Applied and Environmental Microbiology, Mar. 2006, p. 2141–2147 Vol. 72, No. 3
0099-2240/06/$08.00+0 doi:10.1128/AEM.72.3.2141–2147.2006

Concentration-Dependent Patterns of Leucine Incorporation by Coastal Picoplankton

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Received 30 September 2005/Accepted 19 December 2005

Coastal pelagic environments are believed to feature concentration gradients of dissolved organic carbon at a microscale, and they are characterized by pronounced seasonal differences in substrate availability for the heterotrophic picoplankton. Microbial taxa that coexist in such habitats might thus differ in their ability to incorporate substrates at various concentrations. We investigated the incorporation patterns of leucine in four microbial lineages from the coastal North Sea at concentrations between 0.1 and 100 nM before and during a spring phytoplankton bloom. Community bulk incorporation rates and the fraction of leucine-incorporating cells in the different populations were analyzed. Significantly fewer bacterial cells incorporated the amino acid before (13 to 35%) than during (23 to 47%) the bloom at all but the highest concentration. The incorporation rate per active cell in the prebloom situation was constant above 0.1 nM added leucine, whereas it increased steeply with substrate concentration during the bloom. At both time points, a high proportion of members of the *Roseobacter* clade incorporated leucine at all concentrations (55 to 80% and 86 to 94%, respectively). In contrast, the fractions of leucine-incorporating cells increased substantially with substrate availability in bacteria from the SAR86 clade (8 to 31%) and from DE cluster 2 of the *Flavobacteria-Sphingobacteria* (14 to 33%). The incorporation patterns of marine *Euryarchaeota* were between these extremes (30 to 56% and 48 to 70%, respectively). Our results suggest that the contribution of microbial taxa to the turnover of particular substrates may be concentration dependent. This may help us to understand the specific niches of coexisting populations that appear to compete for the same resources.

The pelagic environment is believed to be heterogeneous with respect to the small-scale distribution of dissolved organic molecules. Short-lived point sources of dissolved organic matter may arise directly, e.g., from phytoplankton release (4), viral lysis of algal cells (5), and zooplankton feeding (24), or indirectly from the ectoenzymatic hydrolytic activity of particle-associated microbes (16). In particular, the dissolved free amino acids may account for a substantial fraction of microbial carbon demand in aquatic systems (13, 14), and they also represent an energetically favorable source of nitrogen (49).

Bulk dissolved free amino acid concentrations in seawater are typically low, as set by the high uptake affinities of pelagic microorganisms (reference 45 and references therein). Moreover, their concentrations in coastal temperate waters are maintained within a narrow range regardless of high variability of primary production, likely due to a tight coupling between dissolved free amino acid release and microbial consumption (14, 19). In situ measurements of amino acid incorporation (45) as well as studies on isolates (41) indicate that marine bacteria may harbor several uptake systems for dissolved free amino acids with different kinetic parameters that provide a means of enhanced incorporation over a broad range of substrate concentrations. In addition, the rates of assimilation of amino acids by microbial communities can change rapidly, e.g., during the course of a phytoplankton bloom (47).

Although the importance of dissolved organic matter mineralization by picoplankton has been recognized for decades, the relative contributions of the various phylogenetic groups to the consumption of particular dissolved organic matter fractions in the sea are currently the subject of intense investigation (8, 26, 49). It is theoretically conceivable that some microbial species in pelagic habitats may not be capable of incorporating monomeric substrates such as dissolved free amino acids at all. Alternatively, it is also possible that the relative contributions of different picoplankton taxa to the total turnover of these substrates are governed by their specific incorporation patterns (i.e., affinity, velocity, and saturation). Such differences moreover might have pronounced effects on interspecific competition (6).

Currently, it is unknown whether the above-discussed shifts in community uptake kinetics with changing substrate availability reflect the physiological plasticity of the dominant community members, a succession of specialized genotypes, or both. In past years a promising combination of two single-cell methods has been established (microautoradiography and fluorescence in situ hybridization [MAR-FISH]) that allows direct, semiquantitative tracking of substrate incorporation by specific microbial populations in marine picoplankton (8, 29). Recent modifications of the approach have rendered it more suitable for work with aquatic microbes with low ribosome content (44) and for accurate high-throughput sample processing (2).

We compared in situ substrate incorporation of the pelagic microbial community in coastal North Sea surface waters in a late-winter phytoplankton prebloom situation and during a spring bloom dominated by *Phaeocystis* spp. A widely used tracer of bacterial protein synthesis, leucine (21), was chosen as a model substrate for dissolved free amino acid incorporation. We hypothesized that the individual phylogenetic groups might differ in their concentration-dependent patterns of

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leucine incorporation, and that there might be differences in the numbers of substrate-incorporating cells before and during the bloom. This was studied by MAR-FISH and by the determination of bulk incorporation rates.

MATERIALS AND METHODS

Sampling site. Surface water samples (1-m depth) were collected on 11 March and 6 May 2004 at the sampling station Helgoland Roads (54°11′N, 7°54′E; water depth, 8 m), 50 km offshore in the German Bay of the North Sea. The salinity was comparable at both sampling time points. In March the surface water temperature was 4.2°C and the phytoplankton biomass was low (<10 μg C liter^{-1}). The algal community was dominated by diatoms, in particular *Thalassiosira nitzschioides*. At the second sampling time point the water temperature was 8.3°C and the phytoplankton biomass was >200 μg C liter^{-1}. Although diatoms were still present, the phytoplankton community in May was dominated by colonies of the haptophyte *Phaeocystis* sp., which formed approximately 70% of the total algal biomass (Mursys Umweltreport, www.bsh.de).

Incubations with [^{3}H]leucine. Two sets of incubations were performed for the analysis of concentration-dependent leucine incorporation by the picoplankton assemblage. One set of samples, subsequently referred as bulk incorporation, was used to determine the rate of substrate incorporation by the total microbial community at different concentrations of [^{3}H]leucine. The other set served to track the incorporation patterns of different populations by MAR-FISH. Both sets of samples were incubated with [^{3}H]leucine (specific activity, 2.26 TBq/mmol; Amersham Bioscience, Freiburg, Germany) in a concentration series (0.1, 1, 10, and 100 nM) within 1 h after sample collection. For every treatment type, triplicate 10-ml subsamples plus one control, seawater fixed with freshly prepared buffered paraformaldehyde fixative (final concentration, 1%, vol/vol) (33), were incubated. The incubations were run for 4 h in the dark at ambient water temperatures. Subsequently, paraformaldehyde was added to the incubation mixtures to a final concentration of 1% (vol/vol).

After 1 h of fixation at room temperature the samples were frozen for transport and stored at −20°C. The samples for bulk incorporation were filtered onto cellulose mixed-ester filters (type GSWP; pore size, 0.2 μm; diameter, 25 mm; Millipore, Eschborn, Germany) and rinsed twice with ice-cold trichloroacetic acid and ethanol (20). The samples for MAR-FISH analysis were filtered onto polycarbonate filters (type GTTP; pore size, 0.2 μm; diameter, 25 mm; Millipore, Eschborn, Germany) and rinsed twice with sterile phosphate-buffered saline. All filters were stored at −20°C until further analysis.

Bulk measurements of substrate incorporation. The cellulose filters were placed in 6-ml scintillation vials and dissolved with 0.5 ml of ethyl acetate. Subsequently, 5 ml of Lumasafe Plus scintillation cocktail (Lumac LSC BV, Groningen, The Netherlands) was added to each vial. The amount of radiolabeled substrate assimilated by microbial cells was measured by scintillation counting (Packard TriCarb 2900 TR; Perkin-Elmer, Wellesley, MA). Measurements were corrected for quench (external standard method) and by subtraction of counts from the prefixed controls.

Quantification of populations by FISH. Samples for the determination of the in situ abundance of the different bacterial populations were immediately fixed after collection with paraformaldehyde (final concentration, 1%, vol/vol). The abundance of different microbial taxa was determined by FISH with horseradish peroxidase-labeled oligonucleotide probes and catalyzed reporter deposition (CARD-FISH) (31).

In addition to probe EUBI-III (most *Bacteria*) (9), four specific probes were selected that targeted substantial populations (>1% of total counts) which also scored positively for substrate incorporation: EURY806 (marine group II *Euryarchaeota*) (44), ROS537 (members of the *Roseobacter* clade of α-*Proteobacteria*) (11), SAR86-1245 (members of the SAR86 clade of γ-*Proteobacteria*) (10), and CF6-1267 (members of DE cluster 2 of the *Flavobacteria-Sphingobacteria* group of the phylum *Bacteroidetes)* (23). The eubacterial antisense probe NON338 (3) was used as a negative control. All probes were purchased from Biomers.net (Ulm, Germany). Counterstaining of CARD-FISH preparations was done with 4′,6′-diamidino-2-phenylindole (DAPI; final concentration, 1 μg ml^{-1}). Evaluation of DAPI- and FISH-stained cells was carried out by epifluorescence microscopy (100× magnification, Axioplan; Carl Zeiss, Jena, Germany) as previously described (33), counting a minimum of 1,000 DAPI-stained cells per sample.

MAR-FISH analysis. A recently developed MAR-FISH protocol was applied to study substrate incorporation by specific picoplankton groups (2). The key features of this new protocol are the combination of CARD-FISH staining and microautoradiography of microbes on membrane filters without prior cell transfer onto glass slides and evaluation by semiautomated image acquisition and analysis. Triplicate samples from every treatment type were analyzed by MAR-FISH with the different probes as described previously (2), and controls were evaluated with probe EUB I_III. Altogether, 108 MAR-FISH-stained preparations were evaluated.

The photochemicals for MAR (autoradiography emulsion type NTB-2, developer type Dektol, and fixer) were purchased from Eastman Kodak, Rochester, N.Y. Development of the exposed slides was performed according to the manufacturer's instructions (2 min of development, 10 s of rinsing with deionized particle-free water, 5 min exposure in fixer, washing for 5 min in deionized particle-free water). Different MAR exposure times were used for the evaluation of different treatment types: March samples, 72 h (0.1 nM), 48 h (1 and 10 nM), and 24 h (100 nM); May samples, 72 h (0.1 nM), 24 h (1 and 10 nM), and 12 h (100 nM). This strategy was chosen to obtain the maximal number of cells scored as having silver grains (subsequently referred to as leucine active) at different concentrations of [^{3}H]leucine rather than to quantitatively compare incorporation under one exposure condition (e.g., by determination of grain size area) (7). Images of MAR-FISH preparations were captured with a digital camera (Orca I; Hamamatsu, Herrsching, Germany) mounted on a motorized epifluorescence microscope (Axioplan II Imaging; Carl Zeiss). Evaluation was performed as previously described (2) using the image analysis software KS400 (Carl Zeiss).

Hypotheses for statistical analysis and statistical tests. The following formal hypotheses were tested. (i) The fractions of leucine-active *Bacteria* (as detected by probe EUB I-III) and leucine-active cells affiliated with the SAR86 and *Roseobacter* clades and with marine *Euryarchaeota* would be different between the two sampling times. (ii) The fractions of all leucine-active *Bacteria* and of members of the SAR86 and *Roseobacter* clades, of DE cluster 2 and of marine *Euryarchaeota* would be different at different concentrations of [^{3}H]leucine. This was tested separately for each probe-defined cell population (dependent variable) by two-way linear analysis of variance of the sets of triplicate determinations, with concentrations and sampling time points as independent variables, and by post-hoc pairwise multiple comparisons using Bonferroni corrections. For DE cluster 2, which was present only in May, hypothesis ii was tested by one-way analysis of variance (concentration) and post-hoc comparisons (Bonferroni's method). Calculations were performed using the software SigmaStat (SPSS Inc., Chicago, Ill.).

RESULTS

Community contribution of the study populations. Total cell numbers did not change substantially between the sampling dates: 0.8 (± 0.2) × 10^6 cells ml^{-1} in March and 1.2 (± 0.4) × 10^6 cells ml^{-1} in May. At both time points 84% of all DAPI-stained objects were detected by CARD-FISH (Table 1). *Euryarchaeota* constituted a constant fraction of total counts at both sampling dates. In May, the contribution of *Roseobacter* to all hybridized *Bacteria* was slightly higher. Members of the SAR86 clade formed a similar fraction of hybridized *Bacteria* and of total cell numbers at both months. Members of DE cluster 2 of the *Flavobacteria-Sphingobacteria* group, as targeted by probe CF6-1267, were too rare for accurate quantification in March, and they represented 5% of hybridized *Bacteria* in May.

Incorporation of labeled substrate. Bulk incorporation rates of leucine were higher in May at [^{3}H]leucine concentrations of >0.1 nM (Fig. 1A). In March the amount of incorporated leucine was similar at all concentrations, whereas in May it steeply increased with increasing substrate concentration.

A significantly higher proportion of bacterial cells incorporated tritiated leucine in May than in March ($F = 72.9$, $P < 0.0001$), in particular at the lower substrate concentrations (interaction of variables date and concentration, $F = 9.5$, $P < 0.001$) (Fig. 1B). The proportion of bacterial cells with visible incorporation of [^{3}H]leucine did not significantly increase at concentrations of >10 nM in March and >1 nM in May (Bonferroni pairwise comparisons, $P > 0.05$). Negative controls from prefixed samples yielded similar percentages of false-

TABLE 1. Cell numbers of the different microbial groups and fractions of total (DAPI-stained) cells and of hybridized *Bacteria* (bacterial taxa only)

Group	March			May		
	% of total cells (% DAPI)	% *Bacteria* (% EUB)	Mean abundance, 10^5 cells ml^{-1} (±SD)	% of total cells (% DAPI)	% *Bacteria* (% EUB)	Mean abundance, 10^5 cells ml^{-1} (±SD)
Bacteria	82		6.20 (±1.6)	82		8.40 (±2.5)
Euryarchaeota	2		0.15 (±0.04)	2		0.22 (±0.07)
Roseobacter	7	8	0.50 (±0.12)	8	10	0.97 (±0.29)
SAR86	4	5	0.32 (±0.08)	3	4	0.37 (±0.11)
DE cluster 2	b.d.[a]	b.d.	b.d.	3	4	0.39 (±0.12)

[a] b.d., below detection limit (1% of total cell counts).

positive MAR-active cells irrespective of sampling time point, incubation, and exposure conditions; approximately 1% of cells hybridized with probe EUB I-III (data not shown). In both months, the leucine incorporation rate per leucine-active bacterial cell was fivefold higher at 1 nM than at 0.1 nM added [^{3}H]leucine. In March this parameter did not increase further with concentration, whereas in May it increased by a factor of 30 across the total range of concentrations (Fig. 1B).

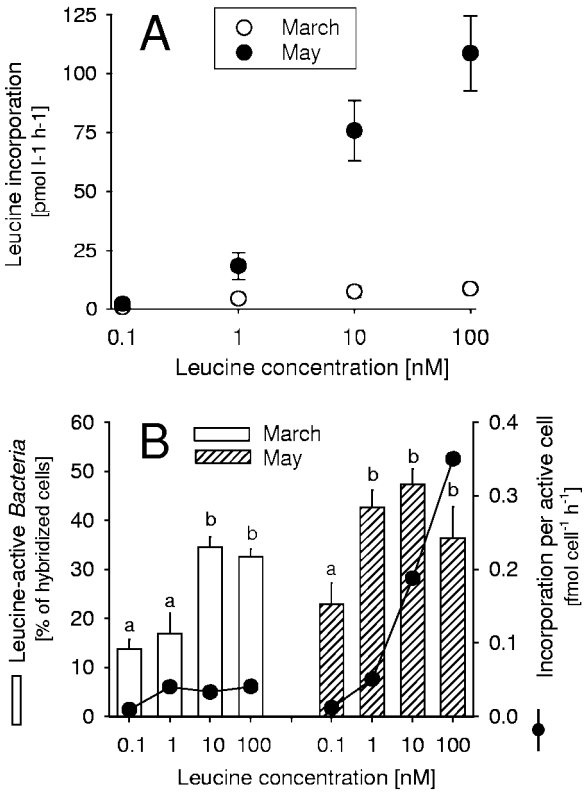

FIG. 1. A: Incorporation rates of [^{3}H]leucine by coastal North Sea picoplankton in a prebloom situation (March) and during spring phytoplankton bloom (May) at different concentrations of offered substrate (mean ± 1 standard deviation). B: Bars show fractions of bacterial cells (i.e., cells hybridized by the probe EUB I-III) with visible incorporation of leucine at different concentrations of offered substrate (mean ± 1 standard deviation). Symbols and lines show average per-cell leucine incorporation normalized to cells with visible substrate incorporation. Identical letters indicate treatments that were statistically indistinguishable.

The majority of members of the *Roseobacter* clade showed visible substrate incorporation at all substrate concentrations (Fig. 2A). A higher fraction of cells from this population incorporated leucine in May than in March, as was observed for the whole bacterial assemblage (Fig. 1B). However, in contrast to *Bacteria*, the proportion of leucine-active cells affiliated to *Roseobacter* was already high at the lowest concentration of added [^{3}H]leucine and there was no (May) or only a little (March) increase in the fraction of *Roseobacter* cells with visibly incorporated [^{3}H]leucine at increasing substrate concentration (Fig. 2A). Although members of the *Roseobacter* clade constituted only 8 and 10% of total hybridized cells in March and May, respectively (Table 1), these bacteria represented >30% (March) and almost 50% (May) of all leucine-active *Bacteria* at the lowest concentration of added [^{3}H]leucine (Fig. 2B).

Members of DE cluster 2 of the *Flavobacteria-Sphingobacteria* group were only present in May. The number of cells with visible

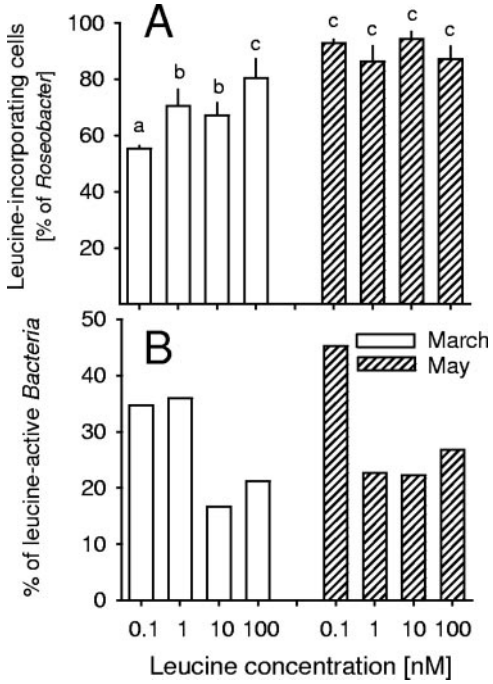

FIG. 2. A: Fractions of cells from the *Roseobacter* clade with visible incorporation of leucine at different concentrations of offered substrate. B: Contribution of bacteria from this lineage to all leucine-assimilating bacterial cells (i.e., cells that hybridized with probe EUB I-III).

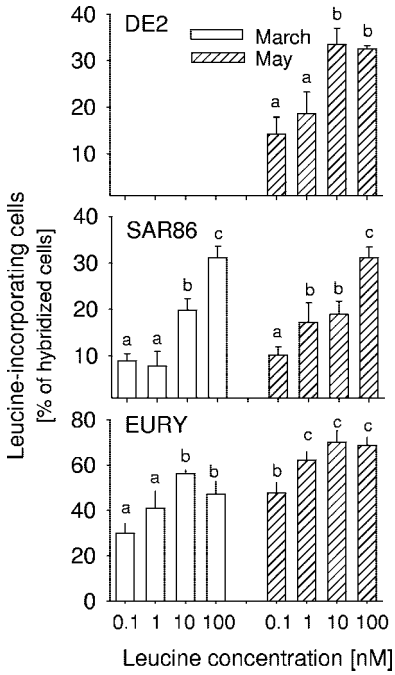

FIG. 3. Fractions of cells from DE cluster 2 (upper panel), the SAR86 clade (central panel), and marine group II *Euryarchaeota* showing visible incorporation of leucine at different concentrations of offered substrate.

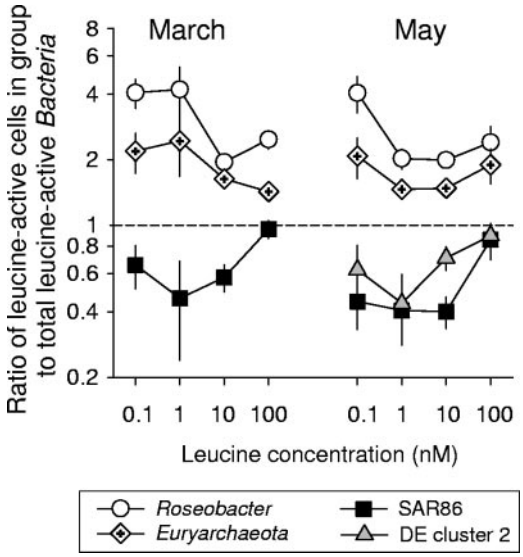

FIG. 4. Ratio of the fraction of leucine-assimilating cells in the phylogenetic groups studied to the fraction of leucine-assimilating *Bacteria*. Errors were estimated assuming additive error propagation.

incorporation of [^{3}H]leucine increased with substrate concentration (Fig. 3). The percentage of leucine-active cells approximately doubled between [^{3}H]leucine concentrations of 1 and 10 nM, and their respective proportions were very similar at the lower two and at the higher two concentrations (Fig. 3). The fraction of cells from the SAR86 clade that incorporated the radiolabeled substrate also increased with increasing concentration (Fig. 3). In contrast to *Bacteria* and to the other populations studied, the proportion of leucine-active SAR86 cells at a given concentration of [^{3}H]leucine was very similar at the two sampling time points. The only exception to this was at 1 nM added [^{3}H]leucine, where the percentage of MAR-positive cells was significantly higher in May (Fig. 3) (Bonferroni pairwise comparisons, $P > 0.05$).

A high proportion of cells from the marine group II *Euryarchaeota* were able to incorporate [^{3}H]leucine at all concentrations (Fig. 3). As observed for *Bacteria*, this fraction was significantly higher in May, when up to 70% of all *Euryarchaeota* were also MAR positive (Fig. 3). The percentage of cells incorporating leucine significantly increased at substrate concentrations >1 nM in March and >0.1 nM in May.

The proportion of cells with visible leucine incorporation was two- to fourfold higher in members of the *Roseobacter* clade than in hybridized *Bacteria*, and it was on average twice as high in *Euryarchaeota* (Fig. 4). This was particularly pronounced in March and at the lowest substrate concentrations. By contrast, the fraction of leucine-incorporating cells in bacteria affiliated with the SAR86 and DE cluster 2 was significantly below the community average at the three lower substrate concentrations. It matched the average fraction of all leucine-active *Bacteria* at a leucine concentration of 100 nM (Fig. 4).

DISCUSSION

Substrate incorporation by the whole microbial assemblage. On average, approximately 30% of all bacterial cells in our samples were able to incorporate [^{3}H]leucine. This has been suggested to be a typical value for aquatic systems (43). However, we also show that the fraction of cells that are "active" with respect to incorporation of this amino acid may be related to the concentration of the offered substrate (Fig. 1B), in particular if specific populations are investigated. If we had used only one concentration of [^{3}H]leucine, we would have concluded that 10% (1 nM), 20% (10 nM), or 30% (100 nM) of bacteria from the SAR86 clade are "active" in March (Fig. 3).

At substrate concentrations of 1 to 10 nM the proportions of leucine-active prokaryotic cells (Fig. 1B) and the rate of leucine incorporation (Fig. 1A) were significantly higher during the bloom than before. This agrees with observations that blooms of *Phaeocystis* may stimulate microbial utilization of monomeric substrates (25). In the prebloom situation the incorporation rate per leucine-active microbial cell remained constant at concentrations of 1 nM added [^{3}H]leucine and above, whereas it steeply increased over the whole range of offered substrate concentrations in May (Fig. 1B). The incorporation rates per leucine-active cell were very similar at the lower two concentrations (0.1 and 1 nM) in both months (Fig. 1B). By contrast, substantially more cells could incorporate the substrate even at low concentrations during the *Phaeocystis* bloom, and these "active" bacteria (and possibly also *Euryarchaeota*) were able to incorporate higher concentrations of substrate at significantly higher rates (Fig. 1B). This suggests a transition from an "oligotrophic" (high affinity) to a more "copiotrophic" (high velocity) substrate incorporation pattern,

as previously observed during a spring bloom in the Chukchi Sea (47).

Substrate incorporation patterns of single picoplankton groups. Incorporation of tritiated leucine was observed in all studied prokaryotic populations at all substrate concentrations (Fig. 2 and 3). We selected different MAR exposure times to obtain a maximum number of leucine-active cells. In our experimental setup a fixed exposure time would have resulted either in underestimation of leucine-active cells at low concentrations of [^{3}H]leucine or in complete blackening of preparations at high concentrations. Potentially, our strategy might have diminished the differences between leucine-active cells at the various substrate concentrations. However, since we nevertheless detected significant differences in the incorporation patterns of the phylogenetic groups studied, the main hypothesis of our investigation could be readily addressed by our approach.

At least two distinct patterns of incorporation could be distinguished. For one, a large fraction of cells from the *Roseobacter* clade were involved in leucine incorporation in May irrespective of the substrate concentration (Fig. 2A). By contrast, a higher number of leucine-active cells were observed in bacteria affiliated to the SAR86 clade and DE cluster 2 at increasing concentrations of [^{3}H]leucine (Fig. 3). *Euryarchaeota* (and *Roseobacter* during the prebloom situation) ranged between these extremes; i.e., the relationship between the proportion of leucine-active cells and substrate concentration was weak but significant (Fig. 2A and 3). Interestingly, none of the bacterial populations studied displayed the pattern that was characteristic of the bacterial assemblage as a whole (Fig. 1B, 2A, and 3). However, the three bacterial groups examined here only accounted for 25 to 50% of all leucine-active bacterial cells. Therefore, it cannot be excluded that other populations more closely resembled the "average" pattern of leucine incorporation.

Members of the *Roseobacter* clade are often found during natural or induced phytoplankton blooms (35, 38, 48). These bacteria are associated with, e.g., diatoms (17, 39) or dinoflagellates (36), and they colonize alga-derived detrital particles (38). In addition, bacteria from this clade also thrive under very low nutrient conditions (34). Our data suggest that members of the *Roseobacter* lineage in coastal North Sea waters are good competitors for leucine across a range of concentrations (Fig. 4) and that these bacteria may thus profit from substrate gradients as encountered during a *Phaeocystis* bloom situation.

Some genotypes within the *Roseobacter* lineage are free-living, whereas others preferentially attach to particles (12, 17). Therefore, it is possible that physiologically distinct subpopulations were responsible for [^{3}H]leucine incorporation at different substrate concentrations in our samples. However, the constantly high percentage of leucine-active cells at all substrate concentrations (Fig. 2A) indicates that the *Roseobacter* population studied behaved as a single ecophysiological unit with respect to leucine assimilation.

SAR86 is a phylogenetic lineage of free-living bacteria in coastal and offshore waters (1, 10, 27). These bacteria have not been described from algal cultures (17, 35, 39), but they have been detected in high quantities during a coccolithophore bloom (15). In the German Bight, SAR86 bacteria grew more rapidly in late summer than during spring (30), and they did not incorporate glucose offered at a concentration of 10 nM (2). The percentage of leucine-assimilating cells from the SAR86 group in our samples was below the community average at all but the highest concentration (Fig. 4). This suggests that SAR86-related bacteria are competitive for leucine only if its supply is high. The similar incorporation patterns of members of the SAR86 clade during the prebloom and bloom situations (Fig. 3) moreover indicate that the activity of SAR86 may not be governed primarily by the availability of alga-derived monomeric substrates.

Marine DE cluster 2 is a monophyletic group of highly similar (>99%) 16S rRNA sequence types closely related to genera such as *Geldibacter* and *Salgentibacter*. It was first described from the Delaware estuary and from the Chukchi Sea (23). Members of this lineage occurred at considerable densities in the coastal North Sea (Table 1). While the three habitats differ in many respects, they also have some common characteristics, e.g., seasonally high primary production, influence of river discharge, and a high exchange between the sediment and the water column (23, 37, 40, 46).

Bacteria of DE cluster 2 were present only during the *Phaeocystis* bloom, whereas they were too rare for FISH quantification in the prebloom situation (Table 1). This suggests that their occurrence may be related to the development of the phytoplankton assemblage. Other members of the *Flavobacteria-Sphingobacteria* group have been found during natural (12) and artificial (17, 35, 38) phytoplankton blooms, including *Phaeocystis*-dominated blooms (42). The significantly higher numbers of leucine-active cells from DE cluster 2 at higher concentrations of added [^{3}H]leucine (Fig. 3) might indicate that these bacteria require the rich conditions provided by the phytoplankton bloom, but also that leucine was not their preferred substrate. Other free-living marine representatives of the *Flavobacteria-Sphingobacteria* group are known to degrade polymers or to preferably incorporate high-molecular-weight dissolved organic matter (8, 22).

The planktonic marine group II *Euryarchaeota* are a common element of microbial assemblages in coastal waters during the growing season of the phytoplankton (28, 32), whereas *Crenarchaeota* in North Sea surface waters only formed larger populations during winter (32). In the upper layers of the central North Atlantic, 20 and 30% of *Euryarchaeota* cells assimilated aspartate and leucine, respectively, and these microbes also incorporated radiolabeled bicarbonate (18, 44). In our samples, the relative abundance of leucine-active *Euryarchaeota* was always higher than that of *Bacteria* and twice as high at the lowest substrate concentration (Fig. 4). Their activity moreover increased at higher substrate concentrations and during the phytoplankton bloom (Fig. 3). Thus, these microbes appear to be highly competitive for dissolved free amino acid across a wide range of concentrations.

Conclusions. We show that several microbial populations that compete for the same substrate differed in their concentration-dependent patterns of leucine incorporation. Thus, their coexistence might be favored by microscale or short-term temporal substrate patchiness. In fact, our observations provide indirect evidence for the ecological relevance of habitat variability. A large fraction of the dissolved free amino acid turnover might be carried out by a few specialists (e.g., *Ro-*

seobacter) at low substrate concentrations, whereas other taxa with lower uptake affinity (e.g., members of DE cluster 2) might participate in this process during phytoplankton bloom situations. Our results moreover agree with the hypothesis that some heterotrophic microbial populations in marine picoplankton may harbor several uptake systems that operate at different ranges of substrate concentration.

ACKNOWLEDGMENTS

Elke Allers and Claudia Piccini are gratefully acknowledged for help during the experiments, and Tim Ferdelman and Citlali Guerra are thanked for assistance during sample evaluation. We also thank Gunnar Gerdts and Melanie Sapp for hosting us on Helgoland during the field work and Rudi Amann for his continuous support.

This work was supported by the European Union (EVK3-2001-00194 BASICS) and by the Max Planck Society.

REFERENCES

1. **Acinas, S. G., J. Antón, and F. Rodríguez-Valera.** 1999. Diversity of free-living and attached bacteria in offshore Western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. Appl. Environ. Microbiol. **65:**514–522.
2. **Alonso, C., and J. Pernthaler.** 2005. Incorporation of glucose under anoxic conditions by bacterioplankton from coastal North Sea surface waters. Appl. Environ. Microbiol. **71:**1709–1716.
3. **Amann, R. I.** 1995. *In situ* identification of microorganisms by whole cell hybridization with rRNA-targeted nucleic acid probes. Mol. Microb. Ecol. Manual **3.3.6:**1–15.
4. **Bell, W. H., and E. Sakshaug.** 1980. Bacterial utilization of algal extracellular products. 2. A kinetic study of natural populations. Limnol. Oceanogr. **25:** 1021–1033.
5. **Brussaard, C. P. D., R. Riegman, A. A. M. Noordeloos, G. C. Cadee, H. Witte, A. J. Kop, G. Nieuwland, F. C. Vanduyl, and R. P. M. Bak.** 1995. Effects of grazing, sedimentation and phytoplankton cell lysis on the structure of a coastal pelagic food web. Mar. Ecol. Prog. Ser. **123:**259–271.
6. **Button, D. K.** 1991. Biochemical basis for whole-cell uptake kinetics—specific affinity, oligotrophic capacity, and the meaning of the Michaelis constant. Appl. Environ. Microbiol. **57:**2033–2038.
7. **Cottrell, M. T., and D. L. Kirchman.** 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware Estuary. Limnol. Oceanogr. **48:**168–178.
8. **Cottrell, M. T., and D. L. Kirchman.** 2000. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. **66:**1692–1697.
9. **Daims, H., A. Bruhl, R. Amann, K. H. Schleifer, and M. Wagner.** 1999. The domain-specific probe EUB338 is insufficient for the detection of all *Bacteria*: development and evaluation of a more comprehensive probe set. Syst. Appl. Microbiol. **22:**434–444.
10. **Eilers, H., J. Pernthaler, F. O. Glöckner, and R. Amann.** 2000. Culturability and in situ abundance of pelagic bacteria from the North Sea. Appl. Environ. Microbiol. **66:**3044–3051.
11. **Eilers, H., J. Pernthaler, J. Peplies, F. O. Glöckner, G. Gerdts, and R. Amann.** 2001. Isolation of novel pelagic bacteria from the German Bight and their seasonal contribution to surface picoplankton. Appl. Environ. Microbiol. **67:**5134–5142.
12. **Fandino, L. B., L. Riemann, G. F. Steward, R. A. Long, and F. Azam.** 2001. Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. Aquat. Microb. Ecol. **23:** 119–130.
13. **Ferguson, R. L., and W. G. Sunda.** 1984. Utilization of amino acids by planktonic marine bacteria—importance of clean technique and low substrate additions. Limnol. Oceanogr. **29:**258–274.
14. **Fuhrman, J. A.** 1987. Close coupling between release and uptake of dissolved free amino acids in seawater studied by an isotope dilution approach. Mar. Ecol. Prog. Ser. **37:**45–52.
15. **González, J. M., R. Simo, R. Massana, J. S. Covert, E. O. Casamayor, C. Pedros-Alio, and M. A. Moran.** 2000. Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. Appl. Environ. Microbiol. **66:**4237–4246.
16. **Grossart, H. P., S. Hietanen, and H. Ploug.** 2003. Microbial dynamics on diatom aggregates in Oresund, Denmark. Mar. Ecol. Prog. Ser. **249:**69–78.
17. **Grossart, H. P., F. Levold, M. Allgaier, M. Simon, and T. Brinkhoff.** 2005. Marine diatom species harbour distinct bacterial communities. Environ. Microbiol. **7:**860–873.
18. **Herndl, G. J., T. Reinthaler, E. Teira, H. van Aken, C. Veth, A. Pernthaler, and J. Pernthaler.** 2005. Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol. **71:** 2303–2309.
19. **Hoppe, H. G., S. J. Kim, and K. Gocke.** 1988. Microbial decomposition in aquatic environments—combined process of extracellular enzyme activity and substrate uptake. Appl. Environ. Microbiol. **54:**784–790.
20. **Kirchman, D.** 2001. Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. Methods Microbiol. **30:**227–237.
21. **Kirchman, D., E. K'nees, and R. Hodson.** 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. **49:**599–607.
22. **Kirchman, D. L.** 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments. FEMS Microbiol. Ecol. **39:**91–100.
23. **Kirchman, D. L., L. Y. Yu, and M. T. Cottrell.** 2003. Diversity and abundance of uncultured *Cytophaga*-like bacteria in the Delaware Estuary. Appl. Environ. Microbiol. **69:**6587–6596.
24. **Lampert, W.** 1978. Release of dissolved organic-carbon by grazing zooplankton. Limnol. Oceanogr. **23:**831–834.
25. **Lancelot, C., and G. Billen.** 1984. Activity of heterotrophic bacteria and its coupling to primary production during the spring phytoplankton bloom in the southern bight of the North Sea. Limnol. Oceanogr. **29:**721–730.
26. **Malmstrom, R. R., M. T. Cottrell, H. Elifantz, and D. L. Kirchman.** 2005. Biomass production and assimilation of dissolved organic matter by SAR11 bacteria in the northwest Atlantic Ocean. Appl. Environ. Microbiol. **71:** 2979–2986.
27. **Mullins, T. D., T. B. Britschgi, R. L. Krest, and S. J. Giovannoni.** 1995. Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnol. Oceanogr. **40:**148–158.
28. **Murray, A. E., A. Blakis, R. Massana, S. Strawzewski, U. Passow, A. Alldredge, and E. F. DeLong.** 1999. A time series assessment of planktonic archaeal variability in the Santa Barbara Channel. Aquat. Microb. Ecol. **20:**129–145.
29. **Ouverney, C. C., and J. A. Fuhrman.** 1999. Combined microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types in situ. Appl. Environ. Microbiol. **65:**1746–1752.
30. **Pernthaler, A., and J. Pernthaler.** 2005. Diurnal variation of cell proliferation in three bacterial taxa from coastal North Sea waters. Appl. Environ. Microbiol. **71:**4638–4644.
31. **Pernthaler, A., J. Pernthaler, and R. Amann.** 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. **68:**3094–3101.
32. **Pernthaler, A., C. M. Preston, J. Pernthaler, E. F. DeLong, and R. Amann.** 2002. A comparison of fluorescently labeled oligonucleotide and polynucleotide probes for the detection of pelagic marine bacteria and archaea. Appl. Environ. Microbiol. **68:**661–668.
33. **Pernthaler, J., F. O. Glöckner, W. Schönhuber, and R. Amann.** 2001. Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. Methods Microbiol. **30:**207–226.
34. **Pinhassi, J., and T. Berman.** 2003. Differential growth response of colony-forming alpha- and gammaproteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. Appl. Environ. Microbiol. **69:**199–211.
35. **Pinhassi, J., M. M. Sala, H. Havskum, F. Peters, O. Guadayol, A. Malits, and C. L. Marrase.** 2004. Changes in bacterioplankton composition under different phytoplankton regimens. Appl. Environ. Microbiol. **70:**6753–6766.
36. **Prokic, I., F. Brummer, T. Brigge, H. D. Gortz, G. Gerdts, C. Schutt, M. Elbrachter, and W. E. G. Muller.** 1998. Bacteria of the genus Roseobacter associated with the toxic dinoflagellate Prorocentrum lima. Protist **149:**347–357.
37. **Radach, G.** 1992. Ecosystem functioning in the German Bight under continental nutrient inputs by rivers. Estuaries **15:**477–496.
38. **Riemann, L., G. F. Steward, and F. Azam.** 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl. Environ. Microbiol. **66:**578–587.
39. **Schäfer, H., B. Abbas, H. Witte, and G. Muyzer.** 2002. Genetic diversity of 'satellite' bacteria present in cultures of marine diatoms. FEMS Microbiol. Ecol. **42:**25–35.
40. **Scheurle, C., D. Hebbeln, and P. Jones.** 2005. An 800-year reconstruction of Elbe River discharge and German Bight sea-surface salinity. Holocene **15:** 429–434.
41. **Schut, F., E. J. De Vries, J. C. Gottschal, B. R. Robertson, W. Harder, R. A. Prins, and D. K. Button.** 1993. Isolation of typical marine bacteria by dilution culture growth maintenance and characteristics of isolates under laboratory conditions. Appl. Environ. Microbiol. **59:**2150–2160.
42. **Simon, M., F. O. Glöckner, and R. Amann.** 1999. Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. Aquat. Microb. Ecol. **18:**275–284.
43. **Smith, E. M., and P. A. del Giorgio.** 2003. Low fractions of active bacteria in natural aquatic communities? Aquat. Microb. Ecol. **31:**203–208.
44. **Teira, E., T. Reinthaler, A. Pernthaler, J. Pernthaler, and G. J. Herndl.** 2004. Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by bacteria and archaea in the deep ocean. Appl. Environ. Microbiol. **70:** 4411–4414.

45. **Unanue, M., B. Ayo, M. Agis, D. Slezak, G. J. Herndl, and J. Iriberri.** 1999. Ectoenzymatic activity and uptake of monomers in marine bacterioplankton described by a biphasic kinetic model. Microb. Ecol. **37:**36–48.
46. **Wild-Allen, K., A. Lane, and P. Tett.** 2002. Phytoplankton, sediment and optical observations in Netherlands coastal water in spring. J. Sea Res. **47:**303–315.
47. **Yager, P. L., T. L. Connelly, B. Mortazavi, K. E. Wommack, N. Bano, J. E. Bauer, S. Opsahl, and J. T. Hollibaugh.** 2001. Dynamic bacterial and viral response to an algal bloom at subzero temperatures. Limnol. Oceanogr. **46:**790–801.
48. **Zubkov, M. V., B. M. Fuchs, S. D. Archer, R. P. Kiene, R. Amann, and P. A. Burkill.** 2001. Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulfoniopropionate in an algal bloom in the North Sea. Environ. Microbiol. **3:**304–311.
49. **Zubkov, M. V., B. M. Fuchs, G. A. Tarran, P. H. Burkill, and R. Amann.** 2003. High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. Appl. Environ. Microbiol. **69:**1299–1304.