

Abundance and activity of *Chloroflexi*-type SAR202 bacterioplankton in the meso- and bathypelagic waters of the (sub)tropical Atlantic

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Summary

The contribution of *Chloroflexi*-type SAR202 cells to total picoplankton and bacterial abundance and uptake of D- and L-aspartic acids (Asp) was determined in the different meso- and bathypelagic water masses of the (sub)tropical Atlantic (from 35°N to 5°S). Fluorescence *in situ* hybridization (FISH) revealed that the overall abundance of SAR202 was $\leq 1 \times 10^3$ cells ml⁻¹ in subsurface waters (100 m layer), increasing in the mesopelagic zone to 3×10^3 cells ml⁻¹ and remaining fairly constant down to 4000 m depth. Overall, the percentage of total picoplankton identified as SAR202 increased from < 1% in subsurface waters to 10–20% in the bathypelagic waters. On average, members of the SAR202 cluster accounted for about 30% of the *Bacteria* in the bathypelagic waters, whereas in the mesopelagic and subsurface waters, SAR202 cells contributed < 5% to total bacterial abundance. The ratio of D-Asp : L-Asp uptake by the bulk picoplankton community increased from the subsurface layer (D-Asp : L-Asp uptake ratio ≈ 0.03) to the deeper layers reaching a ratio of ~ 1 at 4000 m depth. Combining FISH with microautoradiography to determine the proportion of SAR202 cells taking up D-Asp versus L-Asp, we found that $\approx 30\%$ of the SAR202 cells were taking up L-Asp throughout the water column while D-Asp was essentially not taken up by SAR202. This D-Asp : L-Asp uptake pattern of SAR202 cells is in contrast to that of the bulk bacterial and crenarchaeal community in the

bathypelagic ocean, both sustaining a higher fraction of D-Asp-positive cells than L-Asp-positive cells. Thus, although the *Chloroflexi*-type SAR202 constitutes a major bathypelagic bacterial cluster, it does not contribute to the large fraction of D-Asp utilizing prokaryotic community in the meso- and bathypelagic waters of the North Atlantic, but rather utilizes preferentially L-amino acids.

Introduction

Over the past decade, our knowledge on the phylogenetic composition of marine prokaryotic communities in the different depth strata of the oceanic water column and on the relative abundance of specific prokaryotic groups has increased considerably. Applying fluorescence *in situ* hybridization (FISH), it has been shown that bacterial abundance decreases with depth, while *Archaea*, and particularly *Crenarchaeota*, are relatively more abundant in deep than in surface waters (Karner *et al.*, 2001; Teira *et al.*, 2006a; Kirchman *et al.*, 2007; Varela *et al.*, 2008).

Interestingly, there are several bacterial groups with vertical distribution patterns similar to those of planktonic *Crenarchaeota* (DeLong *et al.*, 2006; Moreira *et al.*, 2006). One of these abundant groups is the environmental clade *Chloroflexi*-type SAR202. Since its initial identification at the Bermuda Atlantic Time Series (BATS) station at 250 m depth (Giovannoni *et al.*, 1996), environmental clones related to the SAR202 cluster have been found in several marine environments (Wright *et al.*, 1997; Giovannoni and Rappé, 2000; Bano and Hollibaugh, 2002). Recently, members of the SAR202 cluster were enumerated using a cluster-specific oligonucleotide probe and FISH at the BATS site in the Atlantic and the Hawaiian Ocean Time Series (HOTS) site in the Pacific (Morris *et al.*, 2004). Morris and colleagues (2004) report that the *Chloroflexi*-type SAR202 cluster accounts for about 10% of the bacterioplankton between 500 and 4000 m depth. The available data indicate that members of the thus far uncultured *Chloroflexi*-type SAR202 cluster are ubiquitously distributed in the meso- and bathypelagic waters, suggesting that the SAR202

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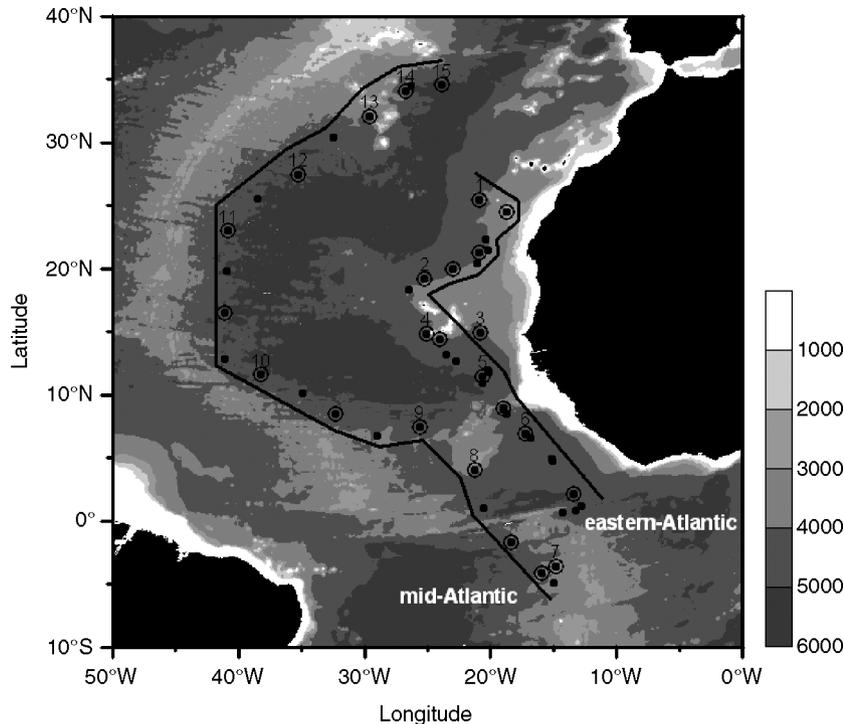


Fig. 1. Map of the study area in the (sub)tropical Atlantic with the mid- and eastern-Atlantic sections (solid lines) and the stations occupied during the ARCHIMEDES-1 and -2 cruises indicated by dots. Stations where FISH data on SAR202 were obtained are indicated by encircled dots. Station numbers indicate stations where D- and L-Asp uptake by bulk picoplankton and the *Chloroflexi*-type SAR202 cluster was measured. The numbers next to the grey scale indicate depth in m.

cluster might play an important role in the deep-ocean biogeochemistry. Its activity levels and substrate requirements, however, remain enigmatic.

While L-enantiomeric amino acids constitute the vast majority of the total amino acid pool in the ocean and are efficiently taken up by the heterotrophic prokaryotic community (Suttle *et al.*, 1991; Ouverney and Fuhrman, 2000; Fuhrman, 2002), D-amino acids are considered refractory. However, it was found that in the meso- and bathypelagic waters of the Atlantic, *Bacteria* and particularly, *Crenarchaeota*, preferentially utilize D-aspartic acid (Asp) over L-Asp (Pérez *et al.*, 2003; Teira *et al.*, 2006b; Varela *et al.*, 2008). Given the high abundance and the ubiquitous presence of *Chloroflexi*-type SAR202 in the deep waters of the Pacific and Atlantic (Morris *et al.*, 2004), we hypothesized that this cluster, together with the marine *Crenarchaeota* Group I, might be responsible for the preferential D-amino acid utilization in the deep waters of the Atlantic.

To test this hypothesis, the abundance of the *Chloroflexi*-type SAR202 cluster was determined in meso- and bathypelagic waters of the eastern (sub)tropical Atlantic (from 35°N to 5°S) as well as the uptake of D-Asp versus L-Asp by combining FISH with microautoradiography (MICRO-FISH, Lee *et al.*, 1999). We found that the *Chloroflexi*-type SAR202 cluster was as abundant as *Crenarchaeota* in the bathypelagic waters of the (sub)tropical Atlantic. Contrary to our original hypothesis, however,

SAR202 did not contribute to the uptake of D-Asp in these waters, but rather utilized exclusively L-Asp.

Results

Physical and chemical characteristics of the meso- and bathypelagic water masses

The water masses along the mid- and eastern-Atlantic sections (Fig. 1) were identified based on their temperature and salinity characteristics (van Aken, 2000a,b). Table 1 summarizes the basic physico-chemical characteristics of the main water masses encountered during the two cruises.

At 4000 m depth, Lower Deep Water (LDW), characterized by low salinity (34.9–35.5) and temperature (2.2–2.6°C), consisted mainly of Northeast Atlantic Deep Water (NEADW) with some Antarctic Bottom Water coming from the south. The NEADW, mainly formed by Iceland Scotland Overflow Water and Labrador Sea Water, is characterized by a temperature between 2.7°C and 4.1°C (Table 1). The core of NEADW was identifiable throughout both the mid- and eastern-Atlantic sections at around 2500 m depth. Two types of intermediate waters were found between 850 and 1100 m depth, the saline Mediterranean Sea Outflow Water (MSOW) and the Antarctic Intermediate Water (AAIW), the latter characterized by lower salinity and temperature than MSOW (Table 1). A local oxygen minimum (< 100 $\mu\text{mol kg}^{-1}$) was found

Table 1. Physical and chemical characteristics of the main water masses sampled during the cruises ARCHIMEDES-1 and -2 based on CTD profiles at the individual stations.

Water mass	Depth (m)	Eastern-Atlantic section			Mid-Atlantic section		
		Temperature (°C)	Salinity	Oxygen (μM)	Temperature (°C)	Salinity	Oxygen (μM)
Subsurface	100	14.2–20.6	35.4–37.1	56.7–221.5	14.3–24.1	35.5–37.3	67.6–211.6
O ₂ -min	380	6.9–15.9	34.6–36.3	44.0–183.9	6.8–17.8	34.6–36.5	62.0–207.2
MSOW	900	6.7–8.3	34.9–35.2	117.1–128.1	7.6–9.6	35.2–35.6	144.8–197.7
AAIW	900	4.5–6.8	34.6–34.9	102.4–151.5	4.4–6.1	35.5–34.8	105.0–152.3
NEADW	2500	2.7–4.1	34.9–35.0	212.3–243.6	2.8–3.9	34.9–35.0	227.9–250.8
LDW	4000	2.2–2.6	34.9	223.7–243.5	2.3–2.6	34.9–35.5	235.1–256.5

Ranges are given for each water mass where samples were collected. O₂-min, oxygen minimum layer.

between 200 and 600 m depth close to the equatorial part of the sections ($\sim 10^\circ\text{N}$).

Contribution of Bacteria to total picoplankton abundance

The contribution of *Bacteria* as determined by Catalyzed Reporter Deposition-FISH (CARD-FISH) using the probe-mix Eub338, Eub338 II and Eub338 III to total picoplankton abundance varied between 40% and about 65% at both the mid- and eastern-Atlantic sections (Fig. 2). Generally, the contribution of *Bacteria* to total picoplankton

increased from north to south while the total picoplankton abundance, determined by 4,6-diamidino-2-phenylindole (DAPI) staining, followed the commonly reported vertical gradient with high abundance in the near-surface waters and declining with depth (Fig. 2).

Chloroflexi-type SAR202 abundance

The abundance of members of the SAR202 cluster was determined by FISH at 13 and 12 stations of the mid- and eastern-Atlantic sections, respectively (see Fig. 1). The

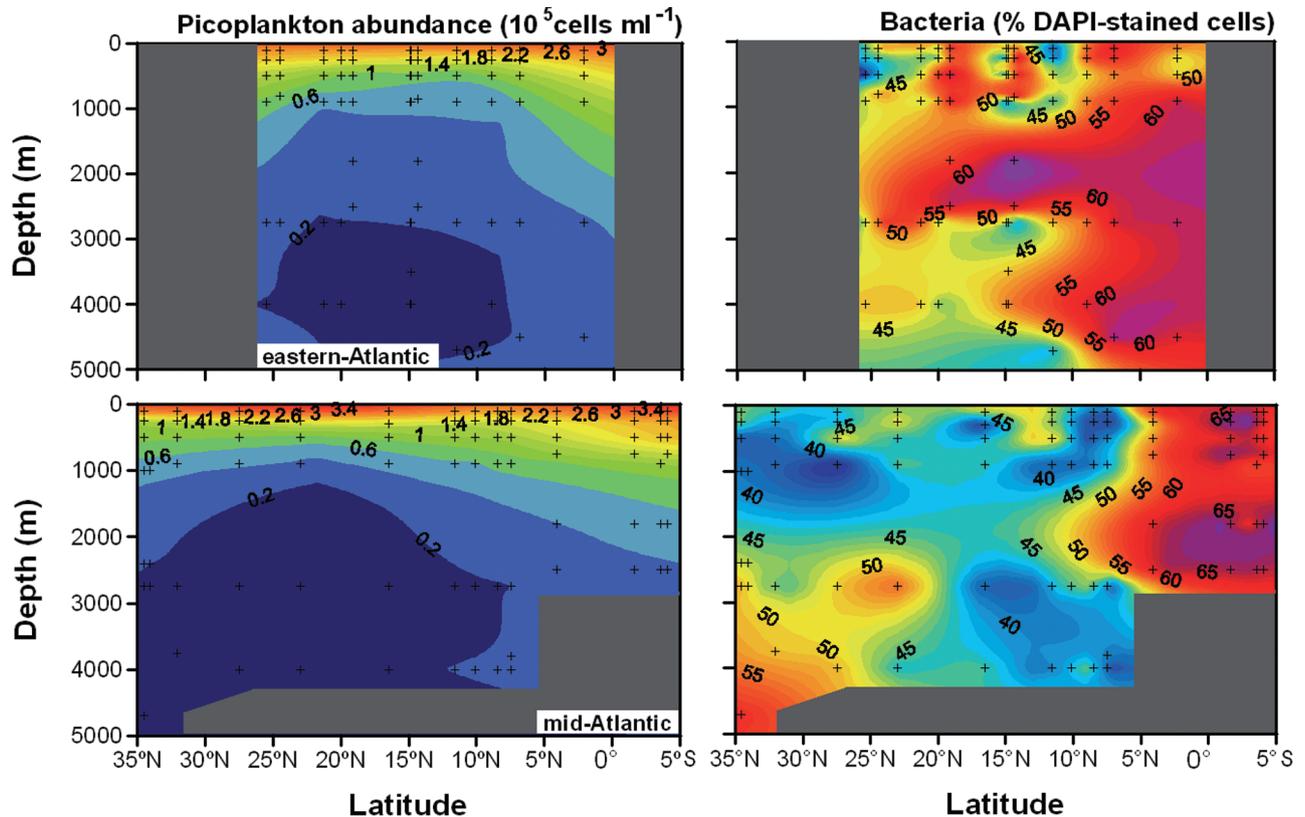
**Fig. 2.** Picoplankton abundance and the relative contribution of *Bacteria* to total picoplankton (% of DAPI-stained cells) abundance along the mid- and eastern-Atlantic transects.

Table 2. Abundance of the *Chloroflexi*-type SAR202 cluster expressed in $N \times 10^3$ cells ml^{-1} and its relative contribution to total picoplankton (% of DAPI-stained cells) and bacterial abundance (% of *Bacteria*) in the different water masses of the mid- and eastern-Atlantic sections.

Water mass	Eastern-Atlantic section			Mid-Atlantic section			<i>n</i>
	$N \times 10^3$ cells ml^{-1}	% DAPI	% <i>Bacteria</i>	$N \times 10^3$ cells ml^{-1}	% DAPI	% <i>Bacteria</i>	
Subsurface	0.50 ± 0.17^a	0.18 ± 0.07^a	0.40 ± 0.16^a	0.47 ± 0.14^a	0.34 ± 0.22^a	0.83 ± 0.58^a	12
O ₂ -min	1.65 ± 0.31^{ab}	1.45 ± 0.33^a	3.47 ± 0.82^a	1.76 ± 0.30^{abc}	1.35 ± 0.25^{ab}	3.12 ± 0.64^a	24
MSOW	2.26 ± 0.44^c	3.37 ± 0.63^a	8.49 ± 1.47^a	3.08 ± 0.30^c	4.10 ± 0.27^b	9.18 ± 0.94^a	5–6
AAIW	0.58 ± 0.14^a	0.85 ± 0.21^a	1.98 ± 0.59^a	1.46 ± 0.18^{ab}	2.30 ± 0.26^{ab}	4.68 ± 0.71^a	6–7
NEADW	1.91 ± 0.14^{bc}	9.47 ± 1.09^b	19.67 ± 3.24^b	2.47 ± 0.16^{bc}	10.72 ± 0.90^c	22.09 ± 2.99^b	13–16
LDW	2.16 ± 0.16^c	14.87 ± 0.83^c	32.97 ± 2.70^c	2.50 ± 0.25^{bc}	12.52 ± 1.19^c	25.82 ± 3.85^b	8–10

n, number of samples analysed. Mean values \pm SE are given. Superscript letters indicate significant differences between the different water masses (Mann–Whitney test, $P < 0.05$).

overall abundance of SAR202 cells ranged between $< 0.5 \times 10^3$ and 1.3×10^3 cells ml^{-1} at 100 m depth and from 1.0×10^3 to 5.3×10^3 cells ml^{-1} in the meso- and bathypelagic waters (Table 2). Overall, no significant differences were found in the SAR202 abundance between the eastern- and mid-Atlantic sections (*t*-test $n = 145$, $P > 0.05$). However, latitudinal trends in SAR202 abundance and differences between several deep-water masses were apparent. The abundance of SAR202 cells decreased throughout the water column from north to south, particularly in the mesopelagic waters where SAR202 abundance declined from around 3×10^3 cells ml^{-1} in the MSOW (from $\sim 25^\circ\text{N}$ to 15°N) to 1×10^3 cells ml^{-1} in the AAIW (from $\sim 10^\circ\text{N}$ towards the equator) (Table 2).

Along the mid-Atlantic section, the abundance of SAR202 was higher in the LDW and MSOW than in the subsurface (≈ 100 m depth), oxygen minimum and AAIW, while no significant differences were found between NEADW and mesopelagic waters (MSOW and oxygen minimum) (Table 2). Similarly, along the eastern-Atlantic section, SAR202 abundance was lower in the subsurface than in the meso- (MSOW) and bathypelagic waters (NEADW and LDW), whereas no significant differences in SAR202 abundance were found between the oxygen minimum and the subsurface layer and between the NEADW and the LDW (Table 2).

The contribution of SAR202 to total picoplankton (% of DAPI-stained cells) and bacterial abundance (% of *Bacteria*) throughout the meso- and bathypelagic waters is shown in Fig. 3. The relative abundance of SAR202 to both total picoplankton and bacterial abundance was not significantly different between the mid- and the eastern-Atlantic sections (*t*-test $P > 0.05$, $n = 144$, $n = 143$ respectively). The percentage of total picoplankton identified as SAR202 increased from $< 1\%$ in subsurface waters to $\approx 20\%$ in the LDW (Fig. 3). Members of the SAR202 cluster constituted between 30% and 40% of the *Bacteria* in the deep waters (NEADW and LDW), whereas in the mesopelagic (MSOW, AAIW and oxygen minimum)

and subsurface waters, SAR202 cells contributed $< 10\%$ to total bacterial abundance (Fig. 3).

The relative contribution of SAR202 to total picoplankton and bacterial abundance was significantly higher in the NEADW and LDW than in the intermediate (MSOW, AAIW and oxygen minimum) and subsurface waters in both the mid- and the eastern-Atlantic sections (Table 2).

D- and *L*-Asp uptake by bulk picoplankton and *Chloroflexi*-type SAR202

The uptake of *D*- and *L*-Asp by the bulk picoplankton and by SAR202 was determined at nine and six stations in the mid- and eastern-Atlantic sections, respectively (see Fig. 1). Mean bulk *L*-Asp uptake rates decreased from about $2.7 \text{ pmol L}^{-1} \text{ h}^{-1}$ at 100 m depth to $0.01 \text{ pmol L}^{-1} \text{ h}^{-1}$ in the LDW (Fig. 4), while *D*-Asp decreased from $0.40 \text{ pmol L}^{-1} \text{ h}^{-1}$ at 100 m depth to $0.001 \text{ pmol L}^{-1} \text{ h}^{-1}$ in the NEADW (data not shown). Generally, the *D*-Asp : *L*-Asp uptake ratio of the bulk picoplankton community increased with depth (Fig. 4). The highest *D*-Asp : *L*-Asp uptake ratios (up to 1.5) were measured in the LDW of the mid-Atlantic section. Overall, the *D*-Asp : *L*-Asp uptake ratio was significantly higher in the mid-Atlantic section than in the eastern-Atlantic section (*t*-test, $P < 0.01$, $n = 87$). This was mainly due to the higher *D*-Asp : *L*-Asp uptake ratio in the oxygen minimum (Mann–Whitney, $P < 0.001$, $n = 28$), MSOW (Mann–Whitney, $P < 0.01$, $n = 8$) and LDW (Mann–Whitney, $P < 0.001$, $n = 12$) of the mid-Atlantic section than in the eastern-Atlantic section (Fig. 4).

The number of SAR202 cells taking up *D*- and *L*-Asp was determined by MICRO-FISH (Fig. 5A). Contrary to the original hypothesis, SAR202 cells essentially did not take up *D*-Asp. Only in three samples (from the oxygen minimum), SAR202 cells took up *D*-Asp amounting to about 5% of the SAR202 present in these specific samples (data not shown). In contrast, the percentage of SAR202 cells taking up *L*-Asp was remarkably high, ranging from about 30% in the oxygen minimum layer to

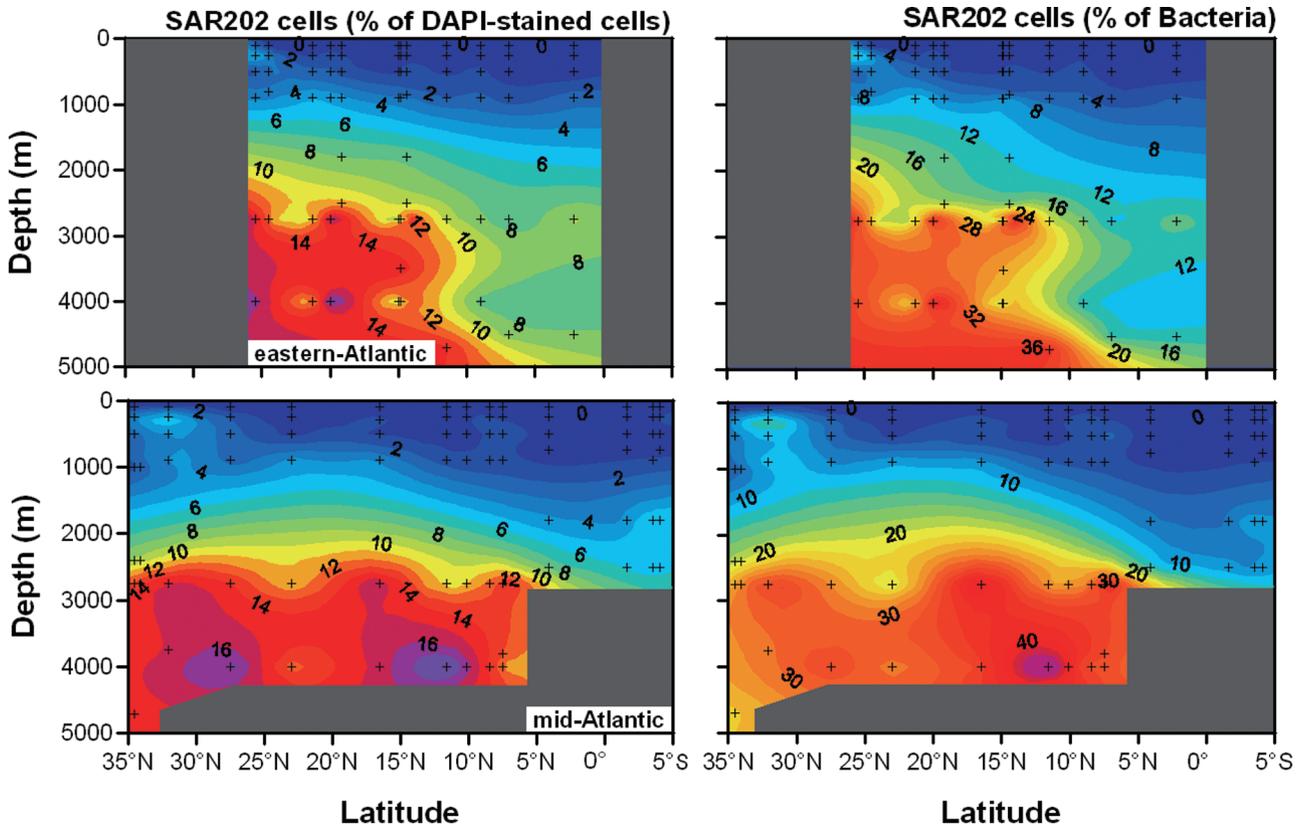


Fig. 3. Relative contribution of the *Chloroflexi*-type SAR202 cluster to total picoplankton (% of DAPI-stained cells) and bacterial (% of *Bacteria*) abundance along the mid- and eastern-Atlantic transects.

≈15% and ≈25% in the deep waters (NEADW and LDW) of the mid- and eastern-Atlantic sections, respectively (Fig. 5A).

Overall, no significant differences in the percentage of SAR202 cells taking up L-Asp were found between the eastern- and the mid-Atlantic sections (Mann–Whitney,

$P > 0.05$, $n = 40$, Fig. 5A). Also, no significant decline in the percentage of SAR202 cells taking up L-Asp with depth was detectable (Fig. 5A), which is in contrast to the pronounced decline in the percentage of L-Asp-positive cells of the bulk bacterial community with depth (Fig. 5B).

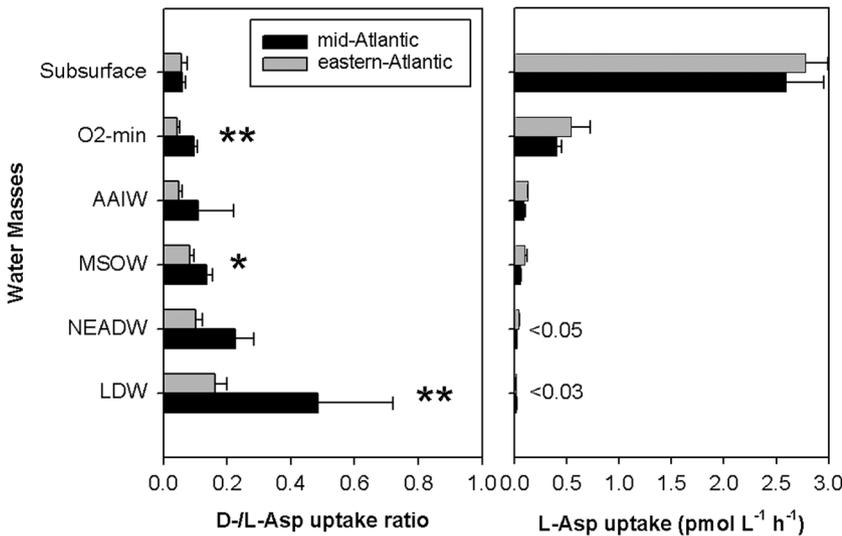


Fig. 4. Mean D-Asp : L-Asp uptake ratio and L-Asp uptake of the bulk picoplankton community in the different water masses of the mid- and eastern-Atlantic sections. Asterisks indicate significant differences in the D-Asp : L-Asp uptake ratio between the mid- and eastern-Atlantic sections in the corresponding water masses (** $P < 0.01$, * $P < 0.05$). Horizontal lines represent SE.

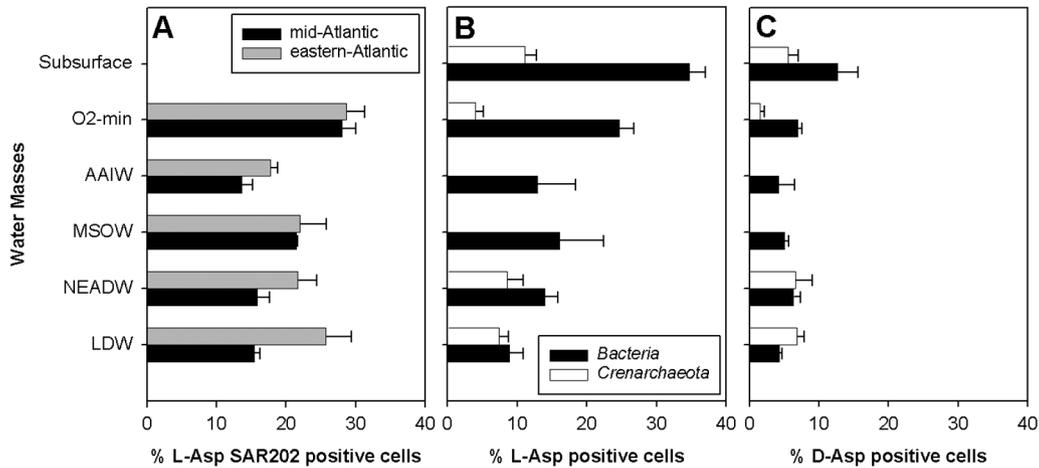


Fig. 5. Mean percentage of (A) *Chloroflexi*-type SAR202 cells (% L-Asp SAR202-positive cells) taking up L-aspartic acid determined by MICRO-FISH in the different water masses of the mid- and eastern-Atlantic sections, and percentage of *Bacteria* and *Crenarchaeota* (% of L- or D-Asp-positive cells) taking up (B) L-Asp and (C) D-Asp in the different water masses of the eastern North Atlantic (data for B and C from Varela *et al.*, 2008). Horizontal lines represent SE; in A, no data are shown for the subsurface water mass as too few L-Asp-positive SAR202 cells were detected.

Discussion

Pronounced latitudinal and vertical gradients were detectable in the abundance of the SAR202 cluster (Table 2, Fig. 3). The abundance of SAR202 decreased from 20°N to 5°N in the mesopelagic zone representing the transition from MSOW to AAIW in both the mid- and eastern-Atlantic sections (Table 2). While total bacterial abundance generally decreases with depth as a result of the increasing bioavailability of dissolved organic matter (DOM) (Fig. 2), the absolute abundance of SAR202 increased from the subsurface to meso- and bathypelagic waters (Table 2). Below 1000 m depth, the absolute abundance of SAR202 remained fairly constant (Table 2). Generally, the abundance of SAR202 reported in our study is similar to that reported by Morris and colleagues (2004) for the meso- and bathypelagic waters of the Atlantic (BATS) and Pacific (HOTS) sites. The mean contribution of SAR202 to the total picoplankton community accounted for 15% and < 5% of DAPI-stained cells in the bathy- (NEADW and LDW) and mesopelagic waters (oxygen minimum, MSOW and AAIW) respectively. However, SAR202 contributes up to 40% to the bacterioplankton in the waters below 2500 m depth (Fig. 3).

For the quantification of SAR202 cells, we used FISH, while *Bacteria* were enumerated by CARD-FISH (Varela *et al.*, 2008). CARD-FISH might yield a substantially higher recovery efficiency than FISH (Pernthaler *et al.*, 2002); however, this is not necessarily always the case. Woebken and colleagues (2007) reported a higher recovery efficiency of *Planktomycetes* anammox with conventional FISH than with CARD-FISH. Differences in the recovery efficiency between both protocols can vary

among different prokaryotic groups (Pernthaler *et al.*, 2002) and environments (Lin *et al.*, 2006; Kirchman *et al.*, 2007). It has been shown that the enzymatic treatment to permeabilize the cell wall can result in changes in the cell morphology of some bacterial strains and in the release of RNA (Furukawa *et al.*, 2006). Alternatively, the penetration of the high-molecular-weight horseradish peroxidase label used for CARD-FISH (\approx 44 kDa) might be inhibited or the permeabilization of the cell wall inefficient in specific bacterial groups. We do not know the actual reason for our failure to detect SAR202 by CARD-FISH, although several formamide concentrations (from 20% to 70%, at 5% intervals, data not shown) were tested. While the detection of archaeal cells is substantially improved using the CARD-FISH protocol (Teira *et al.*, 2004), for *Bacteria* the protocol using the Eub338-horseradish peroxidase (HRP) probe (CARD-FISH) only marginally improves cell detection over that of the monolabelled Eub338I-III probe mix (FISH) (Lin *et al.*, 2006; Kirchman *et al.*, 2007). Based on these findings, we are confident that the high relative contribution of the SAR202 cluster to the bulk bacterial community in the meso- and bathypelagic waters of the (sub)tropical Atlantic reported here is real and not a methodological artefact. Furthermore, the rather low abundance of SAR202 in the subsurface waters where SAR11 is abundant further indicates the stringency of the SAR202 oligonucleotide probe, as it does not hybridize SAR11 cells using the applied FISH protocol.

The obtained D-Asp : L-Asp uptake ratios for the bulk picoplankton community confirm previous findings (Pérez *et al.*, 2003) of increasing D-Asp : L-Asp uptake ratios with depth (Fig. 4). However, differences in the D-Asp : L-Asp uptake ratios between the mid- and the eastern-Atlantic

sections are apparent and may reflect subtle differences in the composition of the DOM pool (Aminot and K erouel, 2004; Yamada and Tanoue, 2006) along the eastern transect, which is closer to the continental slope than the mid-Atlantic transect. Particularly in the oxygen minimum layer, MSOW and LDW, the D-Asp : L-Asp uptake ratios were significantly higher in the mid-Atlantic section than in the eastern-Atlantic section (Fig. 4). D-Asp : L-Asp uptake ratios have been suggested to be an indicator of the diagenetic state of the DOM, with higher uptake ratios indicating the utilization of older, more refractory DOM (P erez *et al.*, 2003; Teira *et al.*, 2006a,b; Kitayama *et al.*, 2007). Similarly, higher D/L ratios of amino acids in the DOM pool are indicative of more refractory DOM (Amon *et al.*, 2001). Following this reasoning, the higher D-Asp : L-Asp uptake ratios in the mid-Atlantic section might indicate the presence of more refractory DOM than in the eastern-Atlantic section. It has been suggested that continental shelf-derived DOM is laterally transported towards the open Atlantic ( lvarez-Salgado *et al.*, 2007), suggesting that DOM becomes more refractory with distance from the continental slope.

Contrary to our original hypothesis that the SAR202 cluster represents a major sink for D-Asp in the meso- and bathypelagic waters, SAR202 cells taking up D-Asp were only sporadically detectable. The fraction of SAR202 cells taking up L-Asp, however, remained remarkably stable throughout the water column (Fig. 5A). These findings are in contrast to the pronounced decrease with depth in the percentage of *Bacteria* (enumerated by Eub3381-III and CARD-FISH) using L-Asp (Fig. 5B). The percentage of *Bacteria* and *Crenarchaeota* taking up D-Asp remains fairly constant throughout the meso- and bathypelagic realm (Fig. 5C, Varela *et al.*, 2008). Taken together, this indicates that the SAR202 cluster is well adapted to exploit the available DOM source in the bathypelagic realm. SAR202 cells are larger than the average bacterioplankton cell at bathypelagic depths (Morris *et al.*, 2004), further supporting the notion that this cluster might be a highly active member of the bathypelagic bacterial community.

Several deep-ocean bacterial adaptations have been revealed recently using metagenomic analyses. DeLong and colleagues (2006) reported a higher abundance of genes encoding for chemotaxis and motility in deep than in surface waters. This might indicate that at least a certain fraction of deep-water *Bacteria* are capable to locate nutrient gradients and likely exploit deep-water hot spots, such as particles which rapidly descended from the surface waters into the deep ocean. It might well be that the thus far uncultured SAR202 cluster represents an r-strategist rapidly exploiting nutrient patches in the dark ocean. Whatever the exact ecological niche of SAR202 might be in the deep ocean, it appears that SAR202 is

maintaining a higher fraction of L-Asp-active cells in the bathypelagic water column than the bulk *Bacteria* and marine *Crenarchaeota* Group I (Fig. 5).

In summary, our data indicate that the *Chloroflexi*-type SAR202 cluster occurs throughout the meso- and bathypelagic (sub)tropical Atlantic, constituting about 15% of the picoplankton and \approx 30% of the total bacterial community. In contrast to the general tendency of an increasing fraction of D-Asp utilizing bacterial and crenarchaeal cells with depth, members of the *Chloroflexi*-type SAR202 cluster are not utilizing D-Asp. Instead, L-Asp is taken up efficiently by the SAR202 cluster without any decreasing tendency with depth, as commonly detected in the bulk bacterial and crenarchaeal communities. This indicates that the SAR202 cluster is well adapted to the prevailing environmental conditions in the dark ocean and that the SAR202 cluster probably contributes a major fraction of the deep-water bacterial activity.

Experimental procedures

Sampling area

Sampling of the meso- and bathypelagic waters was carried out in the eastern Atlantic during the cruises ARCHIMEDES-1 (November/December 2005) and -2 (November/December 2006) on board R/V Pelagia. In total, 22 and 30 stations were occupied at the mid- and eastern-Atlantic sections, respectively. The mid-Atlantic section followed a track from the Azores Basin (35°N) to the Brazil Basin (5°S), and the eastern-Atlantic section from the Canary Basin (25°N) to the Sierra Leona Basin (1°N). Samples were taken from six depths: the subsurface (100 m layer), the oxygen minimum layer (average depth 400 m), the MSOW (average depth 900 m), the AAIW (average depth 900 m), the NEADW (average depth 2500 m) and the LDW (average depth 4000 m).

Samples from the distinct water masses were collected with NOEX bottles (no oxygen exchange, 12 l) mounted in a CTD (conductivity, temperature, depth) frame to determine the abundance of the *Chloroflexi*-type SAR202 cells and their contribution to picoplankton (DAPI-stained cells) and *Bacteria*, the uptake of D- and L-Asp by the bulk picoplankton community and, the potential of the SAR202 cluster to take up D- and L-Asp by MICRO-FISH as described below.

Fluorescence in situ hybridization

Immediately after collecting the samples from the NOEX bottles, 20–80 ml of water was fixed by adding filtered paraformaldehyde at a final concentration of 2% and, subsequently, the samples were stored in the dark at 4°C for 12–18 h. Thereafter, the samples were filtered through 0.2 µm polycarbonate filters (Millipore GTTP, 25 mm filter diameter) supported by cellulose nitrate filters (Millipore, HAWP, 0.45 µm), washed twice with Milli-Q water, and dried and stored in a microfuge vial at –20°C until further processing in the home laboratory.

The hybridization reactions for the SAR202 cluster were performed as described by Morris and colleagues (2004). Reactions were done on one quarter of the membrane at 37°C for 12–15 h in hybridization buffer [900 mM NaCl, 20 mM Tris (pH 7.4), 0.01 (wt/vol) sodium dodecyl sulphate (SDS), 35% formamide] and two Cy3-labelled oligonucleotide probes [SAR202-104R (GTTACTCAGCCGTCTGCC) and SAR202-312R (TGTCTCAGTCCCCCTCTG)] (Morris *et al.*, 2004) specific for the SAR202 cluster. Additionally, a control hybridization reaction was performed with a buffer containing 15% formamide and a Cy3-labelled Non338 probe. All probes had a final concentration of 2 ng µl⁻¹. Optimal hybridization stringency was achieved by washing the membranes in hybridization wash [70 and 150 mM NaCl for SAR202 and Non338 respectively, 20 mM Tris (pH 7.4), 6 mM EDTA, 0.01% SDS] at 58.0°C for 20 min for the SAR202 hybridization reactions and 50°C for the Non338 control hybridization reactions.

The hybridization reactions of the *Bacteria* probe-mix (Eub338, Eub338 II and Eub338 III, Daims *et al.*, 1999) and the negative control probe Non338 were carried out by CARD-FISH (Teira *et al.*, 2004). Filters for CARD-FISH were embedded in low-gelling-point agarose and incubated with lysozyme. Hybridization reactions were carried out with HRP-labelled oligonucleotide probe and tyramide-Alexa488 as described in Teira and colleagues (2004).

Cells were counterstained with a DAPI-mix [5.5 parts of Citifluor (Citifluor), 1 part of Vectashield (Vector Laboratories) and 0.5 part of phosphate-buffered saline with DAPI (final concentration 5 µg ml⁻¹)]. For each microscope field, DAPI-stained, Cy3-positive and Alexa448-positive cells were counted under a Zeiss Axioplan 2 epifluorescence microscope equipped with a 100 W Hg lamp and appropriate filter sets for DAPI, Cy3 and Alexa448. Negative control counts were performed with Cy3-Non338 probes, always amounting to < 5% of DAPI-stained cells and subtracted from SAR202 counts. Negative control counts (hybridization with HRP-Non338) were always < 1% of DAPI-stained cells. More than 600 DAPI-stained cells were counted per sample.

Uptake of D- and L-Asp by the bulk picoplankton community

To measure the uptake of D- and L-Asp by the bulk picoplankton community, 20–40 ml of duplicate water samples and one formaldehyde-killed blank (2% final concentration) were spiked with either D-[2,3-³H]-Asp or L-[2,3-³H]-Asp (Amersham, SA: D-Asp, 38 Ci mmol⁻¹; L-Asp, 32 Ci mmol⁻¹) at a final concentration of 1 nM and incubated in the dark at *in situ* temperature for 12–48 h. After terminating the incubations by adding formaldehyde (2% final concentration), the samples were filtered through 0.2 µm polycarbonate filters (Millipore, GTTP, 25 mm filter diameter) supported by 0.45 µm cellulose nitrate filters (Millipore, HAWP, 25 mm filter diameter) and rinsed twice with 0.2 µm filtered sea water, dried and placed in scintillation vials. Scintillation cocktail (8 ml Canberra-Packard Filter Count) was added and, after 18 h, counted in a liquid scintillation counter (LKB Wallac Model 1212). The disintegrations per minute (DPM) of the formaldehyde-fixed blank were subtracted from the mean DPM of the respective samples and the resulting DPM converted into D- or L-Asp uptake rates.

A final concentration of 1 nM of radiolabelled Asp was used because the concentration of dissolved free Asp in the deep waters is < 5 nmol l⁻¹ and, according to Pérez and colleagues (2003), the uptake rates increase from 0.1 to 10 nmol l⁻¹ final concentration of added Asp by a factor of 10.

The MICRO-FISH with D- and L-Asp

Duplicate water samples of 20–40 ml were spiked with either D-[2,3-³H]-Asp or L-[2,3-³H]-Asp (Amersham, SA: D-Asp, 38 Ci mmol⁻¹; L-Asp, 32 Ci mmol⁻¹) at a final concentration of 1 nM and incubated in the dark at *in situ* temperature for 12–48 h. One control per sample was used, fixed with 2% paraformaldehyde (final concentration) 15 min prior to radiotracer addition. Incubations were terminated with paraformaldehyde (2% final concentration) and, subsequently, the samples stored at 4°C in the dark for 12–18 h. Thereafter, the samples were filtered onto 0.2 µm polycarbonate filters (Millipore, GTTP, 25 mm filter diameter) supported by cellulose nitrate filters (Millipore, HAWP, 0.45 µm), rinsed twice with Milli-Q water, dried, and stored in a microfuge vial at –20°C until further processing in the home laboratory.

The FISH protocol for SAR202 was carried out as described above. Autoradiographic development was conducted by transferring previously hybridized filter sections onto slides coated with photographic emulsion (type NTB-2 melted at 43°C for 1 h). The slides were then placed in a light-tight box containing a drying agent and incubated for exposure at 4°C for 7 days. Finally, the slides were developed and fixed following Kodak's specifications [2 min in Dektol developer (1:1 dilution with Milli-Q water), 10 s in Milli-Q water, 5 min in fixer and 2 min in Milli-Q water]. Before completely dried, filter sections were removed and cells were counterstained with a DAPI-mix and examined under a Zeiss Axioplan 2 epifluorescence microscope. The presence of silver grains surrounding the cells was checked in the transmission mode of the microscope. For each microscope field, we examined SAR202 probe-positive cells and silver grain halos associated with individual cells. Between 20 and 50 SAR202 probe-positive cells were counted per sample. In the killed controls, < 1% of the SAR202 probe-positive cells were associated with silver grain halos.

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References

- van Aken, H.M. (2000a) The hydrography of the mid-latitude Northeast Atlantic ocean: I, the deep water masses. *Deep Sea Res Part I* **47**: 757–788.

- van Aken, H.M. (2000b) The hydrography of the mid-latitude Northeast Atlantic ocean: II, the intermediate water masses. *Deep Sea Res Part I* **47**: 789–824.
- Álvarez-Salgado, X.A., Aristegui, J., Barton, E.D., and Hansell, D.A. (2007) Contribution of upwelling filaments to offshore carbon export in the subtropical Northeast Atlantic Ocean. *Limnol Oceanogr* **52**: 1287–1292.
- Aminot, A., and Kérouel, R. (2004) Dissolved organic carbon, nitrogen and phosphorus in the N-E Atlantic and the N-W Mediterranean with particular reference to non-refractory fractions and degradation. *Deep Sea Res Part I* **51**: 1975–1999.
- Amon, R.M.W., Fitznar, H.-P., and Benner, R. (2001) Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnol Oceanogr* **46**: 287–297.
- Bano, N., and Hollibaugh, J.T. (2002) Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean. *Appl Environ Microbiol* **68**: 505–518.
- Daims, H., Brühl, 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.
- DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.-U., et al. (2006) Community genomics among stratified microbial assemblages in the ocean's interior. *Science* **311**: 496–503.
- Fuhrman, J.A. (2002) Community structure and function in prokaryotic marine plankton. *Antonie Van Leeuwenhoek* **81**: 521–527.
- Furukawa, K., Hoshino, T., Tsuneda, S., and Inamori, Y. (2006) Comprehensive analysis of cell wall-permeabilizing conditions for highly sensitive fluorescence in situ hybridization. *Microbes Environ* **21**: 227–234.
- Giovannoni, S.J., and Rappé, M.S. (2000) Evolution, diversity, and molecular ecology of marine prokaryotes. In *Microbial Ecology of the Oceans*. Kirchman, D.L. (ed.). New York, NY, USA: John Wiley & Sons, Inc, pp. 47–84.
- Giovannoni, S., Rappé, M.S., Vergin, K.L., and Adair, N.L. (1996) 16S rRNA genes reveal stratified open ocean bacterioplankton populations related to the green non-sulfur bacteria. *Proc Natl Acad Sci USA* **93**: 7979–7984.
- Karner, M.B., Delong, E.F., and Karl, D.M. (2001) Archaeal dominance in the mesopelagic of the Pacific Ocean. *Nature* **409**: 507–510.
- Kirchman, D.L., Elifantz, H., Dittel, A.I., and Malmstrom, R.R. (2007) Standing stocks and activity of *Archaea* and *Bacteria* in the western Arctic Ocean. *Limnol Oceanogr* **52**: 495–507.
- Kitayama, K., Hama, T., and Yanagi, K. (2007) Bioreactivity of peptidoglycan in seawater. *Aquat Microb Ecol* **46**: 85–93.
- Lee, N., Nielsen, P.H., Andreasen, K.H., Juretschko, S., Nielsen, J.L., Schleifer, K.H., and Wagner, M. (1999) Combination of fluorescent in situ hybridization and microautoradiography – a new tool for structure-function analyses in microbial ecology. *Appl Environ Microbiol* **65**: 1289–1297.
- Lin, X., Wakeham, S.G., Putnam, I.F., Astor, Y.M., Scranton, M.I., Chistoserdov, A.Y., and Taylor, G.T. (2006) Comparison of vertical distributions of prokaryotic assemblages in the anoxic Cariaco Basin and Black Sea by use of fluorescence in situ hybridization. *Appl Environ Microbiol* **72**: 2679–2690.
- Moreira, D., Rodriguez-Valera, F., and López-García, P. (2006) Metagenomic analysis of mesopelagic Antarctic plankton reveals a novel deltaproteobacterial group. *Microbiology* **152**: 505–517.
- Morris, R.M., Rappé, M.S., Urbach, E., Connon, S.A., and Giovannoni, S.J. (2004) Prevalence of the *Chloroflexi*-related SAR202 bacterioplankton cluster throughout the mesopelagic zone and deep ocean. *Appl Environ Microbiol* **70**: 2836–2842.
- Ouverney, C.C., and Fuhrman, J.A. (2000) Marine planktonic *Archaea* take up amino acids. *Appl Environ Microbiol* **66**: 4829–4833.
- Pérez, M.T., Pausz, C., and Herndl, G.J. (2003) Major shift in bacterioplankton utilization of enantiomeric amino acids between surface waters and the ocean's interior. *Limnol Oceanogr* **48**: 755–763.
- Pernthaler, A., Pernthaler, J., and Amann, R. (2002) Fluorescence in situ hybridization and catalyzed reported deposition for the identification of marine bacteria. *Appl Environ Microbiol* **68**: 3094–3101.
- Suttle, C.A., Chan, A.M., and Fuhrman, J.A. (1991) Dissolved free amino acids in the Sargasso Sea: uptake and respiration rates, turnover times and concentration. *Mar Ecol Prog Ser* **70**: 189–199.
- Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G.J. (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.
- Teira, E., Lebaron, P., van Aken, H., and Herndl, G.J. (2006a) Distribution and activity of *Bacteria* and *Archaea* in the deep waters masses of the North Atlantic. *Limnol Oceanogr* **51**: 2131–2144.
- Teira, E., van Aken, H., Veth, C., and Herndl, G.J. (2006b) Archaeal uptake of enantiomeric amino acids in the meso- and bathypelagic waters of the North Atlantic. *Limnol Oceanogr* **51**: 60–69.
- Varela, M., van Aken, H., Sintes, E., and Herndl, G.J. (2008) Latitudinal trends of *Crenarchaeota* and *Bacteria* in the meso- and bathypelagic waters of the eastern north Atlantic. *Environ Microbiol* **10**: 110–124.
- Woeckel, D., Fuchs, B.M., Kuypers, M.M.M., and Amann, R. (2007) Potential interactions of particle-associated anammox *Bacteria* with bacterial and archaeal partners in the Namibian upwelling system. *Appl Environ Microbiol* **73**: 4648–4657.
- Wright, T.D., Vergin, K.L., Voyd, P.W., and Giovannoni, S.J. (1997) A novel delta-subdivision proteobacterial lineage from the lower ocean surface layer. *Appl Environ Microbiol* **63**: 1441–1448.
- Yamada, N., and Tanoue, E. (2006) The inventory and chemical characterization of dissolved proteins in oceanic waters. *Prog Oceanogr* **69**: 1–18.