reducers, was one of the three most abundant groups, with high numbers of more than 1.5×10^8 cells ml^{-1} also in the anoxic layers up to a depth of 4.75 cm. Oxygen profiles from a close station off the coast of Svalbard with a similar water depth indicated an oxygen penetration depth of about 7 mm (29). Calculations of cellular rRNA contents of *Cytophaga-Flavobacterium* cells made by combining FISH-detected cell numbers and the detected rRNA revealed relatively constant cellular rRNA contents with depth (range, 0.1 to 0.2 fg of rRNA per cell). *Planctomycetales* made up between 1.2 and 3.9% of DAPI-stained cells down to a depth of 9.5 cm, with a maximum in their proportional contribution at 2.25 cm. These data support the hypothesis that these bacterial groups are multiplying even in anoxic zones in the sediment.

A reliable quantification of *Planctomycetales* rRNA was not possible because of the cross-hybridization of probe PLA886 with a wide variety of *Eucarya*. A comparison of the slot blot profiles for probes PLA886 and EUK1379 showed similar shapes and maxima. Therefore, there was presumably a very high contribution of eukaryotic rRNA to PLA886 target rRNA. Since not all organisms targeted by EUK1379 are also targeted by PLA886, a simple subtraction of the values is not possible.

Sulfur-oxidizing bacteria isolated from marine sediments are often members of the genera *Thiomicrospira* (5, 6, 32) or *Thiobacillus* (54). In addition, *Beggiatoa* and *Thioploca* spp. have often been found in sediments and are used for ecophysiological studies (23, 30, 41). *Thiomicrospira* and *Thiobacillus* spp. were often retrieved from most-probable-number (MPN) dilution series for chemolithoautotrophic sulfur-oxidizing bacteria, but only in maximal numbers of 1.4×10^6 cells ml of $sediment^{-1}$ (5, 60). In MPN dilution series of Smeerenburgfjorden sediments, the growth of chemolithoautotrophic sulfur-oxidizing bacteria was observed to 10^{-3} dilutions. This result contradicts the idea that they might be numerically abundant. Using the new probe GAM660 which is specific for 16S rDNA clone sequences affiliated with free-living or endosymbiotic sulfur-oxidizing bacteria retrieved from several marine sediments (7, 35, 36, 52, 67), an abundance of up to 1.1×10^8 cells ml^{-1} was demonstrated in Smeerenburgfjorden sediment. In Wadden Sea sediments, this group was also detected by FISH and accounted for up to 4.6×10^7 cells ml^{-1} sediment (up to 2.3% of total DAPI cell counts; S. Kolb and K. Ravenschlag, unpublished data). Further functional studies of GAM660 target organisms are needed to find out if these abundant bacteria are really sulfur oxidizers. Possible experiments include large-insert DNA libraries (55, 64) of GAM660 target cells for the identification of genes involved in the sulfur oxidation or the combination of microautoradiography with

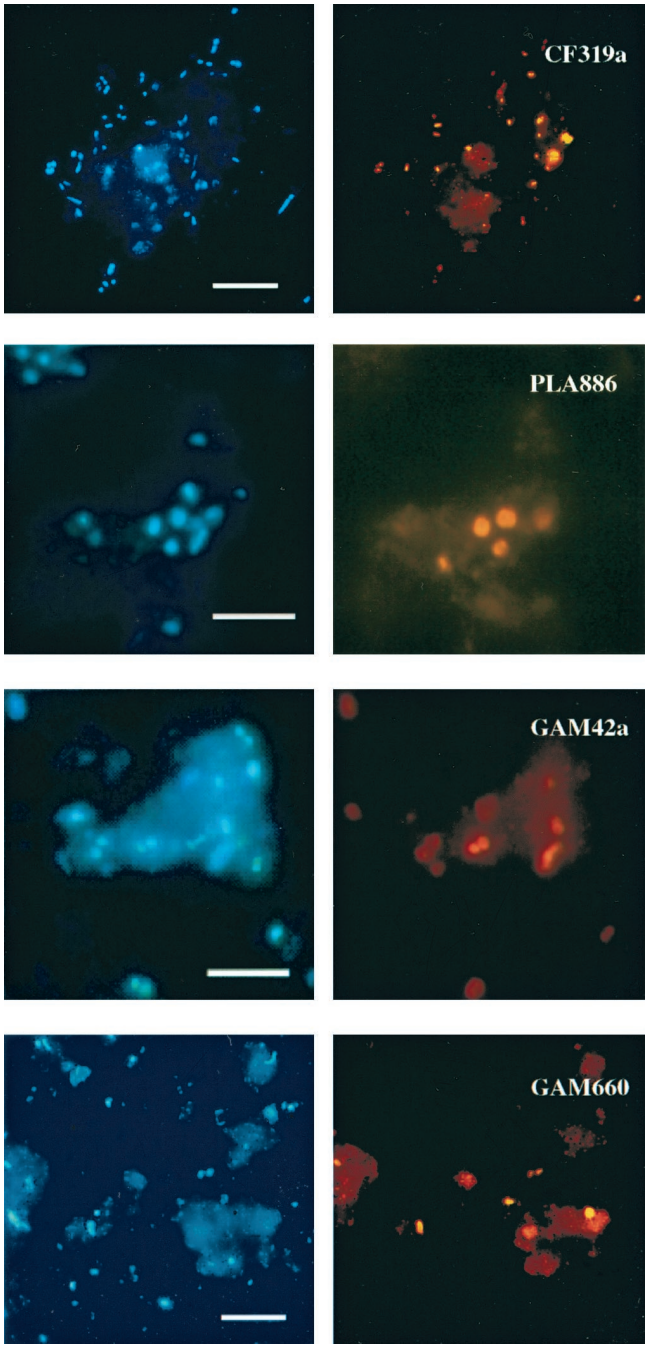

FIG. 2. Epifluorescence micrographs of bacteria in sediment samples from Smeerenburgfjorden (Svalbard). Specific hybridizations for the *Cytophaga-Flavobacterium* cluster (CF319a), for *Planctomycetales* (PLA886), the γ-proteobacteria (GAM42a), and a γ-proteobacterial subgroup which is affiliated with free-living and symbiotic sulfur-oxidizing bacteria (GAM660) and corresponding DAPI staining (same microscopic field). Bar, 5 μm (all panels).

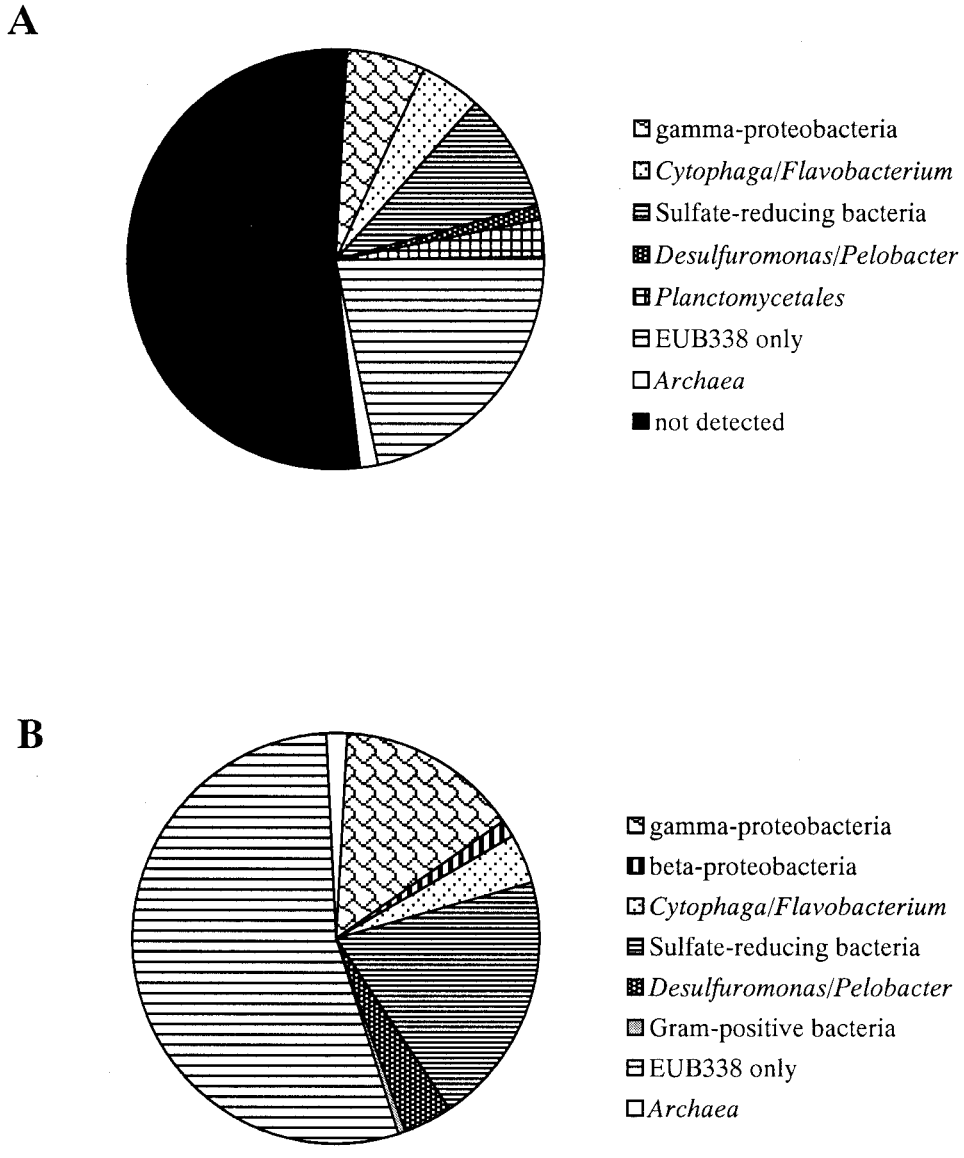

FIG. 3. Overview of the microbial community structure of Smeerenburgfjorden sediments as revealed by FISH (A) and quantitative rRNA slot blot hybridization (B). For the individual phylogenetic groups mean of the abundance along the vertical profile was calculated. Fractions shown indicate the percentage of total DAPI-stained cells (FISH) and the relative percentage of prokaryotic rRNA (slot blot), respectively. Since probe PLA886 targeting *Planctomycetales* is also targeting a wide variety of eukaryotes (see the text), the results are not included in part B. Due to the FISH results it can be expected that a fraction of the unidentified EUB338 target rRNA is formed by *Planctomycetales*.

FISH, allowing the assignment of radiotracer uptake to specific phylogenetic groups (8, 34, 47).

In some layers, detection of the subgroup GAM660 target rRNA was even higher than the rRNA yield of total γ-proteobacterial rRNA. Due to the stringent washing temperature, hybridization with nontarget organisms having one mismatch to the probe sequence can be excluded. However, the discrepancy cannot currently be clarified, because GAM42a targets 23S rRNA. GAM660 targets mostly uncultivated organisms for which the 23S rRNA sequences are yet unknown and cannot be determined easily.

The relative contribution of GAM660 rRNA was significantly higher than for FISH-detected cells (2.4- to 32.3-fold). GAM660 also targets chemoautotrophic symbionts from several bivalve molluscs and tubeworms. Thus, the high relative percentage of GAM660-rRNA could mean a contribution of rRNA derived from endosymbiotic bacteria of bivalves or other eukaryotic hosts. Such bacteria would not have been counted in the FISH analysis due to exclusion during pipetting or sedimentation in dilution steps. The rRNA of these organisms and their hosts, however, might be included in the extracted rRNA used for slot blot hybridization. Chemoautotrophic symbionts have not yet been cultured from their hosts, nor has a free-living stage of the symbionts been isolated from the environment. There is evidence that some hosts obtain their symbionts via environmental transmission (21, 22, 33), which involves the reinfection of the new host generation from an environmental stock of free-living symbiont forms as done by,

for example, *Codakia orbicularis* (21). GAM660 target cells could potentially represent such a free-living symbiont form. The vertical profiles of GAM660-detected rRNA, and GAM660 target cells showed no stratification as might be expected for aerobic chemoautotrophic organisms. However, nitrate respiration has been demonstrated in several endosymbionts, for example, from *Solemya reidi* (71), *Riftia pachyptila* (27), and *Lucinoma aequizonata* (26), as well as in the ectosymbionts of nematodes (25). For the endosymbiotic bacteria, motility of the hosts might be another explanation for the lack of zonation.

We did not screen for α-proteobacteria because the available probes, ALF1b (40) and ALF968 (45), also target a wide variety of δ-proteobacterial sequences, including sulfate-reducing bacteria and members of the genera *Pelobacter*, *Geobacter*, *Desulfuromonas*, *Synthrophus*, and *Polyangium-Chondromyces*. In this sediment, δ-proteobacteria contributed up to 34.5% of prokaryotic rRNA and up to 17.5% of total cell counts (51) and therefore have greatly affected the detection of α-proteobacteria.

Adding up the mean detection rates along a vertical profile for the different bacterial groups (including the large fraction of sulfate-reducing bacteria (51), 57.8% ± 12.7% of total detectable bacterial cells (23.9% ± 7.5% of total DAPI cell counts), and 44.9% ± 5.5% of bacterial rRNA could be assigned to specific phylogenetic groups (Fig. 3). One explanation for the relatively large "black box" could be the limited coverage with the current probe set, which has been shown by the rapid growth of the 16S rRNA sequence database to be rather incomplete. Furthermore, there are certainly other bacterial groups which make up a quantitatively important fraction in the Smeerenburgfjorden sediments. For example, 16S rDNA sequences affiliated with the order *Verrucomicrobiales* (24) were repeatedly found in clone libraries from marine sediments (52, 67) or marine snow (50), and sequences related to *Arcobacter* spp. or other ε-proteobacteria were repeatedly retrieved from marine sediments (4, 35, 36). Furthermore, the genus *Arcobacter* accounted for up to 1.6% of total cell counts in Wadden Sea sediments (37). An ability to carry out nitrate reduction and sulfide oxidation has been reported for *Arcobacter* spp. (66). Further studies will be needed to investigate the quantitative contribution of *Verrucomicrobium* spp., *Arcobacter* spp., and as-yet-unknown phylogenetic groups to microbial communities of marine sediments.

This study reports the first rRNA profiles for major phylogenetic groups in marine sediments and compares these data with abundances determined by FISH. More combined quantitative studies of microbial community structures in marine sediments are needed to identify common benthic features. Furthermore, studies are needed to identify the organisms contributing to the large "black box." A major goal for future work will be to combine these data with measurements of microbial activities to address the functional role of abundant phylogenetic groups in the microbial community.

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