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Identification In Situ and Dynamics of Bacteria on Limnetic Organic Aggregates (Lake Snow)

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Microbial assemblages on large organic aggregates (lake snow) of Lake Constance, Germany, were analyzed with rRNA-directed fluorescent oligonucleotide probes specific for the domain *Bacteria* and the α-, β-, and γ-subclasses of the class *Proteobacteria*. Lake snow aggregates were either collected in situ by SCUBA diving or in a sediment trap at 50 m or formed of natural lake water incubated in rolling cylinders under simulated in situ conditions. For the latter aggregates, the time course of the microbial colonization was also examined. The natural aggregates and those made in rolling cylinders were composed of the particulate organic material present in the lake and thus reflected the composition of the ambient plankton community. All types of lake snow aggregates examined were heavily colonized by microbial cells and harbored between 0.5 × 10^6 and >2 × 10^6 cells aggregate^{-1}. Between 55 and 100% of the microbial cells stained with 4′,6-diamidino-2-phenylindole (DAPI) could be visualized with the domain *Bacteria*-specific probe. In most samples, β-subclass proteobacteria dominated the microbial community, constituting 27 to 42% of total cells as counted by DAPI staining, irrespective of the composition of the aggregates. During the time course experiments with the laboratory-made aggregates, the fraction of β-subclass proteobacteria usually increased over time. Except for a few samples, α- and γ-subclass proteobacteria were far less abundant than β-subclass proteobacteria, constituting 11 to 25 and 9 to 33% of total cells, respectively. Therefore, we assume that a specific aggregate-adapted microbial community was established on the aggregates. Because the compositions of the microbial assemblages on natural and laboratory-made aggregates were similar, we conclude that aggregates made in rolling cylinders are a good model system with which to examine the formation and microbial colonization of macroscopic organic aggregates.

Macroscopic organic aggregates (>0.5 mm long) are important for the cycling and flux of particulate organic matter in the ocean and have been shown to constitute a dominant fraction of the carbon flux from the ocean's surface to its depth (4, 13). Biological and biochemical transformations on aggregates have a profound influence on the quantity and quality of particulate as well as dissolved organic matter. Marine snow, the main type of large aggregates, is highly enriched with nutrients (36) and microorganisms (3, 10) and forms microhabitats which allow high rates of turnover of organic matter (23, 27, 34) and unique heterotrophic processes in a nutrient-depleted environment. Because of the colonization by metabolically highly active microbial cells (23, 34), aggregates can form oxygen-depleted microzones (1) harboring even methanogens (8).

Recently, large organic aggregates have also been found in lakes (lake snow), where they are of trophic significance similar to that in the ocean (18, 19). Despite the significance of aggregate-associated microbial cells in the carbon flux in lakes and the ocean, very little is known about their phylogenetic affiliation and the quantitative occurrence of specific populations on aggregates. Potential phylogenetic lineages in which relevant aerobic heterotrophic aquatic bacteria occur include the classes *Cytophaga*, *Planctomyces*, and *Proteobacteria*. Within the class *Proteobacteria*, the α-, β-, and γ-subclasses harbor the majority of the gram-negative members of the domain *Bacteria* routinely found in aquatic environments. The γ-subclass encompasses, e.g., the genera *Acinetobacter*, *Aeromonas*, *Legionella*, and *Vibrio*, the family *Enterobacteriaceae*, and the true pseudomonads (RNA group I). The genera *Comamonas*, *Hydrogenophaga*, and *Acidovorax* (all formerly included in the genus *Pseudomonas*), the sheathed bacteria affiliated with *Sphaerotilus natans*, and the ammonium-oxidizing bacteria of the genus *Nitrosomonas* are typical members of the β-subclass, whereas the members of the family *Rhizobiaceae* and the genera *Paracoccus*, *Caulobacter*, *Rhodospirillum*, and *Nitrobacter* are examples of the α-subclass. Today it is well known that cultivation-dependent enumeration techniques may strongly overestimate the abundance of rapidly growing members of the γ-subclass, e.g., *Acinetobacter* organisms, compared with other aquatic bacteria (38). The lack of more-detailed information about the quantitative occurrence of either of these groups in pelagic ecosystems is mainly due to the fact that usually <1% of total cells can be cultured by standard techniques, such as plating on agar (20, 29). The cells therefore escape traditional microbiological identification. On the basis of clones from PCR-amplified genes coding for rRNA (11) and profiles of low-molecular-weight rRNA (9), some qualitative differences between aggregate- and particle-associated bacteria and free-living bacteria have been shown. Recently, fluorescent rRNA-targeted oligonucleotide probes have been introduced as a new tool for the in situ identification of bacterial cells (12; for a review, see reference 6). On the basis of phylogenetic relationships, these probes can be designed to be complementary to species-, group-, or kingdom-specific target sites. Probes specific for the domain *Bacteria* and the α-, β-, and γ-subclasses of *Proteobacteria* have been successfully applied for study of the microbial colonization of activated sludge (38) and biofilms (25), indicating that the majority of total

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microbial cells in these microenvironments can be detected and identified with these probes. Because the fluorescent signal conferred by the probe is positively correlated with the rRNA content of the cells (12), small and slowly growing cells, such as free-living planktonic forms, are frequently close to or below the detection limit of this method (21). Particle-associated microbial cells have been shown to be larger than free-living cells (2, 31) and metabolically highly active (see above). Therefore, they should provide an attractive system for the application of fluorescent oligonucleotide probes.

In this study, we examined the colonization of natural lake snow aggregates in a large mesotrophic lake by using probes specific for the domain *Bacteria* and the α-, β-, and γ-subclasses of *Proteobacteria*. Furthermore, we studied the dynamics of the microbial colonization of large aggregates formed from natural lake water in rolling tanks (30) under simulated in situ conditions and incubated for 6 to 13 days.

MATERIALS AND METHODS

Sampling. Individual aggregates >3 mm in length were sampled by SCUBA diving in Lake Constance, Germany, a large mesotrophic prealpine lake, between 23 June and 23 August 1994 at a depth of 20 to 25 m. Trophodynamics of the plankton community and the sinking flux in Lake Constance have been studied extensively in the recent past (15, 16, 19, 32). Aggregates were collected in 10-ml plastic syringes with a wide opening and kept in a cooling box until further processing in the laboratory, usually within 2 h. The aggregates were carefully transferred into 2 ml of filtered (0.2-μm pore size) lake water and stored at −20°C until further processing. From 27 to 30 June 1994, a sediment trap was deployed at 50 m to collect sinking particulate organic matter, the microbial colonization of which was examined as well. For details of the sediment traps, see the description of Simon et al. (32).

Formation and incubation of aggregates in rolling tanks. Water samples, collected from the RV *Robert Lauterborn* at 6 m in Lake Constance between 27 April and September 1993, were incubated in 1.4-liter Plexiglas cylinders rolling horizontally (2.5 rpm) at in situ temperature in a light-dark cycle of 12-12 h (30). In total, 8 to 12 cylinders were incubated for one experiment. After synchronous formation of aggregates >3 mm in length in all tanks, the aggregates of one cylinder (one to five aggregates) were harvested every day or every other day for a period of 6 to 13 days. The first experiment of 27 April was already terminated on day 2 after aggregates had formed. The aggregates were carefully withdrawn from the cylinder in a widely opened 1-ml plastic pipette and stored at −20°C until further processing.

In situ hybridization. Five to ten aggregates collected in situ on the same day were pooled and then subdivided into three aliquots for hybridization. In the time series experiments, all aggregates harvested on one day from one cylinder were also pooled for hybridization but not subdivided further. The pooled aggregates were sonicated (2 s, 3 W), and 20-μl aliquots were pipetted onto gelatin-coated microscopic slides with a Teflon coating (Paul Marienfeld KG, Bad Mergentheim, Germany), air dried at 46°C, and fixed with 40 μl of a 4% paraformaldehyde solution for at least 4 h at 4°C (5). Sonication was done to disrupt the aggregates and evenly distribute the cells on the slides for a reliable quantitative determination of cell numbers (see below). The following oligonucleotide probes were used: (i) EUB 338, complementary to a region of the 16S rRNA specific for the domain *Bacteria* (5′-GCTGCCTCCCGTAGGAGT-3′) (7); (ii) ALF 1b, complementary to a region of the 16S rRNA conserved in the α-subclass of *Proteobacteria* and some other bacteria (5′-CGTTCGYTCT GAGCCAG-3′) (24); (iii) BET 42a, complementary to a region of the 23S rRNA specific for the β-subclass of *Proteobacteria* (5′-GCCTTCCCACTTCGTTT-3′) (24); and (iv) GAM 42a, complementary to a region of the 23S rRNA conserved in the γ-subclass of *Proteobacteria* (5′-GCCTTCCCACATCGTTT-3′) (24). Labelling with tetramethylrhodamine-5-isothiocyanate (TRITC) (Molecular Probes, Eugene, Oreg.) or 5(6)-carboxy-fluorescein-*N*-hydroxysuccinimide ester (FLUOS) (Boehringer, Mannheim, Germany) was performed as described previously (5). Hybridization according to standard protocols (5, 24) was done simultaneously with EUB 338 and ALF 1b and with BET 42a and GAM 42a.

Enumeration of total and hybridized cells. For enumeration of total microbial cells, the protocol of Hicks et al. (21) for dual staining with DAPI (4′,6-diamidino-2-phenylindole) and fluorescent rRNA probes was modified. After hybridization and washing, staining was performed directly on the slides for 5 min at a final concentration of 0.1 μg of DAPI per ml. Cells on the aggregates collected in situ were counted for three replicate samples with an epifluorescence microscope (Nikon Labophot 2), using standard filter sets for the fluorochromes used. Cells on the aggregates from the time series experiments were counted for 10 replicate randomly chosen viewfields. At least 500 cells per sample were enumerated. This counting procedure was applied for the DAPI-stained samples as well as those stained by the fluorescent probes.

Chlorophyll *a*. Chlorophyll *a* concentrations were determined spectrophotometrically after hot-ethanol extraction (70°C, 90%) as described by Simon and Tilzer (33).

RESULTS

Typical abundances of aggregates in Lake Constance between 23 June and 23 August 1994 were 5 to 20 liter^{-1}, with lengths of 3 to 10 mm. The aggregates were of true pelagic origin and composed of various types of phytoplankton present in the lake and/or zooplankton molts or carcasses. Those collected on one date had fairly similar compositions and the same size. Aggregates occurring in July and August were larger than those occurring in June. Total cell numbers of procaryotes per aggregate ranged from 0.5×10^6 to 2×10^6 (Fig. 1A), and the cell size ranged from 0.2 to 0.5 μm^3 (mean, 0.35 μm^3). The number of cells per aggregate increased with the aggregate size. Fifty-five to 100% of the total cells were detected by the *Bacteria*-specific probe (Fig. 1B). Thus, cells of this size obviously contained enough target sites for fluorescent probes for the majority of the microbial assemblage to be identified. The β-subclass of *Proteobacteria* always dominated the bacterial community. This dominance was greatest on 19 July and 23 August, when this subclass composed ca. 70% of the total cells (Fig. 1C). The γ-subclass of *Proteobacteria* was least abundant and accounted for only 8 to 18% of the total cells. The α-subclass of *Proteobacteria* was fairly abundant, in particular on 27 June and 12 July. In order to analyze the temporal dynamics of the aggregate-associated microbial community, particulate organic matter was collected in a sediment trap at 50 m on 30 June, 3 days after lake snow aggregates had been sampled 30 m above the sediment trap. Interestingly, the microbial assemblage on the sediment trap material was also dominated by β-subclass proteobacteria, and the relative distributions of the three proteobacterial subclasses were fairly similar to those on aggregates collected 3 days earlier (Fig. 1C). However, the total fraction of cells detected by the *Bacteria*-specific probe was 82% and thus 1.5 times higher than that for aggregates collected 3 days earlier (55%; Fig. 1B).

In order to study the microbial colonization of aggregates in more detail, naturally derived aggregates were formed in rolling cylinders in the laboratory. One to five days after the cylinders were filled with natural lake water, aggregates had formed (Table 1). The time of formation was short at high chlorophyll *a* concentrations during the phytoplankton spring bloom and in summer (Fig. 2). In June, during the clear-water phase and chlorophyll *a* concentrations of <2 μg liter^{-1}, the time of formation was enhanced. The aggregates were composed mainly of various algae or algal debris and zooplankton molts and carcasses and reflected the composition of the suspended particulate organic material present in the lake during the typical seasonal situations (Table 1 and Fig. 2). The initial cell abundance on the naturally derived aggregates was between 0.2×10^7 and 2×10^7 cells aggregate^{-1} (Fig. 3) and was thus higher by about 1 order of magnitude than that on natural aggregates. Between 64 and 95% of total cells were detected by the *Bacteria*-specific probe (Fig. 3A). At this time, usually the β-subclass proteobacteria dominated the microbial community by 25 to 57% (Fig. 3B). Only in two experiments (27 April and 5 July) the α-subclass proteobacteria initially exceeded the numbers of the β-subclass. At the beginning of the experiment of 4 May, α- and β-subclass proteobacteria constituted similar fractions. It is interesting that on 27 April, β-subclass proteobacteria made up only 5% of the total cells, the lowest percentage we found. On this day, all proteobacteria together (α-, β-, and γ-subclasses) accounted only for 55% of the *Bac-*

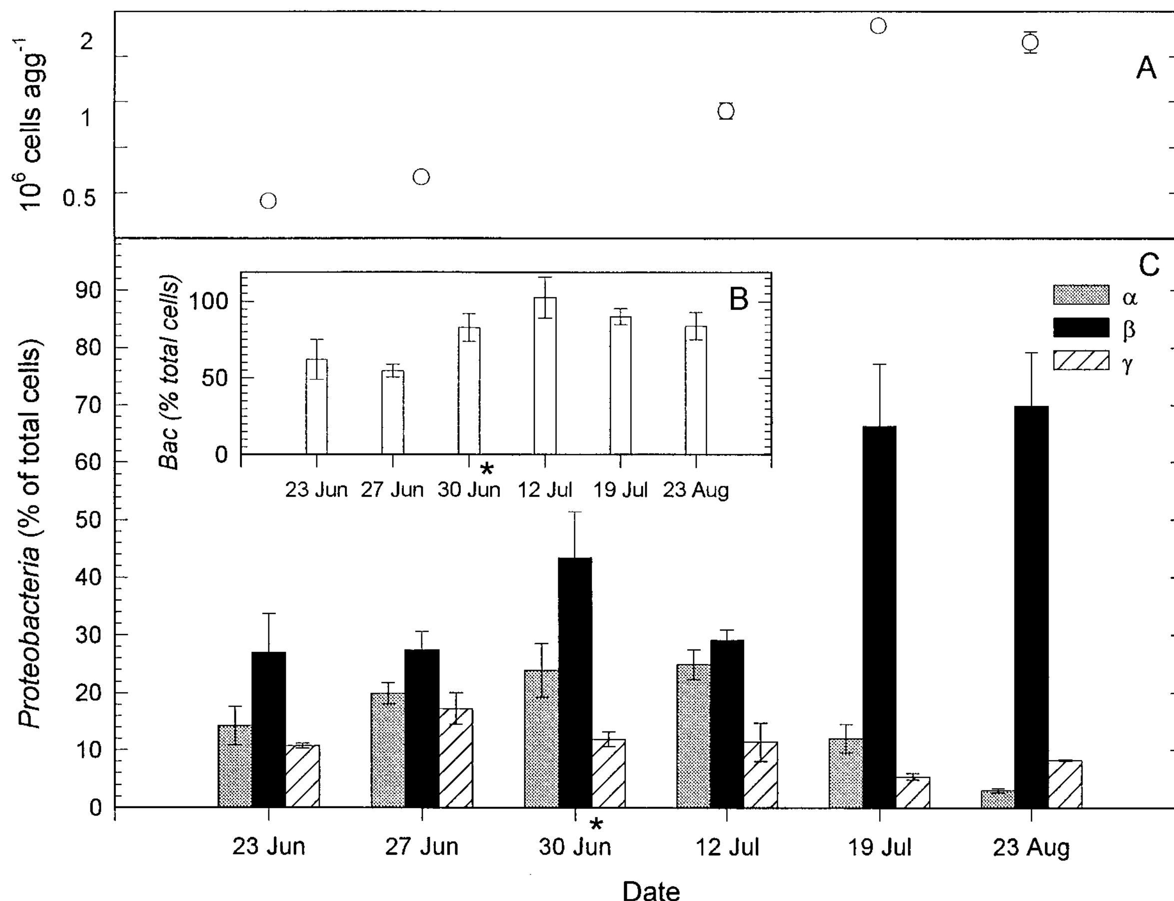

FIG. 1. Microbial colonization of natural aggregates collected in Lake Constance by SCUBA diving at 20 to 25 m in 1994 and of particulate organic matter collected in a sediment trap deployed at 50 m from 27 to 30 June 1994 (*). (A) Total cell numbers per aggregate (agg); (B) cells detected by a *Bacteria*-specific probe (percentages of total cells); (C) cells detected by probes specific for the α-, β-, and γ-subclasses of *Proteobacteria* (percentages of total cells). Error bars indicate the standard deviations of three replicates. In panel A, where error bars are not shown, the standard deviation is less than the diameter of the circle.

teria, whereas in all other cases total proteobacteria accounted for >70% of the cells.

Total cell numbers on aggregates increased substantially during the first 5 to 9 days of incubation (Fig. 4), when the size of the aggregates also increased to >10 mm. Growth rates of the total microbial assemblage during this time ranged from 0.30 to 1.49 day^{-1} (Table 2) and were in the same range as growth rates of the proteobacterial subclasses. Declines in cell numbers late in the experiments were due to grazing by aggregate-associated flagellates. The very strong decrease in cell numbers on days 6 and 10 during the experiments started on 10 May and 24 June (Fig. 4A and B, respectively), in addition, was also affected by the variation in aggregate size and thus by total bacterial numbers in the replicate cylinders. During the course of the experiments, except for that of 10 May, the proportion of β-subclass proteobacteria increased over time and exceeded those of α-subclass and γ-subclass proteobacteria by three- to fivefold, even though this subclass did not exhibit the highest growth rates (Fig. 4 and Table 2). On 10 May, in contrast to all other experiments, γ-subclass proteobacteria became most abundant during the initial phase and even remained the dominating subclass until day 7. γ-subclass proteobacteria were also fairly abundant during the experiments in June. During this period, they were the second most abundant proteobacterial subclass as a mean for the entire incubation period (Table 1). As a seasonal mean and a mean during the course of all experiments, β-subclass proteobacteria dominated the microbial assemblage on aggregates by 27 to 42%, whereas α-subclass proteobacteria constituted 11 to 25% and γ-subclass proteobacteria constituted 9 to 33% of total cells (Table 1).

DISCUSSION

Our results demonstrate that the microbial cells colonizing limnetic macroscopic organic aggregates are predominantly members of the domain *Bacteria*, which make up at least 60% of total microbial cells but often >80%. This was true for natural aggregates collected in situ, for sediment trap material, and for naturally derived aggregates formed in rolling cylinders. This high fraction of cells detected by the bacterial probe EUB 338 gave us confidence that the application of fluorescent oligonucleotide probes is a powerful tool with which to study the colonization of limnetic and presumably also of marine macroscopic organic aggregates by microbial populations.

In most samples analyzed, β-subclass proteobacteria dominated the microbial community. Interestingly, the relative fraction of β-subclass proteobacteria on the sediment trap material was substantially higher than that on lake snow aggregates collected 3 days earlier and ca. 30 m above the sediment trap. We assume that this higher fraction of β-subclass proteobacteria reflects the older organic material collected in the trap and harboring the propagating aggregate-associated microbial

TABLE 1. Composition and microbial colonization of aggregates formed in rolling cylinders under in situ conditions[a]

Sample date	Time (days)[b]	Composition	Total DAPI-stained cells (10^7 agg^{-1})[c]	% of total cells[d]						
				Bacteria	*Proteobacteria*					
					α-subclass		β-subclass		γ-subclass	
					Range	Mean	Range	Mean	Range	Mean
4 May	2	Zooplankton molts, green algae, diatoms	2.2 ± 1.8	72 ± 6	12–32	25 ± 7	20–39	27 ± 6	6–19	9 ± 5
10 May	2	Zooplankton molts and carcasses	3.9 ± 3.9	81 ± 4	6–24	16 ± 7	31–50	39 ± 6	14–42	33 ± 10
3 June	5	Zooplankton molts and carcasses	1.9 ± 1.0	79 ± 8	5–19	11 ± 5	24–60	36 ± 13	8–21	14 ± 4
17 June	4	Diatom floc	1.4 ± 0.7	79 ± 7	3–38	15 ± 11	20–63	38 ± 15	10–68	23 ± 19
24 June	2	Diatom floc	2.4 ± 2.2	80 ± 8	3–23	11 ± 5	26–55	40 ± 8	8–40	22 ± 9
1 July	1	Diatom floc	1.9 ± 2.0	76 ± 8	7–31	17 ± 7	30–59	42 ± 10	7–14	10 ± 3
5 July	1	Zooplankton molts, green algae, diatoms, *Dinobryon* sp.	3.0 ± 2.4	78 ± 10	4–37	16 ± 10	18–44	34 ± 7	4–41	12 ± 11
15 July	2	Diatom floc	2.6 ± 2.1	80 ± 5	12–21	17 ± 3	14–41	30 ± 9	8–18	11 ± 4
6 September	1	*Ceratium hirundinella* and zooplankton molts	1.3 ± 0.4	83 ± 8	17–27	23 ± 4	39–45	41 ± 3	6–13	10 ± 3

[a] The ranges of data and means (± standard deviations) over the entire incubation period of one experiment are given.
[b] Time until aggregates ≥3 mm long were formed in the cylinders.
[c] agg, aggregate.
[d] Values are given as relative percentages on the basis of total cells counted by epifluorescence microscopy.

community. Lake snow aggregates in Lake Constance are formed mainly in the upper 10 m and settle out of this layer with sinking rates of 10 to 15 m day^{-1} except in late summer, when they are dominated by filamentous cyanobacteria (19). Hence, the aggregates collected by SCUBA diving in 20 to 25 m were presumably a few days old and reached the depth of the sediment traps (50 m) approximately 3 days later. The few days after aggregate formation obviously were sufficient for colonization with a microbial community dominated by β-subclass proteobacteria and adapted to the polymer-rich substrate conditions on the aggregates. It appeared that during the subsequent sinking through the water column, this specialized microbial community propagated even more, as shown by the increased fractions of *Bacteria* and of the β-subclass of *Proteobacteria* (Fig. 1).

These patterns of colonization were supported by the more detailed analysis of the structure of the microbial community on the naturally derived aggregates formed in rolling cylinders. The microscopical examination of these aggregates indicated that their morphology and composition closely resembled those of the ambient plankton community in Lake Constance in the various seasonal situations (Table 1). Therefore, we think that the aggregates made in rolling cylinders under simulated in situ conditions were a good model system for studying the microbial colonization of lake snow aggregates, even though the shear rate and turbulence in those tanks may differ

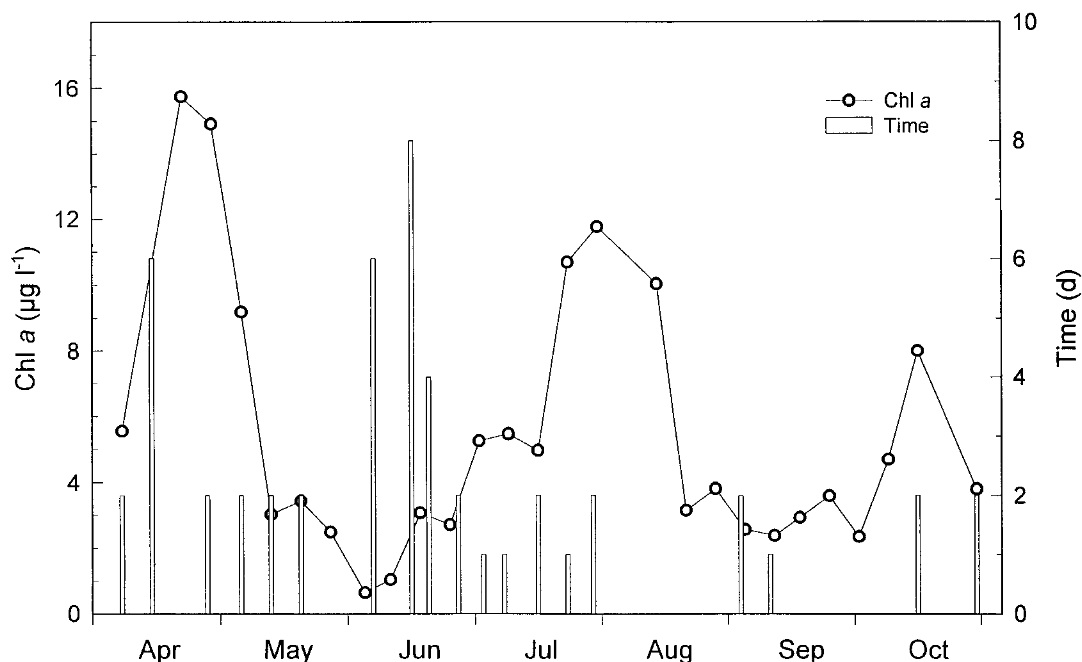

FIG. 2. Time of formation of aggregates in rolling cylinders and chlorophyll *a* (Chl *a*) concentrations (means of 0 to 8 m) for samples from Lake Constance from April to October 1993.

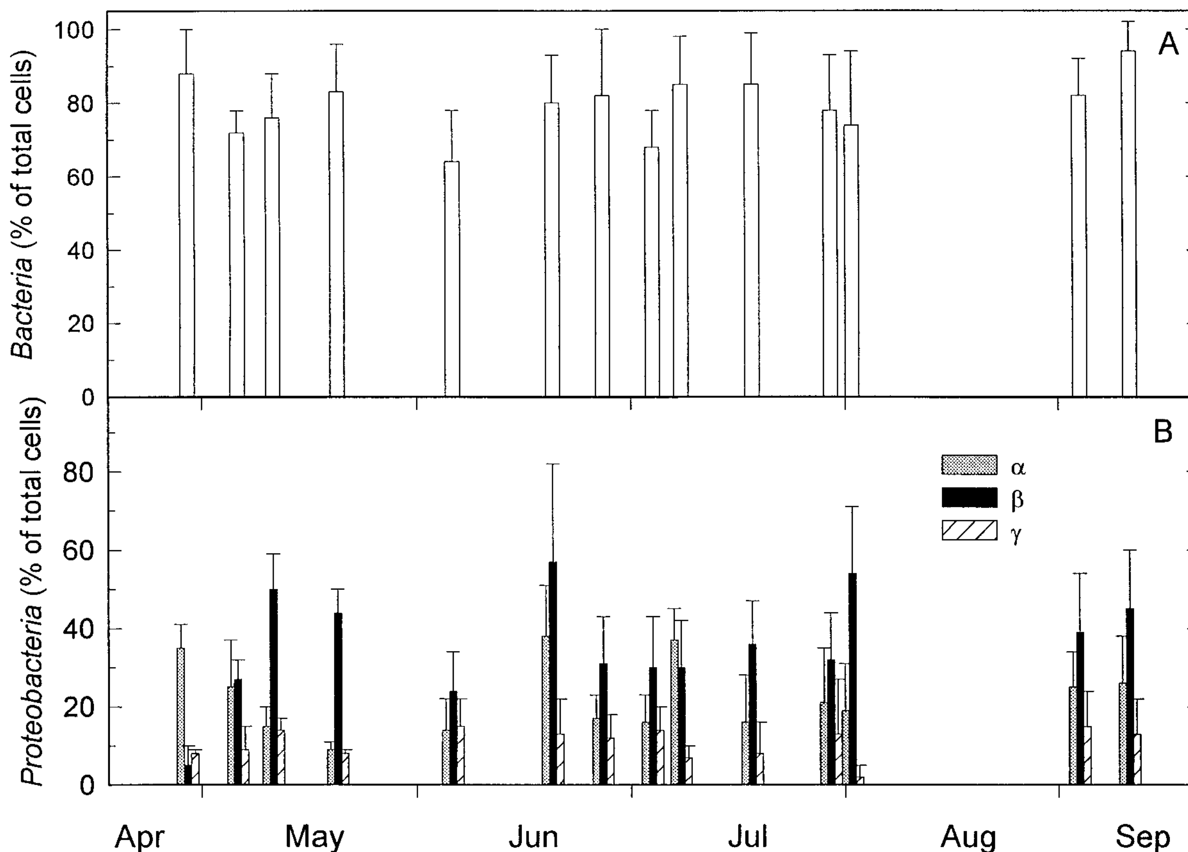

FIG. 3. Microbial colonization of macroscopic organic aggregates made in rolling tanks from natural water samples collected from Lake Constance at 6 m. Colonization patterns of the day at which aggregates had formed are presented. (A) Cells detected by a *Bacteria*-specific probe; (B) cells detected by probes specific for the α-, β-, and γ-subclasses of *Proteobacteria*.

from natural conditions (22). This assumption was also supported by the similar colonization patterns of the natural aggregates and those made in rolling tanks. The time course of the microbial colonization of the latter aggregates further indicated that β-subclass proteobacteria dominated either already when the aggregates had been formed or after a few days of incubation and, therefore, presumably have a key role in microbial dynamics on lake snow aggregates. In contrast to the varying component particle compositions of the aggregates during the different seasons, the microbial colonization patterns remained fairly stable, with a dominance of β-subclass proteobacteria. This observation suggests that the microhabitat of the lake snow aggregates was fairly uniform over the different seasons, irrespective of its composition. It further indicates that there is a specific bacterial community adapted to this substrate-rich microhabitat. The structure of this bacterial community presumably differs from that in the surrounding water, in which substrate concentrations are much more diluted. A structural difference between particle-associated and free-living bacterial communities has been shown by Bidle and Fletcher (9), who analyzed a few samples from the Chesapeake Bay, Maryland, on the basis of low-molecular-weight-RNA patterns. For a mid-bay sample, they found that the particle-associated bacterial community was less diverse than that of the free-living bacteria, even though these findings do not allow any identification of the dominating populations of either community. Structural differences between microbial communities of marine snow-associated cells and free-living cells in the surrounding water have also been found by DeLong et al. (11). On the basis of PCR-amplified 16S rRNA genes, those authors showed that marine snow harbored several clones related to the genera *Cytophaga* and *Planctomyces* and the γ-subclass of *Proteobacteria*, which were not found in the surrounding water. On the other hand, only one clone related to the genus *Cytophaga* and the γ-subclass of *Proteobacteria* was detected in the surrounding water. In addition, two clones of the α-subclass of *Proteobacteria* which were completely absent on marine snow were detected in the surrounding water. Interestingly, no clones of the β-subclass of *Proteobacteria* have been detected in this study, either on marine snow or in the surrounding water.

It would be interesting to know whether the β-subclass of *Proteobacteria* is indeed absent from marine snow aggregates. It would be quite surprising, because the trophic significances of marine snow and lake snow are similar, and microbial communities play a key role in the decomposition of both types of aggregates. Even though this absence has not been examined specifically, it is noteworthy that to our knowledge, in sequence analyses of marine planktonic bacterial 16S rRNA genes, no β-subclass proteobacteria have been detected, either (14, 17, 26). Hence, future studies must examine carefully whether this absence may be a general phenomenon in marine environments. β-subclass proteobacteria have been found, however, in other freshwater environments. Using the same sets of fluorescent oligonucleotide probes as in this investigation, Manz et al. (24) reported that β-subclass proteobacteria were also the

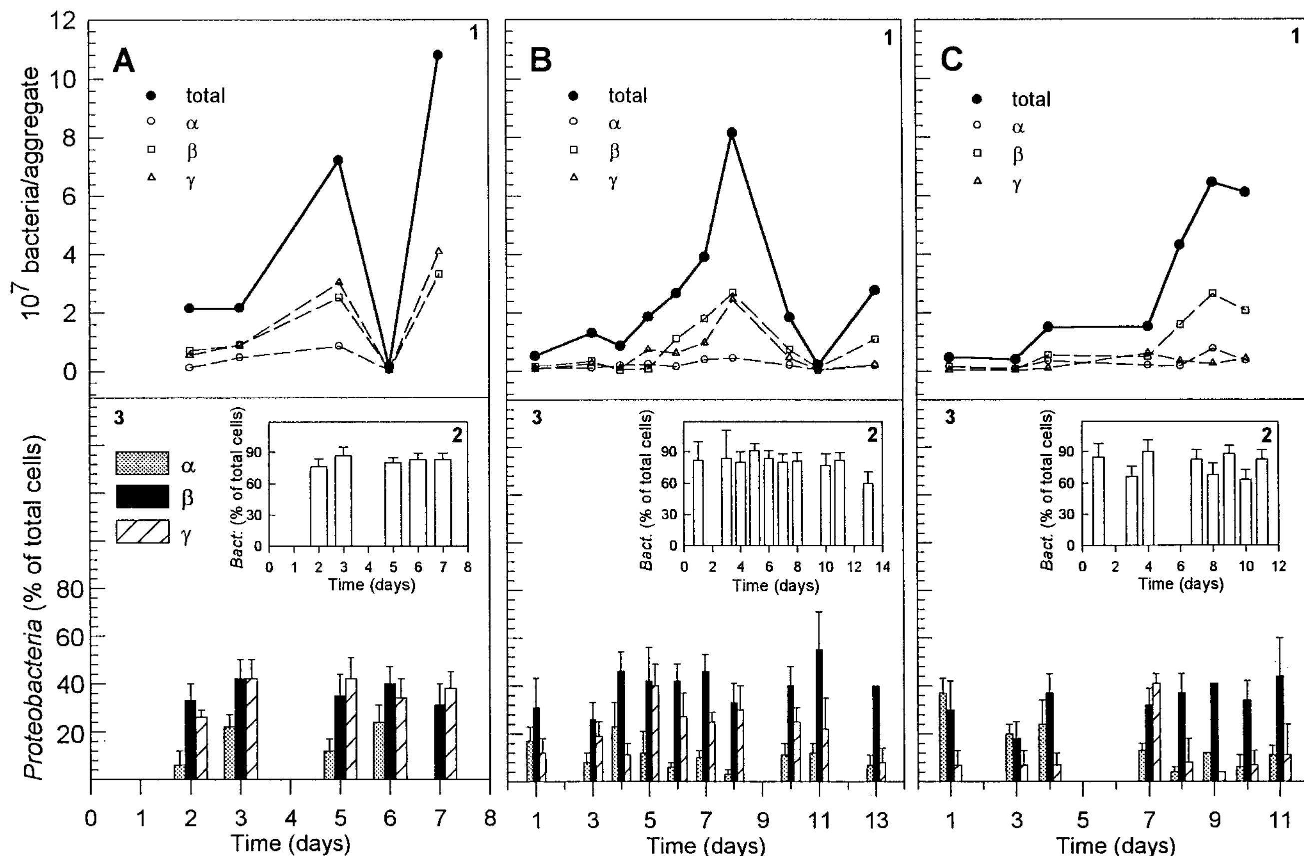

FIG. 4. Time course of the microbial colonization of macroscopic organic aggregates made from natural water samples of Lake Constance. Samples were collected at 6 m and incubated in rolling cylinders under simulated in situ conditions. Analysis started on the day at which aggregates had formed. Results of experiments with lake water collected on 10 May (A), 24 June (B), and 5 July (C) are shown. *Bact.*, cells detected with a *Bacteria*-specific probe.

dominating bacterial population on biofilms in a drinking water distribution system. Wagner et al. (37, 38) showed that β-subclass proteobacteria constituted the dominating population of microbial cells in activated sludge. These similarities in microbial colonization and the fact that all three habitats are relatively rich in organic and inorganic nutrients suggest that the three types of environments share features important for their microbial colonization.

An important implication of these findings concerns the selection of bacteria on aggregates and the question of how different from the planktonic microbial community the aggregate-associated community is. So far, however, there is no information available about the qualitative and quantitative structure of limnetic planktonic microbial communities and which proteobacterial subclasses, if any, dominate. With respect to the dominance of the β-subclass of *Proteobacteria* on lake snow aggregates and to their functional role in aggregate decomposition, an important further question concerns their genus or species affiliation. This can be addressed, for example, with a set of more-specific oligonucleotide probes that is under development. Wagner et al. (37) designed oligonucleotide probes specific for *S. natans*- and *Leptothrix discophora*-related bacteria which belong to the β1-subgroup of the β-subclass of *Proteobacteria*. They found that these organisms made up substantial fractions of the microbial populations on activated sludge (37). Preliminary experiments with these oligonucleotide probes indicated that organisms of this subgroup are also the dominant β-subclass proteobacteria on lake snow aggregates (29a). These preliminary observations suggest that the microenvironment of lake snow aggregates may have close similarities to activated sludge with respect to microbial colonization. If these observations hold true, they support the hypothesis that lake snow aggregates have a role in degrading organic matter in lacustrine systems similar to that of activated sludge in wastewater treatment plants. Lake snow is also highly enriched with organic and inorganic nutrients which are readily turned over and released (18, 19). Since marine snow exhibits nutrient enrichment and release properties similar to those of lake snow aggregates (23, 27, 34, 36), it would be very inter-

TABLE 2. Population growth rates of total cells and subclasses of *Proteobacteria* on aggregates formed in rolling cylinders

Sample date	Growth rate/day[a]			
	Total cells	Proteobacteria of subclass:		
		α	β	γ
4 May	1.49	1.57	1.52	1.29
10 May	0.58	0.61	0.52	0.84
24 June	0.37	0.23	0.47	0.49
1 July	0.30	0.27	0.31	0.28
5 July	0.32	0	0.37	0.33

[a] Growth rates were calculated during the growth phases on the basis of the exponential-growth model.

esting to examine the microbial colonization of marine snow by similar techniques.

Even though β-subclass proteobacteria dominated on most aggregates we analyzed, we want to point out that on several samples the α-subclass also made up relatively high proportions of the total cells, such as on natural aggregates of the 12 July experiment (Fig. 1) or on naturally derived aggregates of the experiment of 4 May (Table 1). So far, we do not have any information on the subgroup or genus affiliation and function of these bacteria. γ-subclass proteobacteria usually were less abundant than α- and β-subclass proteobacteria. However, on natural aggregates of the experiment of 27 June and naturally derived aggregates of the experiments of 10 May and during the period between days 3 to 5 and 5 to 8 of the experiments whose results are shown in Fig. 2, γ-subclass proteobacteria for a short time exceeded α-subclass proteobacteria and in several cases also exceeded β-subclass proteobacteria. The results of Wagner et al. (38) for activated sludge demonstrate that γ-subclass proteobacteria selectively and readily grow on organic substrates which are present in high concentrations and are readily available, such as on agar plates. Hence, the dominance of γ-subclass proteobacteria on aggregates suggests that large amounts of labile organic substrates are available under the conditions mentioned above, favoring the growth of bacteria of this proteobacterial subclass for a short period. Towards the end of the incubation period and on the sediment trap material, however, fractions of γ-subclass proteobacteria were always fairly low, supporting the idea that they preferentially grow on readily available labile organic substrates.

Adding up all three proteobacterial subclasses and comparing the total fraction of the class *Proteobacteria* with that of the domain *Bacteria* indicate that usually 85 to >95% of the total cells of *Bacteria* on aggregates belonged to this major bacterial phylogenetic unit. This leaves little room for cells other than proteobacteria as important colonizers of lake snow aggregates. Only for the natural aggregates of 12 July and naturally derived aggregates of the experiments of 3 June and 5 and 15 July were the mean ratios of α- plus β- plus γ-subclass *Proteobacteria* to *Bacteria* <0.80 (Table 1). There was only one sample, the initial value of the experiment of 27 April, in which all proteobacteria together composed only 58% of the *Bacteria* and other unknown bacterial cells were nearly as important as proteobacteria as colonizers of the aggregates.

In most samples analyzed, however, a fraction of total microbial cells which was not detected by the *Bacteria*-specific probe remained. This fraction was 37 and 44% on natural aggregates collected on 23 and 27 June, respectively, and in particular during the initial stage of aggregate formation and microbial colonization in rolling tanks. During this initial stage, the microbial cells often were smaller than later during the incubations, as examined periodically. Fairly small cells were also found on the natural aggregates collected on 23 and 27 June. This observation suggests that a fraction of the cells were smaller cells of the domain *Bacteria* containing not enough ribosomes and consequently not enough target sites for visual detection with a microscope. However, we cannot rule out that the cells were not permeabilized for the oligonucleotide probes by the applied fixation protocol or that they are not *Bacteria*. However, in several cases in which we examined aggregates with a fluorescent probe specific for *Archaea* (35), we could not visualize specifically stained cells (39).

In conclusion, our results demonstrate that rRNA-targeted fluorescent oligonucleotide probes are an attractive and powerful means of studying the dynamics of microbial assemblages on large organic aggregates. β-subclass proteobacteria were usually the dominant bacteria colonizing lake snow aggregates. Rolling tanks have been proven to be well suited to production of aggregates from natural water samples which mimic natural aggregates reliably. Further studies with more-specific probes are needed to determine in more detail the relationship between the structural properties of aggregate-associated microbial populations and their physiological function.

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