APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1996, p. 2138–2144 Vol. 62, No. 6
0099-2240/96/$04.00+0

Community Analysis of the Bacterial Assemblages in the Winter Cover and Pelagic Layers of a High Mountain Lake by In Situ Hybridization

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Received 20 November 1995/Accepted 5 April 1996

The bacterial community structure in the winter cover and pelagic zone of a high mountain lake was analyzed by in situ hybridization with fluorescently labeled rRNA-targeted oligonucleotide probes. Cells fixed on membrane filters were hybridized with a probe specific for the domain *Bacteria* as well as with probes for the alpha, beta, and gamma subclasses of the class *Proteobacteria* and the *Cytophaga-Flavobacterium* group. The fraction of bacteria detectable after hybridization with the bacterial probe EUB ranged from 40 to 81% of 4′,6-diamidino-2-phenylindole (DAPI) counts. The bacterial assemblage varied considerably between and within different habitats (snow, slush, and lake water) but was in most cases dominated by members of the beta subclass (6.5 to 116% of bacteria detectable with probe EUB). The sum of bacteria hybridizing with group-specific probes was usually lower than the fraction detectable with probe EUB. Image analysis was used to characterize morphology and the size-specific biomass distribution of bacterial assemblages, which clearly separated the three habitats. Although the measured secondary production parameters and the fraction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride-reducing bacteria varied by more than an order of magnitude in the different slush and pelagic layers, detectability with the fluorescent probe EUB was constantly high. Physiological strategies of bacteria under nutrient limitation and at low temperatures are discussed in the context of the ribosome content of single cells. This study confirms the suitability of fluorescently labeled rRNA-targeted probes for the characterization of bacterial population structures even in oligotrophic habitats.

Our knowledge about the structure and dynamics of aquatic microbial communities is strongly hampered by the difficulties in characterizing their taxonomic composition. Therefore, microbial studies have usually been done from the perspective of a taxonomist or an ecologist, but both viewpoints have rarely been considered in combination (32), and so far bacteria are still treated as a black box in most ecological studies. The lack of a complex morphology generally does not allow the in situ identification of individual bacterial cells by microscopy. A physiological identification based on pure cultures is of little ecological use because the vast majority of heterotrophic bacteria from aquatic environments resist cultivation (16, 21), and activity parameters should be measured under natural conditions (6).

In recent years, molecular biological techniques, particularly rRNA sequence analysis for the phylogenetic characterization of microorganisms (43), have allowed investigations about the diversity within complex microbial assemblages (13, 31). However, comparing rRNA sequence patterns from mixed bacterial communities is too time-consuming to attempt for the fine spatial or temporal resolution which is required for the study of ecological processes. Furthermore, this taxonomic information cannot be combined easily with results from well-established methods of aquatic microbial ecology such as epifluorescence microscopy (33, 35).

Whole-cell hybridization with fluorescently labeled, rRNA-targeted oligonucleotide probes permits the microscopic determination of absolute abundances and cell morphologies or sizes of different bacterial taxa (2, 8). Such probes have found several successful applications in aquatic ecosystems, e.g., studies of eutrophic freshwater (15), activated sludge (42), and biofilms (4, 26).

However, until now in oligotrophic systems only a small fraction of the total 4′,6-diamidino-2-phenylindole (DAPI) counts has been detectable with fluorescent probes (1). It has been argued that low cellular ribosome content or low cell wall permeability may limit detection sensitivity (3). This appeared to be a serious limitation of the method. Attempts to increase the signal strength by simultaneous applications of multiple rRNA-targeted fluorescent probes succeeded in labeling up to 75% of all bacteria in marine coastal waters (23). Because of the relatively high conservation of rRNA molecules, however, it is hardly possible to find target sites at the group, genus, or species level with identical specificity for five or more probes. Hybridization with digoxigenin-labeled (44) or enzyme-labeled (5) oligonucleotide probes and the use of 23S rRNA-targeted multiply labeled polynucleotide probes (41) result in increased sensitivity without reducing specificity. However, these probes are no ready solution for the problem of low detectability yet, as they are often too large to penetrate fixed microbial cells (3).

The aim of this study was to introduce an improved technology of in situ hybridization with monolabeled fluorescent oligonucleotide probes. We attempted to split the bacterial community in the winter cover and the pelagic zone of an oligotrophic high-mountain lake into several phylogenetic groups. We used probes specific for all members of the domain

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Bacteria; for the alpha, beta, and gamma subclasses of the class *Proteobacteria*; and for the *Cytophaga-Flavobacterium* group.

We selected a system in which low temperatures and nutrient limitation may prevent cells from reaching high metabolic activities. On the other hand, there are reports about highly active microbial communities in the slush and snow layers of high mountain lakes (11). Therefore, we also measured several microbial activity parameters. We expected to encounter both high and low levels of microbial activity in the studied system and thus gain information on how this will affect the applicability of the improved in situ hybridization method. Finally we also collected data about the size structure and morphology of the studied bacterial assemblages and compared this information with the results from in situ hybridization.

MATERIALS AND METHODS

Samples were taken on 11 May at Gossenköllesee in the Tyrolean Alps (2,417 m above sea level; area, 1.7 ha; maximum depth, 9.9 m). This oligotrophic lake is weakly buffered and covered by ice and snow for over half of the year. Details of the study site are given elsewhere (11).

We took a profile from the winter cover and the upper 3 m of the water column. Snow was collected from the surface and deeper levels. It was left to melt overnight at 4°C before further processing. Water was pumped from four distinct slush layers and from the lake itself (0- and 3-m depths) with a device described by Felip et al. (11).

Whole-cell in situ hybridization. One hundred milliliters of sample was used for hybridization with fluorescent oligonucleotide probes. The water was prescreened (20-μm mesh size) in order to reduce the amount of chlorophyll-containing detritus and filtered on white 0.2-μm-pore-size polycarbonate membrane filters (Poretics Corp.; 50-mm diameter). Bacterial cells were fixed by overlaying the filters with 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.2) for 30 min at room temperature. The fixative was removed by applying a gentle vacuum, and the filters were rinsed with PBS and bidistilled water prior to removal from the filtration apparatus. Next, the filters were air dried and stored at −20°C until further processing.

The following five oligonucleotide probes were used for a preliminary analysis of bacterial community composition: ALF, complementary to the 16S rRNA (positions 19 to 35) of alpha-subclass *Proteobacteria*; BET for beta subclass *Proteobacteria* (23S rRNA, positions 1027 to 1043); GAM for gamma subclass *Proteobacteria* (23S rRNA, positions 1027 to 1043); CF for the *Cytophaga-Flavobacterium* group (16S rRNA, positions 319 to 336); and EUB for members of the domain *Bacteria* (16S rRNA, positions 338 to 355) (3). Probes were labeled with the indocarbocyanine fluorescent dye CY3 (Biological Detection Systems, Pittsburgh, Pa.) (3) and stored at −20°C.

Prior to in situ hybridization, filters were cut into smaller sections (0.5 to 1 cm^2). These sections were placed on coverslips, covered with 16 μl of hybridization buffer containing 2 μl (50 ng ml^{-1}) of the respective fluorescent probe, and hybridized in jars with slip-on lids (Brand, Wertheim am Main, Germany) at 46°C for 90 min. A piece of blotting paper soaked in hybridization buffer was put into the jars in order to prevent the filter sections from drying out during the hybridization process. Hybridization buffers consisted of 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate (SDS), and a variable concentration of formamide (EUB, 0%; ALF, 20%; BET and GAM, 35%; CF, 15%) (24, 25). Afterwards, the filter sections were incubated in 20 ml of prewarmed washing buffer at 48°C for 15 min, rinsed with distilled water, and air dried. The washing solution consisted of 20 mM Tris-HCl (pH 7.4), 5 mM EDTA, 0.01% SDS, and a variable concentration of NaCl (EUB, 0.9 M; ALF, 0.225 M; BET and GAM, 80 mM; CF, 80 mM). In order to determine total bacterial abundance, the filter sections were stained for 5 min with DAPI (final concentration, 2 to 5 μg/ml) at room temperature prior to microscopic examination.

Preparations were put on glass slides, mounted in glycerol medium (Citifluor, Canterbury, England), and inspected by epifluorescence microscopy (Axioplan; Zeiss, Oberkochen, Germany). Filter sets for DAPI were BP 365, FT 395, and LP 397 (Zeiss 01), and those for CY3 were BP 535-550, FT 565, and LP 610-675 (HQ 41007; Chroma Technology Corp., Brattleboro, Vt.).

Between 400 and 600 bacteria stained with fluorescent probes and 400 to 1,000 bacteria stained with DAPI were counted on each hybridized filter (magnification, ×1,000 to ×1,250). As the fluorescence of CY3-stained bacteria and chlorophyll autofluorescence were similar in color, we determined a background level of orange fluorescent bacterium-sized particles (cyanobacteria, chlorophyll-containing detritus, and dust particles, etc.) by inspecting unhybridized, DAPI-stained filter sections from each layer in green and UV excitation.

To test the staining efficiency of fluorescent oligonucleotide probing in snow, slush, and water samples, counts from hybridizations with the bacterial probe were compared with DAPI counts from the respective filter section. This portion of cells visualized with oligonucleotide probe staining was used as a reference for the subsequent community analysis with group-specific probes (expressed as percentage of EUB338-detectable cells [$\%_{EUB}$]).

Bacterial abundance and biomass. To confirm that bacteria can be enumerated accurately from any region of a large filter without introducing a bias, 10 randomly selected sections were cut from one filter and stained with DAPI and at least 400 bacteria were counted from each section. The distribution was statistically evaluated by calculating a dispersion index (the quotient of the variance and mean from each count), which allowed the performance of a χ^2 test (10).

In order to provide a reference to established techniques, parallel samples were fixed with formalin and cells from each layer were enumerated on black membrane filters (33). From these samples, size measurement of 400 to 600 DAPI-stained cells per layer was carried out with a semiautomated image analysis system (22) at ×1,000 to ×2,000 magnification and mean bacterial volumes were calculated (35). An allometric relationship between bacterial cell volume and carbon content (40) in its recalculated version (30) was used for biomass estimation: $C = 120 \cdot V^{0.72}$, where C is femtograms of carbon per bacterial cell and V is cell volume.

The distribution of cell biomass in different size classes (interval: 0.2 μm of cell length) was calculated as the product of abundance and mean carbon content per cell in the respective size class.

Bacterial secondary production and viable fraction of bacterioplankton. Bacterial in situ growth rates and protein production were estimated from all but the snow layers via dual labeling with [^{3}H]thymidine and [^{14}C]leucine (modified from the procedure in reference 40). Triplicate 20-ml subsamples were incubated for 24 h with [*methyl*-^{3}H]thymidine (70 to 90 Ci $mmol^{-1}$; 10 nM final concentration) and [^{14}C]leucine (310 mCi $mmol^{-1}$; 40 nM final concentration) at the in situ temperature. Incorporation was stopped by the addition of prefiltered (0.2-μm pore size) buffered formalin (2% [final concentration]). A time series experiment confirmed linear uptake of radiotracers only after 20 h of incubation (data not shown) in slush layers 3 and 4. In pelagic samples incorporation rates were so low that reliable measurements required 24 h of incubation. Prefixed blanks were processed for each set of samples to correct for tracer adsorption on particles. Cells were filtered on cellulose nitrate filters (Sartorius, Göttingen, Germany; 0.2-μm pore size) which had been prerinsed with 5 ml of ice-cold trichloroacetic acid (5% concentration) and distilled water. Samples were incubated for 5 min with ice-cold trichloroacetic acid (5% concentration) for cell disruption. Afterwards, filters were again washed three times with trichloroacetic acid, air dried, and put into 20-ml polyethylene vials. Fifteen milliliters of liquid scintillation cocktail (Kiessler Diagnostics) was added to dissolve the filters, and samples were counted two times for 10 min in a Beckman LS 6000 IC liquid scintillation counter. No calculation of cell division rates and bacterial carbon production from radiotracer incorporation rates was attempted, as the standard conversion factors have been derived from systems that differ greatly from the study site (for thymidine, see reference 18).

Viable, i.e., respiring, cells were quantified through incubation with 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT; Sigma Chemicals; final concentration, 0.02% [vol/vol]) (modified from the procedure in reference 45). The frequency of respiratory active bacteria was determined only from liquid samples (slush water and lake water) because the melting of snow samples prior to INT incubation might introduce an unpredictable bias with respect to bacterial activity. Thirty milliliters of sample was incubated after prefiltration (10-μm pore size) at the in situ temperature for 24 h. After incubation, samples were fixed with prefiltered (0.2-μm pore size) Formol (4% final concentration). Prefixed samples were used as controls. Ten milliliters of sample was filtered onto white cellulose nitrate filters (Sartorius; 0.2-μm pore size). Filters were mounted with glycerin, and INT grains were counted with normal incident light at a magnification of ×1,600.

RESULTS

Hybridization efficiency and bacterial community structure. Cell counts from 10 different sections cut from one filter were much more similar than would be expected if numbers were varying within a random (Poisson) distribution ($\chi^2 = 0.076$; $n = 10$; $P < 0.05$). Hybridization of different sections of one large filter and subsequent counting do not seem to introduce an error due to patchy distribution of bacteria on large filters.

On unhybridized filters, orange fluorescent particles ranged between 0 and 0.26% of DAPI counts. This background value may lead—if unaccounted for—to a slight overestimation of fluorescent probe counts for rare groups of bacteria. Only the sample snow 1 did not allow reliable cell counts for hybridizations with probes CF, ALF, and GAM because of the amount of orange fluorescent particles that were unrelated to hybridization. To account for the occurrence of such fluorescent particles (which usually do not stain with DAPI) that could be confused with stained bacteria, at low abundances of particular bacterial groups the observer should frequently switch between

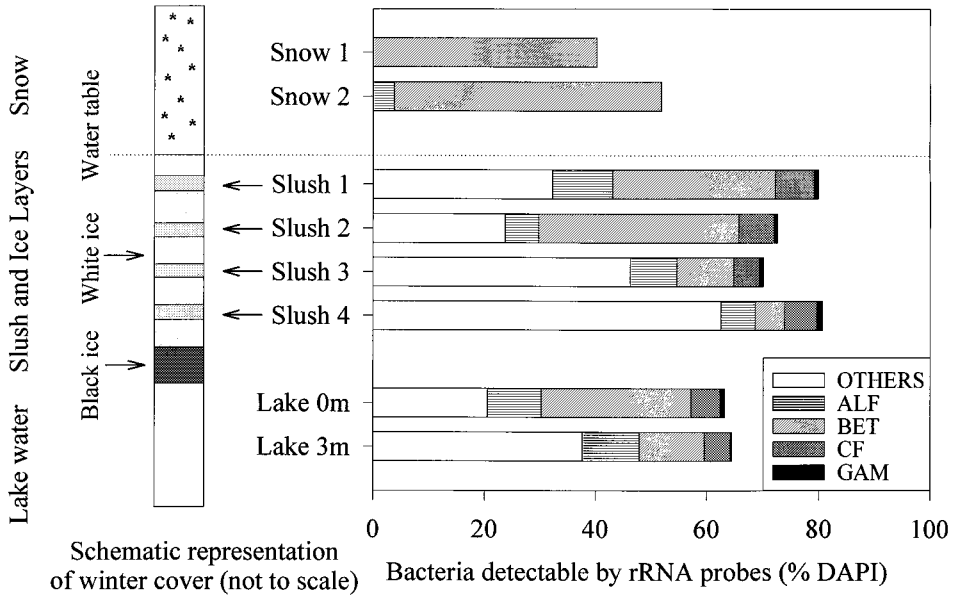

FIG. 1. Composition of the bacterial assemblages in the snow and slush layers of the winter cover and in two pelagic layers of a high mountain lake, as determined by in situ hybridization with group-specific fluorescent probes. Total bar length: fraction of DAPI cell counts detectable by probe EUB in the respective layer.

green and UV excitation. Six parallel counts from one filter (slush 1) hybridized with EUB showed detection levels between 70 and 85% of DAPI counts (mean: 76.3 ± 5.8 standard deviations).

Figure 1 shows the vertical distribution of the different bacterial groups as a fraction of total DAPI counts. The label "OTHERS" refers to the share of bacteria detectable by probe EUB that could not be explained by the sum of bacteria detected with the four group-specific probes used in this investigation. Figure 2 depicts typical morphotypes of bacteria detectable with EUB and the group-specific probes. Table 1 is a listing of different cell shapes observed after hybridization with group-specific probes, some of which are also visible in the micrographs (Fig. 2, left panels). Figure 2 (right panels) shows images of the same cells in DAPI staining. Some cells appear larger when stained with fluorescent probes than when stained with DAPI (c.f. Fig. 2E and F), which could be observed in the microscopic image as well. This was very clear in samples from the snow layers hybridized with probe BET (e.g., Fig. 2E).

In the snow cover of Gossenköllesee the percentages of DAPI-stained cells that could be visualized microscopically with probe EUB were 40.2% in snow 1 and 41.2% in snow 2. The majority of bacteria from the four different slush layers hybridized with the eubacterial probe (from 70.1 to 80.7% of total bacterial counts). In lake water samples 64.5% (0 m) and 63.2% (3 m) of DAPI cell counts were detected with EUB (Fig. 1).

In the snow cover virtually all bacteria detectable with probe EUB also hybridized with probe BET (snow 1, 99.9%$_{EUB}$; snow 2, 116.5%$_{EUB}$). These cells were typically rod shaped (1.2- to 2-μm length) and of bright fluorescence (Fig. 2; Table 1). Our data from image analysis confirm that cells of this morphotype indeed constituted the main share of bacterial biomass in the snow cover, separating this habitat clearly from the other ones (see Fig. 4).

In the different slush layers microbial community structure varied considerably. Bacteria more frequently hybridized with probe BET in the upper slush layers, slush 1 (36.5%$_{EUB}$) and slush 2 (49.4%$_{EUB}$), than in the deeper samples (slush 3, 14.6%$_{EUB}$; slush 4, 6.5%$_{EUB}$). In the two bottom slush layers we observed a shift towards bacteria that did not hybridize with any of the group-specific probes. This change was coupled with the increase of cells of intermediate size (1.2 to 2 μm) (see Fig. 4), higher bacterial biomass, higher incorporation rates of radiolabeled compounds, and an increase in the fraction of respiring bacteria (Fig. 3).

Lake water samples showed a higher share of beta proteobacteria at 0 m (42.7%$_{EUB}$) than at 3 m (18.2%$_{EUB}$), where the major part of bacteria did not hybridize with any of the group-specific probes (Fig. 1).

Members of the alpha proteobacterial group were found in all samples, and counts varied between 7.6%$_{EUB}$ (slush 4) and 15.8%$_{EUB}$ (lake 3 m). Only 0.42%$_{EUB}$ (3 m) to 1.34%$_{EUB}$ (slush 4) hybridized with probe GAM in different slush layers and lake water. The CF-positive cells accounted for 4.7%$_{EUB}$ (lake 3 m) to 8.7%$_{EUB}$ (slush 2) of cells visualized by the probe EUB. The abundances of ALF-, GAM-, and CF-positive bacteria were rather similar in all slush and lake water layers.

Size and biomass structure of the microbial community. Whereas total cell counts were highest in the lower slush layers (Fig. 3), a distinct maximum of bacterial biomass was found in the lower snow layer (Fig. 4). In the uppermost snow layer, it was not possible to measure bacteria with image analysis because of numerous dust particles. In snow 2 the main share of bacterial biomass was formed by cells of between 1 and 2 μm (>75%) and large cells of more than 2.6 μm in length (13%). In the slush layers the fraction of large bacteria of >2.6 μm still contributed significantly to total biomass (14 to 26%), but the distribution was shifted towards smaller cells of <1 μm (50 to 60%). This trend was even stronger in samples from lake water, particularly at the 3-m depth (>90% of biomass from cells of <1 μm). In slush layers, biomass was distributed more heterogeneously over size classes. In slush 3 and slush 4, a distinct class of cells between 1.2 and 2 μm in length that was absent in the two upper slush layers could be separated morphologically (Fig. 4).

Bacterial secondary production and the INT-reducing fraction of bacterioplankton. Tritiated thymidine incorporation

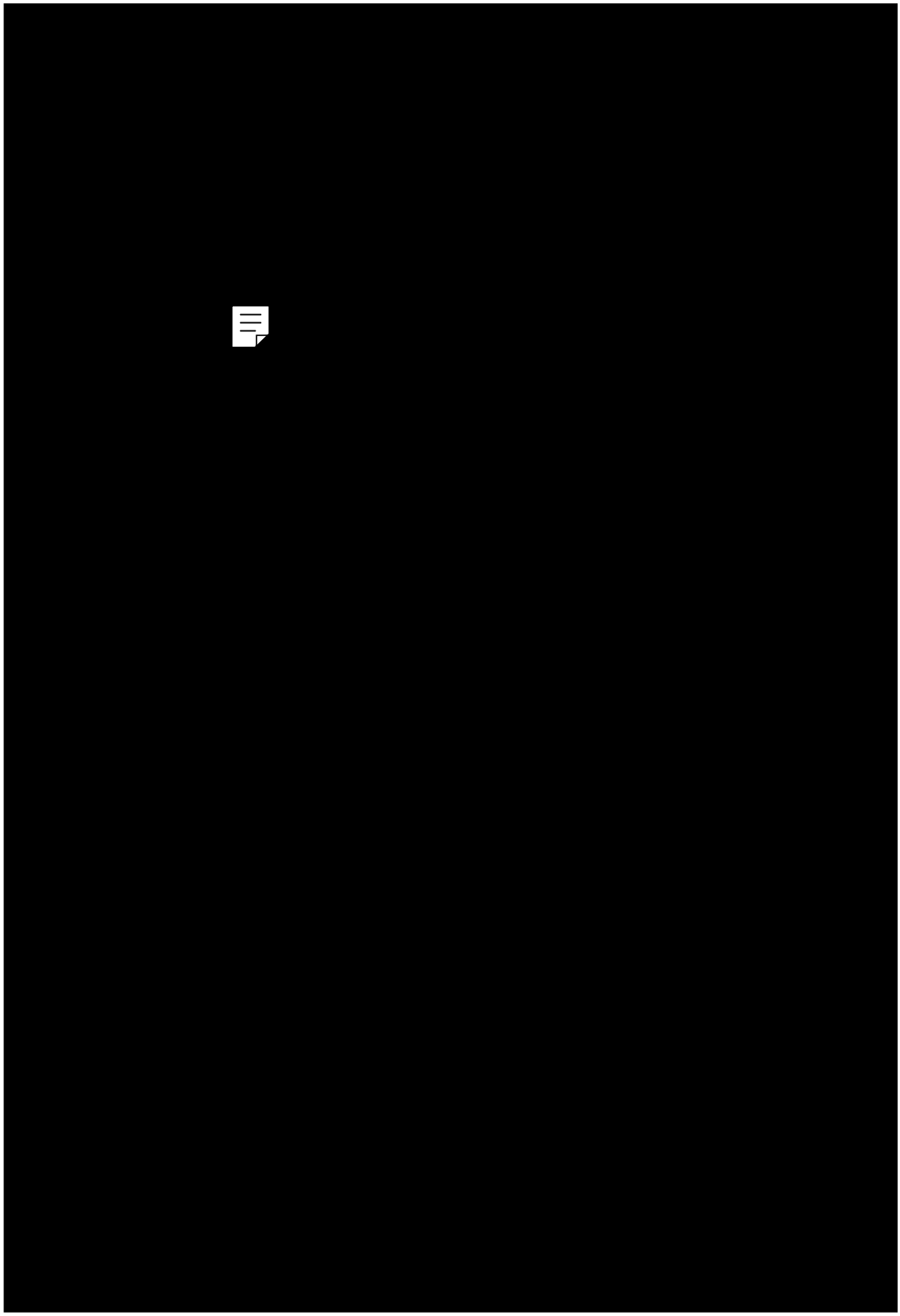

FIG. 2. Typical morphotypes of bacteria hybridizing with CY3-labeled oligonucleotide probes (epifluorescence micrographs). (Left) Green excitation (hybridized cells) with probe EUB (A), with probe ALF (C), with probe BET (E), and with probe CF (G). (Right) The same microscopic fields as on the left with UV excitation (DAPI staining). All photomicrographs are at a magnification of ×1,250. The bar in panel A is 8 μm and applies to all eight photomicrographs.

TABLE 1. Morphological diversity of bacteria hybridized with fluorescent rRNA-targeted oligonucleotide probes

Probe	Source of cell type	Morphology	Example
ALF	Lake water	Small cocci (aggregates)	
		Small cocci (dispersed)	Fig. 2C
	Slush	Rods arranged in star-shaped aggregates	
	Slush and lake water	Short, very bright rods	Fig. 2C
		Long rods	Fig. 2C
		Short threads	
BET	Snow	Large, very bright rods	Fig. 2E
	Slush and lake water	Large, very bright rods	Fig. 2E
		Thin rods of uneven brightness	
		"Vibrio-typed" rods, curved	
		Long, segmented threads	
	Lake water	Oval, very bright cells, in pairs	
GAM	Slush	Short bright rods	
CF	Slush	Long threads, not segmented, dim	Fig. 2G
		Long spirilla	Fig. 2G
		Short rods	Fig. 2G

into bacterial cells ranged from 0.01 to 0.69 pmol liter^{-1} h^{-1}. [^{14}C]leucine uptake was between 1.23 and 44.67 pmol liter^{-1} h^{-1} (Fig. 3). Less than 1% of all bacteria from the pelagic zone were found to produce visible formazan crystals; in slush layers, the fraction of viable, respiring cells was between 1.95 and 17.8% of total bacterial counts. The vertical profiles of the two radioisotopes and the distribution of INT-reducing bacteria matched very closely, with a conspicuous maximum in the deepest slush layer (Fig. 3).

DISCUSSION

Microbial community composition. Hicks et al. (15) reported that in natural bacterioplankton communities between 35 and 67% of DAPI counts were also detectable by in situ whole-cell hybridization with EUB. However, these samples were drawn from artificial, highly eutrophic ponds, about 30% of total counts were lost during the transfer from filters to slides, and no community analysis with more-specific probes was performed. The only other investigation of microbial community composition using oligonucleotide rRNA targeted probes in a natural freshwater system has been carried out on lake snow from Lake Constance (39).

In our study the application of probes specific for the alpha, beta, and gamma subclasses of *Proteobacteria* (26) and the *Cytophaga-Flavobacterium* group (24) showed a very distinct bacterial community composition in the different snow, slush, and pelagic layers. Interestingly, the relationship between bacterial production, activity, and microbial community structure in samples from the pelagic layers differs strongly from the observations in the slush layers. It is quite possible that there is high physiological variability within one of the detected bacterial groups, as our taxonomic analysis pools a wide variety of different bacterial taxa. Alternatively, high metabolic activity might be associated with the increased fraction of EUB-positive cells not classified by the applied group-specific probes. Relying entirely on taxonomical information could lead to premature conclusions about the similarity of the two habitats, which can be avoided if it is put in the context of productivity, activity, and biomass. The biomass distribution over different size classes (Fig. 4) shows a conspicuous shift between the habitats: intermediate-size bacteria are dominant in snow layers and still present in slush but virtually absent in the lake water column.

The gamma subclass of *Proteobacteria* accounted for only about 1% of bacteria visualized by probe EUB in all habitats (Fig. 1). Equally low detection rates of this group were also found in the pelagic environment of another, but less oligotrophic lake, Piburger See (unpublished data). Our results are in contrast to those of investigations on freshwater bacterial community composition based on cultivation techniques, in which this subclass appears to be prominent (14). However, Wagner

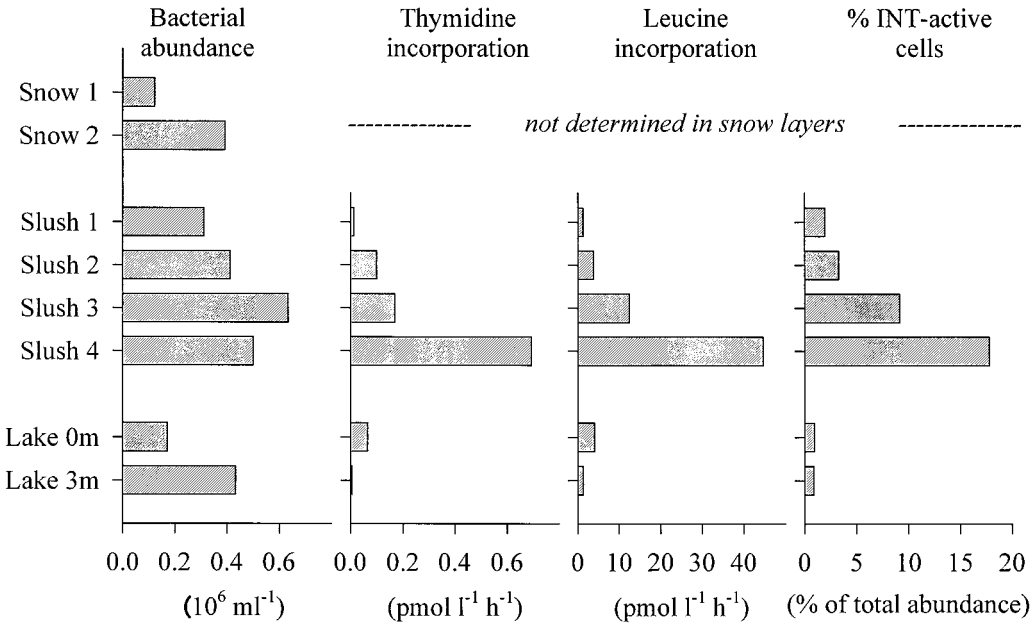

FIG. 3. Bacterial abundance, [^{3}H]thymidine [^{14}C]leucine uptake, and the fraction of INT-reducing cells in the snow, slush, and lake water layers.

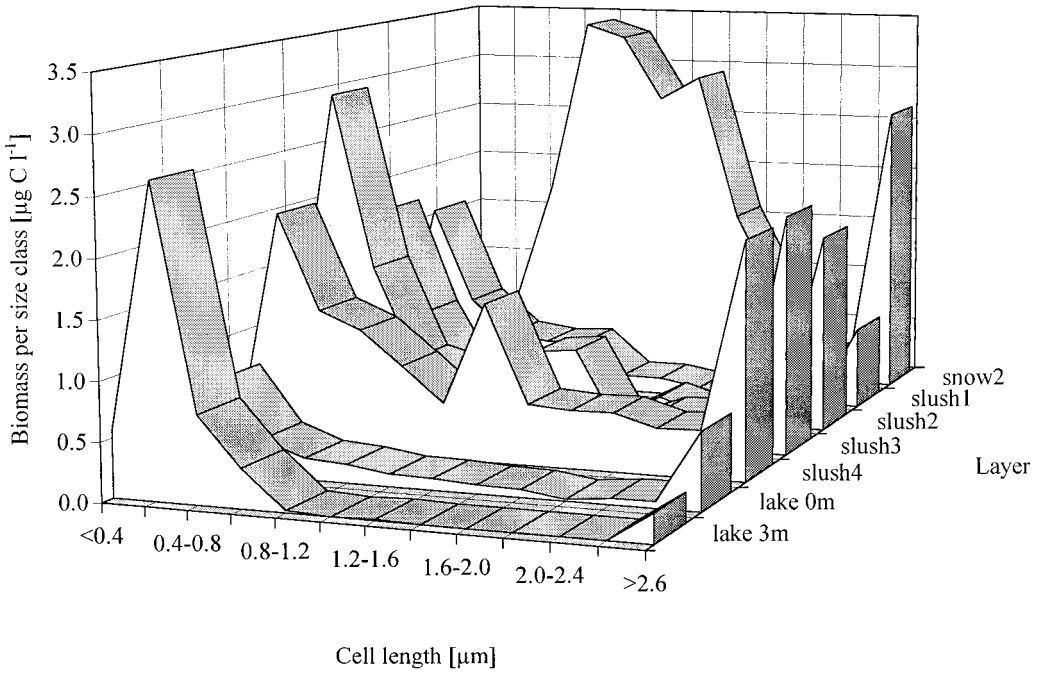

FIG. 4. Distribution of bacterial biomass in size classes (cell length) in the snow, slush, and lake water layers.

et al. (42) could demonstrate that cultivation-dependent shifts even occur in the microbial community structure of activated sludge. Nutrient-rich media favored the growth of organisms from the gamma subclass of proteobacteria and selected against the beta subclass of proteobacteria.

Studies of the diversity of aquatic microbial communities have been carried out almost exclusively in marine systems, and the bacterioplankton in these systems was dominated by cyanobacteria and members of the alpha and gamma subclasses of *Proteobacteria*, although in more recent studies sequences related to other bacterial and archaeal phyla have been found as well (7, 12, 13, 29, 36, 38). In contrast to our findings, there are virtually no reports of marine 16S rRNA sequences that fall into the group of beta-proteobacteria.

Fluorescent probes and bacterial activity. From laboratory studies it is known that the signal emitted from cells hybridized with fluorescent probes is strongest during logarithmic fast growth (8, 37). It has been suggested that a large fraction of pelagic bacteria are in a state of quasi-dormancy (9). Consequently it was assumed that their low metabolism would result in low ribosome contents and restrict the use of rRNA-targeted probes. Until now most applications of monolabeled rRNA-targeted probes have thus been carried out in nutrient-rich environments with rapidly growing bacterial communities (3). We succeeded, however, in detecting an almost equally large proportion of DAPI-stainable bacteria in samples where the fractions of INT-reducing bacteria change from 1.9 to 17.8% and radiotracer incorporation rates vary by more than 1 order of magnitude (slush 1 to slush 4, Fig. 1 and 3). We assume that this independence of the hybridization efficiency from bacterial activity is partially a consequence of improved methodology and the superior signal strength of the fluorescent dye CY3. Hybridization with probes conjugated to other fluorescent molecules resulted in much lower detection levels (data not shown).

It is theoretically possible to quantify the rRNA content of single cells with fluorescent probes (8, 34, 37). Our discussion of bacterial activity, however, is based solely on detection rates of hybridized cells, which gives us the percentage of bacteria that contain a minimal amount of ribosomes.

Although secondary production and activity parameters varied by more than an order of magnitude in different slush and pelagic layers (Fig. 3), detection levels with CY3-labeled fluorescent probes remained fairly constant (Fig. 1). There is evidence from laboratory experiments that rRNA and ribosomes may exist in excess in starved marine *Vibrio* cells (19). Moyer and Morita (28) found that the DNA/RNA ratio of a marine psychrophilic bacterium was shifted towards RNA during starvation. A fast physiological response to sudden changes in nutrient concentrations could thus be realized by nongrowing bacteria (20) by maintaining a relatively high number of ribosomes.

Kemp et al. (17) stress the importance of taxonomic classification to use RNA content as a predictor of growth rates. This agrees with our observation that bacteria of certain morphotypes hybridizing with a subgroup-specific probe were always of similar brightness. For example, filamentous cells hybridizing with CF were rather dim (Fig. 2G), whereas large rod-shaped bacteria stained with BET were of a brightness comparable to that of cultured cells harvested during logarithmic growth (Fig. 2E; Table 1). On the other hand, we also found morphotypes that looked identical that were very similar in brightness but hybridized with different subgroup-specific probes (e.g., short bright rods in Fig. 2C and E), which shows that morphological and physiological information cannot replace taxonomic classification. However, since certain cells gave signals slightly above the detection limit, penetration efficiency of fluorescent probes into bacterial cells and other hybridization-related factors might influence detection rates as well. Still, these observations strongly indicate that there is physiological as well as phylogenetic diversity within subgroups that remains to be analyzed with more specific oligonucleotide probes and activity-specific fluorescent dyes (27).

ACKNOWLEDGMENTS

We thank Ruben Sommaruga for helpful comments on the manuscript.

A.A. and J.P. were financed by grants from a project by the Fonds zur Förderung der wissenschaftlichen Forschung (P10184-MOB to R.P.); the study was also supported by the Deutsche Forschungsgemeinschaft (AM 73/2-2 to R.A.) and the European Community (MRAMI-2 to R.A.).

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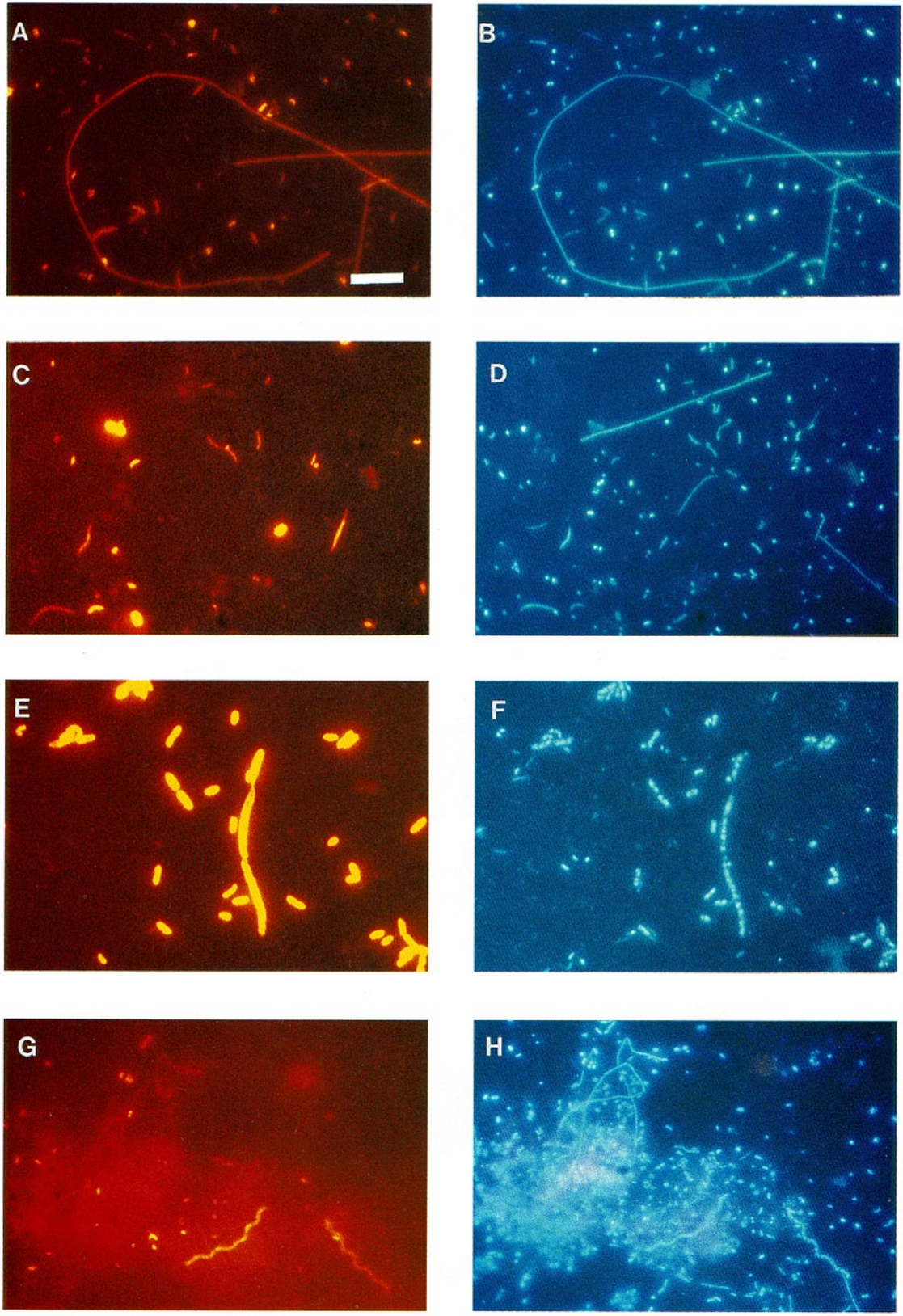
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