

***Roseobacter* and SAR11 dominate microbial glucose uptake in coastal North Sea waters**

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Summary

Bacterial assemblages in coastal pelagic environments are exposed to pronounced temporal and spatial fluctuations in the availability of monomeric substrates. Little is known about the response of particular bacterial groups to such variability. We studied glucose incorporation at various concentrations (0.1–100 nM) by bacteria related to *Roseobacter*, SAR11, *Gammaproteobacteria* and *Cytophaga-Flavobacteria* in coastal North Sea waters in late winter and during a spring phytoplankton bloom dominated by *Phaeocystis* sp. (March and May 2004 respectively). Both the fraction of glucose-assimilating bacterial cells and the rate of substrate incorporation per active cell were higher in May. The respective contributions of the studied groups to all glucose-assimilating *Bacteria* were related to substrate concentration. The majority of glucose-incorporating bacterial cells at the lower concentrations were members of the *Roseobacter* and SAR11 clades. At both time-points the two groups formed approximately equal fractions of all glucose-incorporating bacteria. This was due to a small population of highly active *Roseobacter* cells and high abundances of SAR11 bacteria with low proportions of glucose-incorporating cells. By contrast, the proportion of active cells from the *Cytophaga-Flavobacteria* lineage substantially increased at higher levels of available substrate. The determination of concentration-dependent substrate incorporation patterns may help to better understand the different ecophysiological niches of bacterioplankton populations.

Introduction

Glucose is typically the most abundant free neutral aldose in seawater (Rich *et al.*, 1996; Skoog *et al.*, 1999). Current estimates of glucose concentrations in the seawater range

from undetectably low (< 1 nM) to approximately 100 nM (Rich *et al.*, 1996; Skoog *et al.*, 1999; Kirchman *et al.*, 2001). The direct determination of glucose concentrations usually matches calculations based on the uptake kinetics of heterotrophic bacteria (Skoog *et al.*, 1999), indicating a close coupling between release and uptake. The turnover of the (monomeric and polymeric) glucose pool in different oceanic regions ranges between days to months, and glucose assimilation in marine surface waters may represent from <1% to 40% of bacterial carbon production (Rich *et al.*, 1996; Skoog *et al.*, 1999; Kirchman *et al.*, 2001).

In marine surface waters glucose mainly originates from autotrophic sources, i.e. phytoplankton photosynthesis (Ittekkot *et al.*, 1981; Mopper *et al.*, 1995; Biddanda and Benner, 1997). It forms a prominent fraction of the storage polymers of some algal groups e.g. *Phaeocystis* sp. (Janse *et al.*, 1996a; van Rijssel *et al.*, 2000). A widespread release of glucose from polysaccharides by the activities of free-living and particle-bound microbes (Janse *et al.*, 1996b; Agis *et al.*, 1998; Becquevort *et al.*, 1998) is suggested by the ubiquity of a range of dissolved beta-glucosidases with contrasting kinetic properties (Christian and Karl, 1995; Arrieta and Herndl, 2002).

The availability of glucose (and other dissolved free carbohydrates) to aquatic microbial assemblages is assumed to be related to changes of primary production, e.g. during spring blooms of *Phaeocystis* in the North Sea (Ittekkot *et al.*, 1981). In addition, there may be considerable short-term temporal fluctuations due to diurnal patterns of photosynthesis (Burney *et al.*, 1982; Skoog *et al.*, 1999) or microscale patchiness, e.g. in the immediate vicinity of senescent or lysed algal cells (Blackburn *et al.*, 1997). Pelagic bacteria in coastal habitats are thus probably exposed to glucose concentrations that range over several orders of magnitude. This is reflected by the presence of multiphasic uptake systems for this substrate both in single species of aquatic microbes (Nissen *et al.*, 1984) and in whole bacterioplankton assemblages (Vaccaro and Jannasch, 1967; Azam and Hodson, 1981; Unanue *et al.*, 1999).

Evidence is accumulating that the different phylogenetic groups of bacteria and archaea in marine waters also differ in their respective contributions to the flux of individual DOC components (Cottrell and Kirchman, 2000; Herndl *et al.*, 2005). For example, Malmstrom and

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coworkers recently demonstrated that bacteria affiliated to one widespread lineage of *Alphaproteobacteria*, SAR11, were responsible for a disproportionately high fraction of glucose assimilation in surface waters of the North-west Atlantic Ocean (Malmstrom *et al.*, 2005). In these experiments, glucose was offered at a concentration of 0.5 nM. This matches the ambient bulk concentrations in the open ocean, but does not reflect the potentially wide range of glucose available in coastal habitats, e.g. before and during bloom situations (Ittekkot *et al.*, 1981; Kirchman *et al.*, 2001). Thus, other groups of bacteria may be more competitive than SAR 11 at different glucose concentrations.

The assimilation of glucose by different phylogenetic lineages of coastal North Sea bacterioplankton was studied at different concentrations of radiolabelled substrate (0.1–100 nM). We compared the incorporation patterns of two groups of *Alphaproteobacteria* (SAR11 and *Roseobacter*), of *Gammaproteobacteria* and of *Cytophaga-Flavobacteria* during a late winter pre-bloom situation and in a phytoplankton spring bloom dominated by *Phaeocystis* sp. Microbial substrate uptake was investigated both by bulk incubations and by microautoradiography combined with fluorescence *in situ* hybridization (MARFISH).

Results

Community composition

The selected probes accounted for 65% and 92% of bacterial cells hybridized with the EUB I–III probe in March and May respectively (Fig. 1). In both months SAR11 cells constituted a high fraction of all *Bacteria*, and *Gammaproteobacteria* and *Cytophaga-Flavobacteria* formed approximately equal fractions of the bacterial assemblage (Fig. 1). In May these three groups represented a higher proportion of all *Bacteria*. By contrast, the proportion of *Roseobacter* cells was similar in both months (Fig. 1). Total cell numbers did not change significantly between both months: $0.8 (\pm 0.2) \times 10^6$ cells ml^{-1} in March and $1.2 (\pm 0.4) \times 10^6$ cells ml^{-1} in May (Alonso and Perntaler, 2006).

Glucose incorporation

More cells were able to incorporate glucose, and higher amounts of substrate were incorporated in May than in March at a given tracer concentration (Fig. 2A, Table 1). The percentage of glucose-incorporating cells, as well as the amount of incorporated tracer, increased with increasing glucose concentration in both months up to a concentration of 10 nM (Fig. 2A, Table 1). The number of glucose-assimilating bacteria in May was approximately double at all concentrations. By contrast, the incorporation rate per active cell steeply increased with the concentration in May (Fig. 2B).

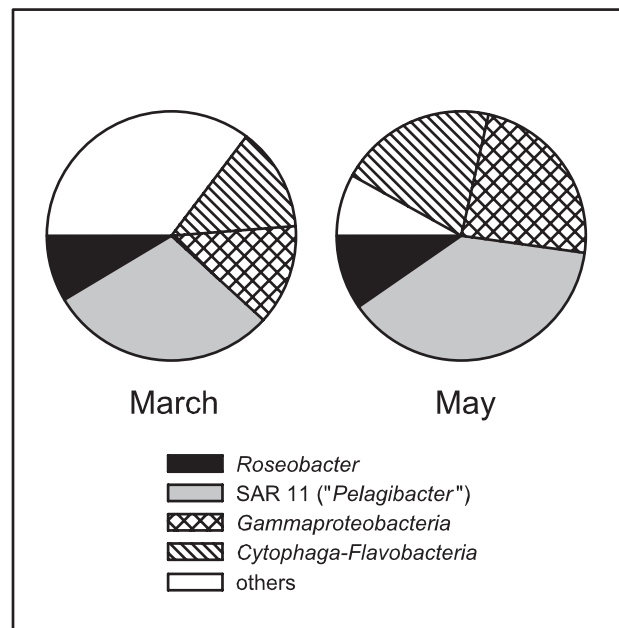


Fig. 1. Contributions of bacteria affiliated to the *Roseobacter* and SAR11 clades, to *Gammaproteobacteria*, and to *Cytophaga-Flavobacteria* to all hybridized bacterial cells in coastal North Sea surface waters during a late winter situation (March 2004) and during a phytoplankton bloom dominated by *Phaeocystis* sp. (May 2004).

A high fraction of cells from the *Roseobacter* clade (60–90%) showed glucose uptake (Fig. 3). A substantially higher proportion of this population incorporated the monomer in May (90%) than in March (71%). The fraction of glucose-incorporating *Roseobacter* cells was similar at all tracer concentrations.

Between 10% and 35% of the bacteria related to SAR11 assimilated glucose. In both months the proportion of SAR11 cells that showed glucose incorporation was maximal at concentrations of 1 nM (Fig. 3). In May, the proportion of glucose-incorporating SAR11 cells was higher at substrate concentrations ≥ 1 nM (Fig. 3).

An increasing number of glucose-incorporating *Gammaproteobacteria* and *Cytophaga-Flavobacteria* were found with increasing glucose concentrations (Fig. 4). The difference between the percentage of glucose-incorporating cells at the highest and lowest concentrations was

Table 1. Incorporation rates of glucose by the whole microbial community at different glucose concentrations in March and May 2004.

Glucose concentration (nM)	Incorporated substrate (pmoles $\text{l}^{-1} \text{h}^{-1}$)	
	March	May
0.1	1.4 ± 0.2	6.7 ± 0.7
1	11.7 ± 7.3	75.1 ± 13.4
10	34.0 ± 13.0	390.3 ± 60.6
100	22.4 ± 8.6	433.0 ± 16.5

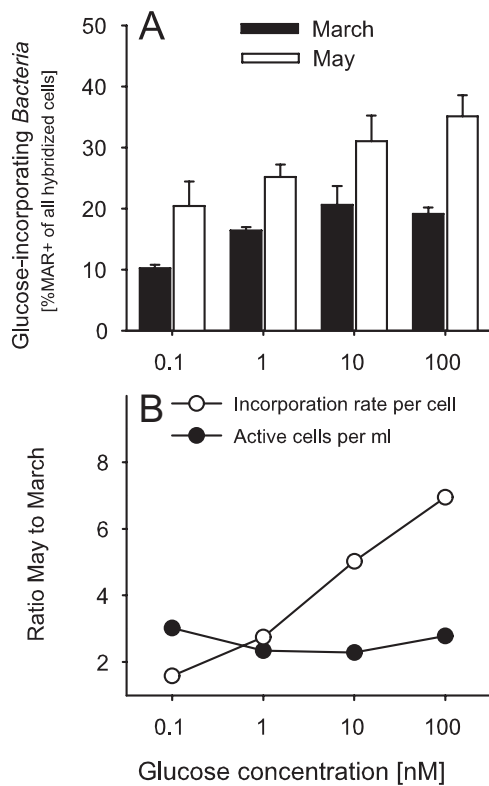


Fig. 2. A. Fractions of bacterial cells with visible uptake of glucose (MAR+ cells) at different concentrations of offered substrate (mean \pm 1 standard deviation). B. Ratios of glucose-incorporating cells (closed symbols) and of incorporation rates per active cell (open symbols) between the pre-bloom (March) and the phytoplankton bloom situation (May).

more pronounced for *Cytophaga-Flavobacteria* (Fig. 4). Similar proportions of glucose-incorporating cells from these two groups were observed at both sampling time-points (10% to 20%) (Fig. 4).

Negative controls yielded similar percentages of false-positive glucose-incorporating cells irrespective of sampling time or incubation conditions, on average less than 1% of cells hybridized by EUB I–III (data not shown).

Contribution of phylogenetic groups to all glucose assimilating bacteria

Considering the combined errors of the FISH and MARFISH methods, virtually all glucose-incorporating bacterial cells could be assigned to the four studied groups (74% to 119% of glucose-incorporating cells hybridized with probe EUB I–III). *Roseobacter* and SAR11 were the main glucose consumers, particularly at the lower concentrations (Fig. 5). *Gamma*proteobacteria and *Cytophaga-Flavobacteria* contributed similarly to glucose-incorporating bacteria along the concentration series (Fig. 5).

The relative contribution of the studied groups to glucose-incorporating bacterial cells was compared with their relative contribution to all bacterial cells. The contribution of *Roseobacter* to total active cells was at least threefold higher than expected from their abundance, particularly in March and at lower substrate concentrations (Table 2). The relative contribution of SAR 11 cells to bacterial glucose uptake was higher than expected from their abundance only at 1 nM concentration (Table 2). The contributions of *Gamma*proteobacteria and *Cytophaga-Flavobacteria* to all glucose consuming cells generally matched or were below their respective relative abundances (Table 2).

Discussion

Uptake of glucose by the whole microbial community

During both months, the number of glucose-assimilating ('active') cells and the rate of glucose incorporation per active cell increased with increasing glucose concentrations (Table 1, Fig. 2A and B). A comparison of the two time-points moreover revealed clear differences between these parameters of microbial activity: while the total numbers of 'active' cells approximately doubled between the two samplings irrespective of substrate concentration (Fig. 2B), the substrate incorporation rate per active cell

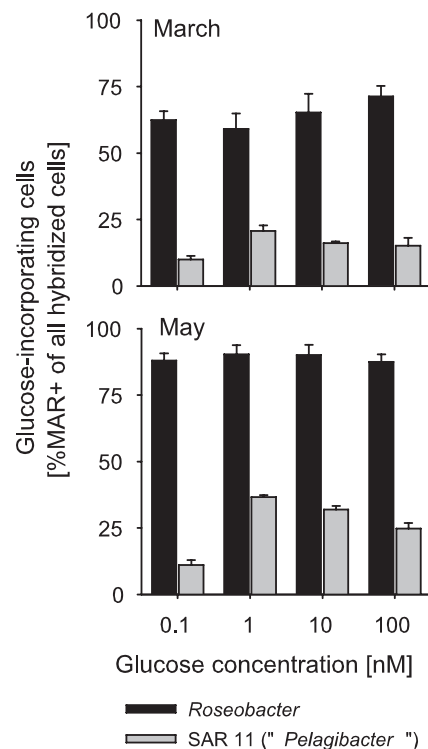


Fig. 3. Fractions of cells from the *Roseobacter* and SAR11 clades with visible incorporation of glucose at different concentrations of offered substrate in March (upper panel) and May (lower panel).

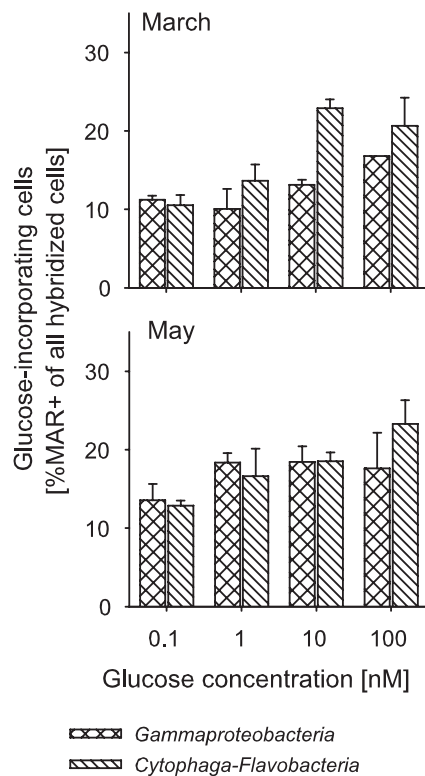


Fig. 4. Fractions of cells affiliated to *Gammaproteobacteria* and *Cytophaga-Flavobacteria* with visible incorporation of glucose at different concentrations of offered substrate in March (upper panel) and May (lower panel).

in May was clearly related to tracer concentration: it was less than twice the March value at the lowest tracer concentration but approximately seven times as high at 100 nM (Fig. 2B).

Thus, the active bacterial cells could readily increase their incorporation rates at higher concentrations of available substrate only during the bloom situation, but not before. This suggests a transition of the bacterial assemblage from an oligotrophic (March) to a copiotrophic (May) type of substrate uptake. Similar observations have been reported for glucose and leucine uptake during spring phytoplankton blooms in the coastal Alaskan Arctic Sea and the North Sea respectively (Yager *et al.*, 2001; Alonso and Pernthaler, 2006).

Table 2. Relative contributions of the studied groups to glucose-incorporating bacterial cells (detected by probe EUB I–III) as compared with their relative contributions to all bacterial cells.

Glucose concentration (nM)	<i>Roseobacter</i>		SAR11		<i>Gammaproteobacteria</i>		<i>Cytophaga-Flavobacteria</i>	
	March	May	March	May	March	May	March	May
0.1	6.1	4.3	1.0	0.5	1.1	0.7	1.0	0.6
1	3.6	3.6	1.3	1.5	0.6	0.7	0.8	0.6
10	3.2	2.9	0.8	1.0	0.6	0.6	1.1	0.6
100	3.7	2.5	0.8	0.7	0.9	0.5	1.0	0.7

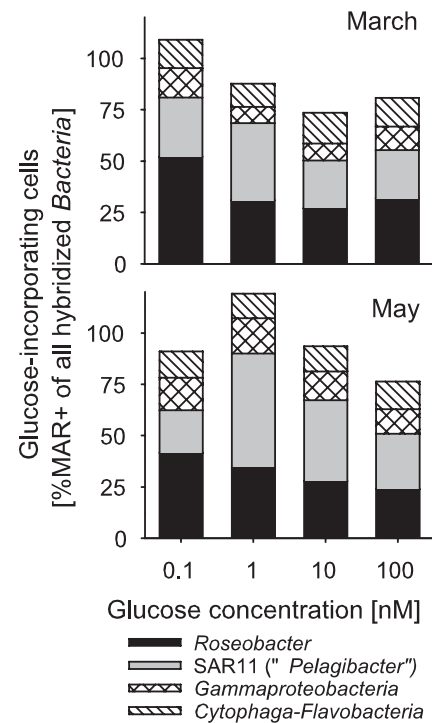


Fig. 5. Relative contributions of glucose-incorporating cells from the studied phylogenetic groups to all glucose-incorporating bacteria.

Phytoplankton bloom events are typically accompanied by a rise in the fraction of particulate organic matter (Riemann *et al.*, 2000). Laboratory studies show that there is a dynamic balance between the colonization and detachment of bacteria from such aggregates (Kiorboe *et al.*, 2003). Thus, some apparently free-living bacteria might be particle-attached during other phases of their life cycle. Unanue and colleagues (1999) found a significantly higher V_{max} for glucose uptake in bacteria associated with particles than in free-living bacteria. Microbes that thrive in pelagic habitats at times when point sources of dissolved organic carbon are common (i.e. during the *Phaeocystis* bloom) would therefore profit from the ability to consume more rapidly substrates that are available at higher concentrations. By contrast, such a feature would be of little use if substrates are evenly distributed, as it is likely during a late winter phytoplankton pre-bloom situation.

Contribution of specific groups to total uptake

Most glucose consuming bacterial cells were members of the *Roseobacter* and SAR11 lineages, particularly at the lower substrate concentrations (Fig. 5, Table 2). Their relative importance diminished at increasing concentration, partly due to a decrease in the proportion of glucose-incorporating cells within the SAR11 clade (Fig. 3), but also due to the rising contribution of the other bacterial groups (Fig. 4). These findings suggest that *Alphaproteobacteria* are the main lineage responsible for glucose uptake at low ambient concentrations in coastal North Sea waters. Our results confirm the proposed role of *Alphaproteobacteria* as specialists for monomers (Cottrell and Kirchman, 2000; Elifantz *et al.*, 2005). In agreement with recent work by Elifantz and colleagues (2005), we observed that *Gammaproteobacteria* and *Cytophaga-Flavobacteria* generally contributed to glucose consuming cells as would be expected from their abundances or even less. This indicates that glucose is not the preferred substrate for these bacteria if present at low concentrations. In particular, the *Cytophaga-Flavobacteria* participated more substantially in glucose turnover at increasing substrate availability: Approximately twice as many bacteria from this lineage exhibited glucose assimilation at the highest substrate concentrations (Fig. 4). Representatives of the highly diverse *Cytophaga-Flavobacteria* group have been previously found to appear during *Phaeocystis*-dominated phytoplankton blooms (Simon *et al.*, 1999) and bacteria from this lineage are frequently encountered in nutrient-rich microenvironments and on organic aggregates (Rath *et al.*, 1998).

At the level of larger subgroups (i.e. summing up *Roseobacter* and SAR11) the relative contributions of *Alphaproteobacteria*, *Cytophaga-Flavobacteria* and *Gammaproteobacteria* to the total numbers of glucose-assimilating cells corresponded well to what would be expected from their cell numbers. This has been interpreted as indication that the same (i.e. 'bottom-up') factors control the abundances and activity of these groups (Cottrell and Kirchman, 2003). However, our results call for a more cautious appreciation of ecologically different microbial populations that may be summed up by such large categories. Specifically, the two studied populations of *Alphaproteobacteria* appeared to be strikingly different in terms of ecophysiology and the 'average' alphaproteobacterial cell did not seem to exist (Fig. 3). The same might hold true for the planktonic *Gammaproteobacteria* and for members of the *Cytophaga-Flavobacteria*.

Substrate uptake patterns of Roseobacter and SAR11

Considering the high contributions of *Roseobacter* and SAR11 to glucose-incorporating bacteria at both sam-

pling time-points (Fig. 5), it is likely that the concentration-dependent differences of community uptake rates in March and May (Table 1, Fig. 2B) reflect the physiological properties of cells from one or both of these lineages. The contrasting uptake behaviour of bacteria at the two sampling time-points might indicate phenotypic plasticity, i.e. that identical genotypes were present at both sampling time-points that differed in their substrate uptake patterns due to the increase in temperature and substrate availability. Alternatively, a change in the dominant genotypes might have been masked by the use of FISH probes for rather large phylogenetic groups. There is evidence that bacteria from the *Roseobacter* and SAR11 lineages might occupy comparable niches in different marine environments. For example, *Roseobacter* can be the main mediator of the flux of dimethyl sulfonopropionate (DMSP) (Zubkov *et al.*, 2002; Malmstrom *et al.*, 2004a; Vila *et al.*, 2004) in coastal waters or during blooms whereas in open ocean samples DMSP was mainly consumed by SAR11 (Malmstrom *et al.*, 2004b). Our study for the first time compared substrate uptake patterns of both populations co-occurring in the same habitat. *Roseobacter* and SAR11 contributed approximately equally to the fraction of cells with visible glucose uptake (Fig. 5). However, a high proportion of active cells was present in the comparatively small *Roseobacter* population (Fig. 1) at all concentrations (Fig. 3). By contrast, the substantial contribution of the SAR11 bacteria to glucose uptake was due to their high abundances (Fig. 1) that compensated for the relatively low fractions of active SAR11 cells (Fig. 3).

These different patterns of glucose incorporation appear to agree with recent findings from the genome analysis of isolates from the two clades, *Silicibacter pomeroyi* (*Roseobacter*) and *Pelagibacter ubique* (SAR11). A similar percentage of the genome codes for transporters and binding proteins in both strains (around 12%) (Moran *et al.*, 2004; Giovannoni *et al.*, 2005). However, the proportion of ABC transporters -known for high substrate affinity- is distinctly higher in *P. ubique* (50% vs. 36%). *Silicibacter pomeroyi* features three rRNA operons, whereas SAR11 possesses only one. The number of rRNA operons has been related to the potential to respond rapidly to changes in resource availability (Klappenbach *et al.*, 2000). This hints at a higher potential of *S. pomeroyi* to adjust to changes in growth conditions and to exploit pulses of substrate concentrations, whereas SAR11 might be better suited for slow but efficient growth in the ambient DOC field (Giovannoni *et al.*, 2005). Moreover, *S. pomeroyi* and other *Roseobacter*-related isolates possess quorum-sensing systems (Gram *et al.*, 2002; Moran *et al.*, 2004), whereas *P. ubique* does not (Giovannoni *et al.*, 2005). Quorum sensing in *S. pomeroyi* has been interpreted to provide regulatory flexibility associ-

ated with the potential alternations between a particle-associated and planktonic life style (Moran *et al.*, 2004). *Roseobacter* cells have been found both free-living and attached, e.g. during phytoplankton blooms (Riemann *et al.*, 2000), or on marine snow (Rath *et al.*, 1998) and have been isolated from rich and poor nutrient conditions (Pinhassi and Berman, 2003). By contrast, SAR11 has only been observed in the free-living fraction of pelagic bacteria (Morris *et al.*, 2002), and bacteria from this lineage do not grow at elevated substrate concentrations or on solid media (Rappe *et al.*, 2002; Simu and Hagström, 2004).

In a previous study in the North-west Atlantic Ocean the fraction of SAR11 cells that incorporated glucose (offered at a concentration of 0.5 nM) clearly exceeded the community average (Malmstrom *et al.*, 2005). Although this was not generally the case in our samples (Table 2), we found the highest numbers of active cells from this clade at glucose concentrations of 1 nM (Fig. 3). Thus, the results of Malmstrom *et al.* fully agree with our observation that low ambient levels of glucose represent a competitive advantage for SAR11 bacteria. On the other hand, SAR11 (as targeted by the FISH probe here applied) represents a phylogenetically highly diversified lineage that should appropriately be regarded as a genus (Acinas *et al.*, 2004). It is therefore also conceivable that different ecotypes with different affinities for glucose might occur in coastal and open ocean habitats.

It should be noted that the distinctly higher abundance of bacteria from the SAR11 clade than of the *Roseobacter* lineage in coastal North Sea waters during spring cannot be explained by their respective affinities to glucose. Other factors, such as top-down control by protistan grazers (Pernthaler, 2005) or viral lysis (Weinbauer and Rassoulzadegan, 2004), need to be taken into account. In our samples *Roseobacter* cells were substantially bigger and more active than cells from the SAR11 clade (Fig. 6), inviting speculations about food-web related population control of *Roseobacter*, e.g. by size-selective grazing on

larger, more active bacteria (Andersson *et al.*, 1986; Sherr *et al.*, 1992).

Experimental procedures

Sampling site

Surface water samples (1-m depth) were collected at the sampling station Helgoland Roads (54°11'N, 7°54'E), in the German Bay of the North Sea. The sampling dates were 11th March 2004 (late winter) and 6th May 2004 (mid-spring). The water temperature in March was 4.2°C and the algae cells were very scarce. In May the water temperature was 8.3°C, and there was a spring bloom of *Phaeocystis* sp. -a typical phenomenon in coastal North Sea waters (Cadee and Hegeman, 2002). It reached its maximal biomass (157 µg C dm⁻³) 5 days after the sampling date. More details about the sampling site and conditions are given in Alonso and Pernthaler (2006).

Glucose incorporation

Triplicate water samples (10 ml) plus one control sample (sea water prefixed with paraformaldehyde) were amended with tritiated glucose (specific activity: 1.29 TBq mmol⁻¹, Amersham, Freiburg, Germany) at different concentrations (0.1 nM, 1 nM, 10 nM and 100 nM) within 1 h after sample collection. Samples were incubated for 4 h in the dark, at 4°C (March) and 10°C (May). Subsequently, freshly prepared buffered paraformaldehyde fixative was added to the samples to a final concentration of 1%. After fixation, the samples were frozen for transport, and later filtered onto polycarbonate filters (type GTTP, pore size, 0.2 µm, diameter, 25 mm, Millipore, Eschborn, Germany). The filters were rinsed twice with sterile phosphate-buffered saline (PBS) and stored at -20°C until further MARFISH analysis. Identical incubations were performed to measure the amount of substrate incorporated by the cells at the different concentrations. These samples were fixed as described above, filtered onto cellulose mixed esters filters (type GSWP, pore size, 0.2 µm, diameter 25 mm, Millipore, Eschborn, Germany), and rinsed twice with ice cold trichloroacetic acid and ethanol (Kirchman, 2001). Filters were stored at -20°C until further processing.

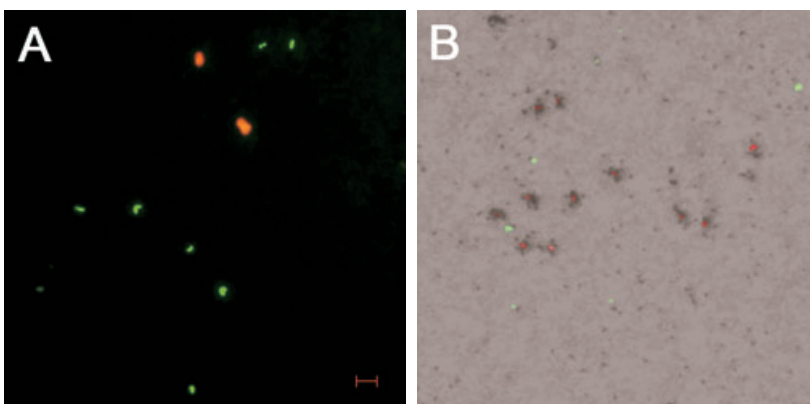


Fig. 6. A. Size comparison of bacteria from the *Roseobacter* (red cells) and SAR11 (green cells) clades after double hybridization with specific probes. Bar, 2 µm.

B. Relative size of MAR grain areas associated with glucose-incorporating *Roseobacter* (red cells) and SAR11 (green cells) bacteria. Note that *Roseobacter* cells appear small in B due to the overlaying MAR grains.

- Cadee, G.C., and Hegeman, J. (2002) Phytoplankton in the Marsdiep at the end of the 20th century; 30 years monitoring biomass, primary production, and phaeocystis blooms. *J Sea Res* **48**: 97–110.
- Christian, J.R., and Karl, D.M. (1995) Bacterial ectoenzymes in marine waters – activity ratios and temperature responses in 3 oceanographic provinces. *Limnol Oceanogr* **40**: 1042–1049.
- Cottrell, M.T., and Kirchman, D.L. (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.
- Cottrell, M.T., and Kirchman, D.L. (2003) Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnol Oceanogr* **48**: 168–178.
- Daims, H., Bruhl, A., Amann, R., Schleifer, K.H., and Wagner, M. (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.
- Eilers, H., Pernthaler, J., Peplies, J., Glöckner, F.O., Gerdts, G., and Amann, R. (2001) Isolation of novel pelagic bacteria from the German Bight and their seasonal contribution to surface picoplankton. *Appl Environ Microbiol* **67**: 5134–5142.
- Elifantz, H., Malmstrom, R.R., Cottrell, M.T., and Kirchman, D.L. (2005) Assimilation of polysaccharides and glucose by major bacterial groups in the Delaware Estuary. *Appl Environ Microbiol* **71**: 7799–7805.
- Giovannoni, S.J., Tripp, H.J., Givan, S., Podar, M., Vergin, K.L., Baptista, D., et al. (2005) Genome streamlining in a cosmopolitan oceanic bacterium. *Science* **309**: 1242–1245.
- Gram, L., Grossart, H.P., Schlingloff, A., and Kiorboe, T. (2002) Possible quorum sensing in marine snow bacteria: production of acylated homoserine lactones by *Roseobacter* strains isolated from marine snow. *Appl Environ Microbiol* **68**: 4111–4116.
- Herndl, G.J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., and Pernthaler, J. (2005) Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean. *Appl Environ Microbiol* **71**: 2303–2309.
- Ittekkot, V., Brockmann, U., Michaelis, W., and Degens, E.T. (1981) Dissolved free and combined carbohydrates during a Phytoplankton bloom in the Northern North-Sea. *Mar Ecol Prog Ser* **4**: 299–305.
- Janse, I., vanRijssel, M., vanHall, P.J., Gerwig, G.J., Gottschal, J.C., and Prins, R.A. (1996a) The storage glucan of *Phaeocystis globosa* (Prymnesiophyceae) cells. *J Phycol* **32**: 382–387.
- Janse, I., vanRijssel, M., Gottschal, J.C., Lancelot, C., and Gieskes, W.W.C. (1996b) Carbohydrates in the North Sea during spring blooms of *Phaeocystis*: a specific fingerprint. *Aquat Microb Ecol* **10**: 97–103.
- Kiorboe, T., Tang, K., Grossart, H.P., and Ploug, H. (2003) Dynamics of microbial communities on marine snow aggregates: colonization, growth, detachment, and grazing mortality of attached bacteria. *Appl Environ Microbiol* **69**: 3036–3047.
- Kirchman, D. (2001) Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. In *Methods in Microbiology*. Paul, J.H. (ed.). London, UK: Academic Press, pp. 227–237.
- Kirchman, D.L., Meon, B., Ducklow, H.W., Carlson, C.A., Hansell, D.A., and Steward, G.F. (2001) Glucose fluxes and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. *Deep-Sea Res Pt II* **48**: 4179–4197.
- Klappenbach, J.A., Dunbar, J.M., and Schmidt, T.M. (2000) rRNA operon copy number reflects ecological strategies of bacteria. *Appl Environ Microbiol* **66**: 1328–1333.
- Malmstrom, R.R., Kiene, R.P., and Kirchman, D.L. (2004a) Identification and enumeration of bacteria assimilating dimethylsulfoniopropionate (DMSP) in the North Atlantic and Gulf of Mexico. *Limnol Oceanogr* **49**: 597–606.
- Malmstrom, R.R., Kiene, R.P., Cottrell, M.T., and Kirchman, D.L. (2004b) Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the North Atlantic ocean. *Appl Environ Microbiol* **70**: 4129–4135.
- Malmstrom, R.R., Cottrell, M.T., Elifantz, H., and Kirchman, D.L. (2005) Biomass production and assimilation of dissolved organic matter by SAR11 bacteria in the Northwest Atlantic Ocean. *Appl Environ Microbiol* **71**: 2979–2986.
- Manz, W., Amann, R., Ludwig, W., Wagner, M., and Schleifer, K.-H. (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of *Proteobacteria*: problems and solutions. *Syst Appl Microbiol* **15**: 593–600.
- Manz, W., Amann, R., Ludwig, W., Vancanneyt, M., and Schleifer, K.-H. (1996) Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum *Cytophaga-Flavobacter-Bacteroides* in the natural environment. *Microbiol* **142**: 1097–1106.
- Mopper, K., Zhou, J.A., Ramana, K.S., Passow, U., Dam, H.G., and Drapeau, D.T. (1995) The role of surface-active carbohydrates in the flocculation of a diatom bloom in a mesocosm. *Deep-Sea Res Pt II* **42**: 47–73.
- Moran, M.A., Buchan, A., Gonzalez, J.M., Heidelberg, J.F., Whitman, W.B., Kiene, R.P., et al. (2004) Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. *Nature* **432**: 910–913.
- Morris, R.M., Rappe, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., Carlson, C.A., and Giovannoni, S.J. (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420**: 806–810.
- Nissen, H., Nissen, P., and Azam, F. (1984) Multiphasic uptake of D-glucose by an oligotrophic marine bacterium. *Mar Ecol Prog Ser* **16**: 155–160.
- Pernthaler, J. (2005) Predation on prokaryotes in the water column and its ecological implications. *Nat Rev Microbiol* **3**: 537–546.
- Pernthaler, A., Pernthaler, J., and Amann, R. (2002) Fluorescence *in situ* hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Appl Environ Microbiol* **68**: 3094–3101.
- Pinhassi, J., and Berman, T. (2003) Differential growth response of colony-forming alpha- and gamma-proteobacteria 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.
- Rappe, M.S., Connon, S.A., Vergin, K.L., and Giovannoni, S.J. (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* **418**: 630–633.
- Rath, J., Wu, K.Y., Herndl, G.J., and DeLong, E.F. (1998) High phylogenetic diversity in a marine-snow associated bacterial assemblage. *Aquat Microb Ecol* **14**: 261–269.
- Rich, J.H., Ducklow, H.W., and Kirchman, D.L. (1996) Concentrations and uptake of neutral monosaccharides along 140 degrees W in the equatorial Pacific: contribution of glucose to heterotrophic bacterial activity and the DOM flux. *Limnol Oceanogr* **41**: 595–604.
- Riemann, L., Steward, G.F., and Azam, F. (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Appl Environ Microbiol* **66**: 578–587.
- van Rijssel, M., Janse, I., Noordkamp, D.J.B., and Gieskes, W.W.C. (2000) An inventory of factors that affect polysaccharide production by *Phaeocystis globosa*. *J Sea Res* **43**: 297–306.
- Sherr, B.F., Sherr, E.B., and McDaniel, J. (1992) Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. *Appl Environ Microbiol* **58**: 4371–4378.
- Simon, M., Glöckner, F.O., and Amann, R. (1999) Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. *Aquat Microb Ecol* **18**: 275–284.
- Simu, K., and Hagström, A. (2004) Oligotrophic bacterioplankton with a novel single-cell life strategy. *Appl Environ Microbiol* **70**: 2445–2451.
- Skoog, A., Biddanda, B., and Benner, R. (1999) Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. *Limnol Oceanogr* **44**: 1625–1633.
- Unanue, M., Ayo, B., Agis, M., Slezak, D., Herndl, G.J., and Iriberry, J. (1999) Ecto enzymatic activity and uptake of monomers in marine bacterioplankton described by a biphasic kinetic model. *Microb Ecol* **37**: 36–48.
- Vaccaro, R.F., and Jannasch, H.W. (1967) Variations in uptake kinetics for glucose by natural populations in seawater. *Limnol Oceanogr* **12**: 540–542.
- Vila, M., Simo, R., Kiene, R.P., Pinhassi, J., González, J.A., Moran, M.A., and Pedros-Alio, C. (2004) Use of microautoradiography combined with fluorescence *in situ* hybridization to determine dimethylsulfoniopropionate incorporation by marine bacterioplankton taxa. *Appl Environ Microbiol* **70**: 4648–4657.
- Weinbauer, M.G., and Rassoulzadegan, F. (2004) Are viruses driving microbial diversification and diversity? *Environ Microbiol* **6**: 1–11.
- Yager, P.L., Connelly, T.L., Mortazavi, B., Wommack, K.E., Bano, N., Bauer, J.E., *et al.* (2001) Dynamic bacterial and viral response to an algal bloom at subzero temperatures. *Limnol Oceanogr* **46**: 790–801.
- Zubkov, M.V., Fuchs, B.M., Archer, S.D., Kiene, R.P., Amann, R., and Burkill, P.H. (2002) Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. *Deep-Sea Res Part II – Top Stud Oceanogr* **49**: 3017–3038.