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Archaeal uptake of enantiomeric amino acids in the meso- and bathypelagic waters of the North Atlantic

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Abstract

We determined the contribution of the three major prokaryotic groups (Bacteria, *Crenarchaeota*, and *Euryarchaeota*) on the uptake of D- and L-aspartic acid (Asp) in the major water masses of the North Atlantic (from 100- to 4,000-m depth) with the use of microautoradiography combined with catalyzed reporter deposition fluorescence in situ hybridization (MICRO-CARD-FISH). The percentage of prokaryotic cells that assimilated D- and L-Asp ranged from <5% to 25%. In the meso- and bathypelagic waters of the North Atlantic, Archaea are more abundant (42% ± 2% of 4′,6′-diamino-2-phenylindole [DAPI]–stained cells) than Bacteria (30% ± 1% of DAPI-stained cells), and more archaeal than bacterial cells are actively incorporating D-Asp (62% ± 2% vs. 38% ± 2% of total D-Asp active cells). In contrast, Bacteria and Archaea almost equally contribute to L-Asp use in the deep waters of the North Atlantic (47% ± 2% vs. 53% ± 2% of total L-Asp active cells). The increase in the D-Asp : L-Asp uptake ratio in the prokaryotic community with depth appears to be driven by the efficient uptake of D-Asp by, especially, the *Crenarchaeota* in the deep waters. Because Archaea, and particularly *Crenarchaeota*, commonly dominate the prokaryotic communities in the ocean's interior, we suggest that they represent a previously unrecognized sink of D–amino acids in the deep ocean.

The formation of the North Atlantic Deep Water (NADW) is the major driving force of the oceanic conveyor belt system that, in turn, influences the global climate (Broecker 1997). The turnover time of this oceanic conveyor belt system is about 2,000 yr, whereas that of the dissolved organic carbon (DOC) in the oceanic deep water is about 6,000–8,000 yr (Williams 2000). Hansell and Carlson (1998) showed that the deep water DOC concentrations decline from the deep North Atlantic (~45 μmol L^{-1}) to the opposite end of the conveyor belt circulation, the deep Pacific (~37 μmol L^{-1}), indicating net removal of DOC.

Despite recent advances in the phylogenetic characterization of deep-water prokaryotic communities, little is known about the metabolically active fraction of the prokaryotic community that drives the biogeochemical cycles in the ocean's interior. Only recently, our knowledge on the richness of marine prokaryotic communities increased considerably by applying molecular tools such as fingerprinting techniques, cloning, and sequencing (*see* review by Giovannoni and Rappé 2000). These molecular techniques revealed that Archaea are widespread among marine prokaryotic plankton (DeLong et al. 1994; Massana et al. 1998; Crump and Baross 2000). Quantitative studies with fluorescence in situ hybridization (FISH) have shown the dominance of Group I Archaea (members of the *Crenarchaeota*) in meso- and bathypelagic waters, whereas Group II Archaea (members of the *Euryarchaeota*) comprise <5–8% of total picoplankton cells in deep waters of the Pacific and in the Antarctic waters (Karner et al. 2001; Church et al. 2003). Use of the recently developed catalyzed reported deposition FISH (CARD-FISH), in combination with the proteinase-K permeabilization of the archaeal cell wall, allowed higher detection rates of *Euryarchaeota* in deep waters of the North Atlantic Ocean (Teira et al. 2004).

In contrast to advances in the phylogenetic characterization of deep-water prokaryotic communities, information on their activity is rather scarce (Dufour and Torréton 1996; Nagata et al. 2000; Tanaka and Rassoulzadegan 2004). Most of these studies focused on the regulation and timescale of bacterial dynamics and on the relation between bacterioplankton abundance or production and particulate organic carbon flux (Nagata et al. 2000; Hansell and Ducklow 2003).

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Recently, Pérez et al. (2003) observed an increase of two to three orders magnitude in the ratio of D- to L–aspartic acid (Asp) uptake by prokaryotic plankton from the surface to deep mesopelagic waters in the North Atlantic. This has been interpreted as an indication that mesopelagic prokaryotic plankton are using bacterial cell wall–derived organic matter efficiently because the bacterial peptidoglycan layer is the only biotic source of significance of D–amino acids in the ocean. The peptidoglycan layer contains four enantiomeric amino acids (alanine, Asp, serine, glutamic acid; Schleifer and Kandler 1972). Pérez et al. (2003) hypothesized that specific prokaryotic communities are present in the mesopelagic realm that use D–amino acids as efficiently as L–amino acids. Major shifts in the prokaryotic community composition between surface and mesopelagic waters support this hypothesis (Karner et al. 2001; Moeseneder et al. 2001). Thus far, no direct evidence has been presented; however, that these shifts in the prokaryotic community composition are responsible for the observed increase in the D-Asp : L-Asp uptake ratio with depth.

The recently developed approach of combining FISH with microautoradiography allows the determination of the uptake potential of specific dissolved organic matter (DOM) compounds by individual members of the prokaryotic community (Ouverney and Fuhrman 2000; Cottrell and Kirchman 2000, 2003). These studies, however, were restricted to prokaryotic communities of the top 200-m layer of the oceanic water column.

In this article, we combined the refined CARD-FISH with microautoradiography (MICRO-CARD-FISH; Teira et al. 2004) to determine the distribution of prokaryotic cells capable of taking up D-Asp versus L-Asp to identify the prokaryotic group responsible for the shift in the D-Asp : L-Asp uptake ratio from surface to deep waters.

Material and methods

Sampling of meso- and bathypelagic waters was carried out along a more than 4,000-km-long transect from Bermuda to east of Greenland following the western branch of the NADW to its origin (on board RV *Pelagia*, May/June 2003). Samples were taken at 16 stations out of a total of 35 stations occupied during the TRANSAT-2 cruise (Fig. 1) from five or six depths: the 100-m layer (subsurface) and, if present, the oxygen-minimum (O_2-min) layer (average depth 400 m); the Labrador Sea water (LSW, average depth 1,300 m); the NADW (average depth 2,600 m), and the Denmark Strait overflow water (DSOW, average depth 3,200 m). Water masses were identified on the basis of their salinity and temperature characteristics (Aken 2000*a*,*b*). Water from the distinct water masses was collected with NOEX-bottles (12 liters) mounted on a CTD (conductivity, temperature, depth) frame to determine the uptake of D-Asp and L-Asp by the bulk prokaryotic community and the cell-specific D-Asp and L-Asp uptake by Bacteria, *Crenarchaeota* and *Euryarchaeota* by combining microautoradiography with CARD-FISH (MICRO-CARD-FISH) as follows.

Uptake of D-*Asp and* L-*Asp by the bulk prokaryotic community*—To measure the uptake of D-Asp and L-Asp by the bulk prokaryotic community, 20–40 mL of duplicate water samples and one formaldehyde-killed blank (2% final concentration) were spiked with either D-[2,3-^{3}H]-Asp or L-[2,3-^{3}H]-Asp (Amersham, SA: D-Asp, 481 GBq mmol^{-1}; L-Asp, 1,369 GBq mmol^{-1}) at a final concentration of 1 nmol L^{-1} and incubated in the dark at in situ temperature for 4–7 h. After terminating the incubations by adding formaldehyde (2% final concentration), the samples were filtered through 0.22-μm cellulose nitrate filters (Millipore, 25-mm filter diameter), rinsed twice with 0.2-μm filtered seawater, and stored in scintillation vials at −20°C until analysis. Back in the laboratory, filters were dissolved in 1 mL of ethyl acetate (Riedel de Haen), and after 10 min, 8 mL of scintillation cocktail (Insta-gel plus II, Canberra Packard) was added. After 18 h, the radioactivity of the filters was assessed with a liquid scintillation counter by counting each sample for 10 min. The disintegrations per minute (DPM) of the formaldehyde-fixed blank were subtracted from the corresponding samples, and the resulting DPM was converted into D-Asp and L-Asp uptake rates. The coefficient of variation between replicates was 10% ± 9% and 8% ± 9% of the mean D-Asp and L-Asp uptake rate, respectively, and the DPM of the blanks were always <40% of the mean DPM of the respective duplicate samples. Final concentration of radiolabeled Asp (1 nmol L^{-1}) was used for bulk D-Asp and L-Asp uptake measurements because the concentration of dissolved free Asp in the NADW is <5 nmol L^{-1}, and Pérez et al. (2003) showed that uptake rates increase from 0.1 to 10 nmol L^{-1} final concentration of added Asp by a factor of 10. The D-Asp : L-Asp uptake ratio, however, was not affected by the concentration of added Asp (Pérez et al. 2003). Thus, for MICRO-CARD-FISH analyses (see following), a final concentration of 10 nmol L^{-1} Asp was used to facilitate enumeration of Asp-positive cells.

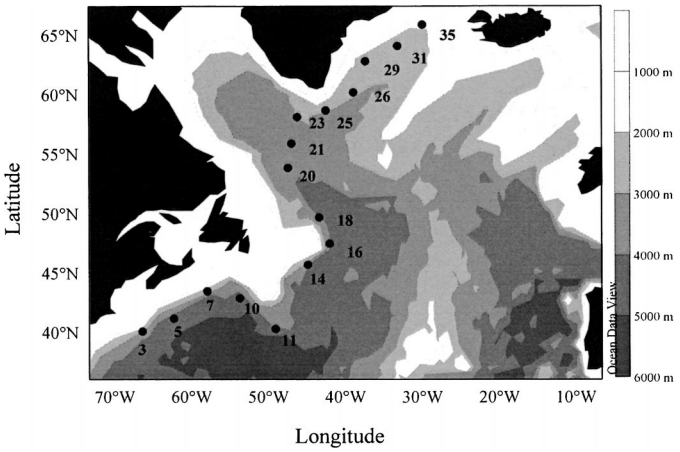

Fig. 1. Location of sampling stations in the North Atlantic following the flow of the western branch of the NADW during the TRANSAT-2 cruise.

MICRO-CARD-FISH with D-*Asp and* L-*Asp*—Water samples of 20–40 mL were spiked with either D-[2,3-^{3}H]-Asp or L-[2,3-^{3}H]-Asp (Amersham, SA: D-Asp, 481 GBq mmol^{-1}; L-Asp, 1,369 GBq mmol^{-1}) at a final concentration of 10 nmol L^{-1} and incubated in the dark at in situ temper-

ature for 8–10 h. Controls consisted of 20–40-mL samples killed with 2% paraformaldehyde 20 min before addition of the radiotracer. Incubations were terminated by adding paraformaldehyde (2% final concentration), and subsequently, the samples were stored at 4°C in the dark for 12–18 h. Thereafter, the sample was filtered through a 0.2-μm polycarbonate filter (Millipore, GTTP) supported by a cellulose nitrate filter (Millipore, HAWP, 0.45 μm), washed twice with Milli-Q water, dried, and stored in a microfuge vial at −20°C until further processing in the home laboratory.

Filters for CARD-FISH were embedded in low–gelling point agarose and incubated either with lysozyme (for the Bacteria probe Eub338 and for the negative control probe Non338; Amann et al. 1995) or proteinase-K (for the *Euryarchaeota* probe Eury806 [5′-CACAGCGTTTACACCT-AG-3′] and for the *Crenarchaeota* probe Cren537 [5′-TGACCACTTGAGGTGCTG-3′]; Teira et al. 2004). Filters were cut in sections and hybridized with horseradish peroxidase (HRP)–labeled oligonucleotide probes and tyramide-Alexa488 for signal amplification following the protocol described in Teira et al. (2004). 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 36–48 h. Finally, the slides were developed and fixed following Kodak's specifications (2 min in Dektol developer [1 : 1 dilution with Milli-Q water], 10 sec 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 counter-stained with a DAPI mix (5.5 parts of Citifluor [Citifluor, Ltd.], 1 part of Vectashield [Vector Laboratories, Inc.], and 0.5 parts of phosphate-buffered saline with DAPI [final concentration 1 μg mL^{-1}]).

The slides were examined under a Zeiss Axioplan 2 microscope equipped with a 100-W Hg lamp and appropriate filter sets for DAPI and Alexa488. The presence of silver grains surrounding the cells was checked with the use of the transmission mode of the microscope. In the killed controls, <0.5% of the total DAPI-stained cells were associated with two or more silver grains. More than 800 DAPI-stained cells were counted per sample. For each microscope field, four different categories were enumerated: (1) total DAPI-stained cells, (2) cells stained with the specific probe, (3) DAPI-stained cells with >3 associated silver grains, and (4) cells labeled with the specific fluorescent probe and >3 silver grains associated with individual cells. Negative control counts (hybridization with HRP-Non338) averaged 1.5% and were always <5% of DAPI-stained cells. The counting error, expressed as the percentage of standard error between replicates, was <2% for DAPI counts and <9% for FISH counts. The corresponding error for microautoradiography counts averaged (mean ± SE) 25% ± 1%.

Statistical analysis—To check for differences between three or more groups in terms of a given variable, a nonparametric test (Kruskal–Wallis) was applied because of the nonnormal distribution of the variables considered. The nonparametric Mann–Whitney test was used to compare two independent samples. The Bonferroni correction was applied to the Mann–Whitney test for multiple comparisons to control errors of type I.

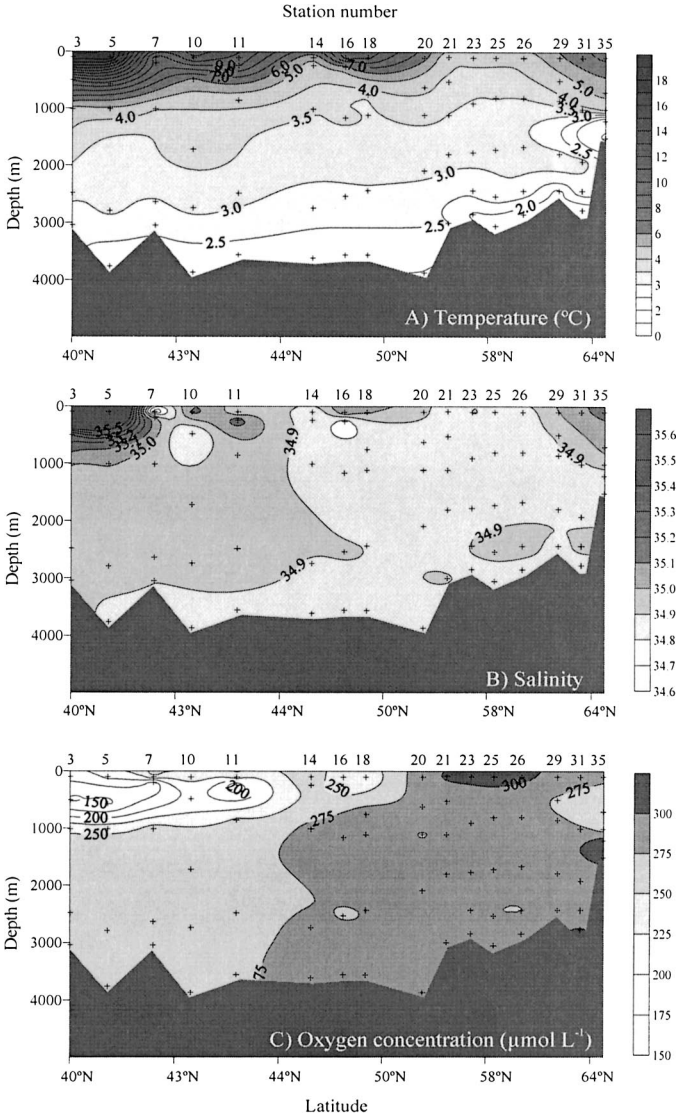

Fig. 2. (A) Distribution of temperature, (B) salinity, and (C) oxygen concentration along the transect in the western basin of the North Atlantic from Bermuda to east of Greenland.

Results

Water mass characteristics—The vertical distribution of temperature, salinity, and oxygen concentration along the S–N transect from Bermuda to east of Greenland is shown in Fig. 2. The basic physicochemical characteristics of the main water masses sampled during this study are summarized in Table 1. LSW was clearly identifiable at depth levels between 800 and 2,100 m throughout the transect, except in the southern part of the transect (approximately between Sta. 3 and 11). The NADW originating in the eastern basin and entering the western Atlantic through the Charlie–Gibbs fracture zone (Dobrolyubov et al. 2003) is identified by high salinity (>34.9) and a seawater temperature between 2.6 and

Table 1. Basic physical and chemical characterisitics of the main water masses sampled in the North Atlantic. Ranges are given for each water mass where samples were collected. LSW, Labrador sea water; NADW, North Atlantic Deep Water; DSOW, Denmark Strait overflow water.

Water mass	Depth (m)	Temperature (°C)	Salinity	Oxygen (μmol kg^{-1})
Subsurface	88–103	4.1–18.8	34.6–36.6	206–305
O_2 minimum	200–626	4.3–10.1	34.8–35.3	144–257
LSW	809–2,060	3.1–3.7	34.87–34.90	274–291
NADW	2,435–2,790	2.6–3.2	34.90–34.95	265–282
DSOW	1,216*–3,869	0.8–2.4	34.81–34.90	271–309

* At Sta. 35.

3.2°C (Table 1). NADW was identifiable throughout the transect between 2,400- and 2,800-m depth, except at two stations in the north (Sta. 29, 35) and at Sta. 20 and 21. The DSOW underlying the NADW constitutes the densest component of the NADW masses studied, with a seawater temperature between 0.8°C and 2.4°C and a salinity of <34.9 (Table 1). We could sample DSOW at all the stations except at Sta. 3 and 7. A local oxygen minimum (<250 μmol L^{-1}) was found between 200 and 600 m in the southern part of the transect (from Sta. 3 to 20; Fig. 2). The oxygen minimum layer was most pronounced at Sta. 3 and 11 (<200 μmol L^{-1}).

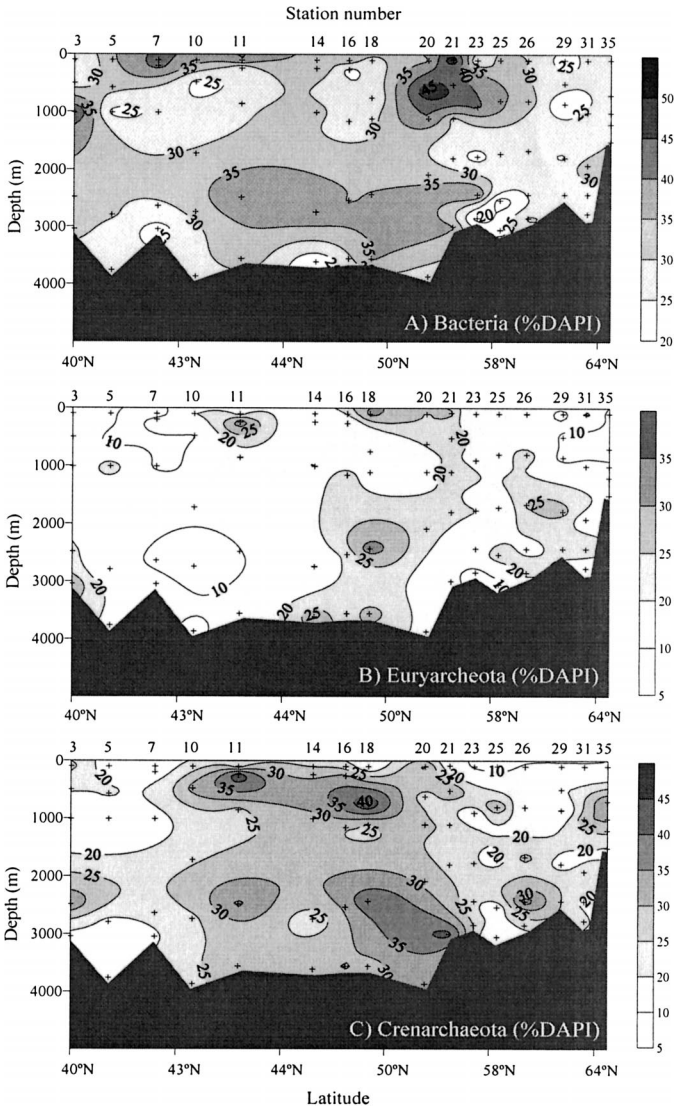

Fig. 3. (A) Contribution of Bacteria, (B) *Euryarchaeota*, and (C) *Crenarchaeota* to total prokaryotic abundance along the transect in the western basin of the North Atlantic expressed as percentage of DAPI-stained cells.

Prokaryotic community composition—Bacterial, crenarchaeotal, and euryarchaeotal abundance was determined by CARD-FISH with HRP oligonucleotide probes. For each sample, counts obtained with each of the specific probes were related to the abundance of DAPI-stainable cells, referred to as picoplankton abundance. Figure 3 shows the vertical distribution of these three main prokaryotic groups along the S–N transect.

Picoplankton abundance ranged between 1.5 and 4.4 × 10^5 cells mL^{-1} at 100-m depth, and between 0.1 and 1.3 × 10^5 cells mL^{-1} in the meso- and bathypelagic region of the western North Atlantic Ocean (*see Table 2* for mean values in the different water masses). The bacterial contribution to total picoplankton abundance ranged from 20% to 30% in the oxygen-minimum layer and the bathypelagic zone to >50% in subsurface and mesopelagic waters (Fig. 3). Generally, the vertical distribution was rather homogeneous and lacked a clear latitudinal pattern. *Crenarchaeota* dominated the picoplankton community in the NADW and the DSOW with >30% of DAPI-stainable cells. At a local oxygen minimum (Sta. 11), *Crenarchaeota* contributed >40% to the picoplankton community; however, as was true also for Bacteria, no distinct latitudinal trend was detectable for *Crenarchaeota*. *Euryarchaeota* exhibited a more patchy distribution throughout the transect. The percentage of total picoplankton identified as *Euryarchaeota* ranged from <10% to >30% in subsurface waters (Sta. 18) and the NADW (Sta. 18; Fig. 3). In general, *Euryarchaeota* contributed <15% to the total picoplankton community at the 100-m layer in the northern part of the transect (Sta. 29–35) and throughout the water column from Sta. 5 to 11. The sum of the abundance of Bacteria, *Crenarchaeota* and *Euryarchaeota* averaged 71% ± 2% (n = 70) of total picoplankton cells.

Because no clear latitudinal trend could be identified and the three main prokaryotic groups were associated with specific depths and water masses, we pooled the abundance data according to the different water masses sampled: subsurface, oxygen minimum, LSW, NADW, and DSOW (Table 2). All

Table 2. Prokaryotic abundance (as obtained by DAPI counts) and the relative contribution of Bacteria, *Crenarchaeota*, and *Euryarchaeota* to the total prokaryotic community in the different water masses. Mean ± SE is given. For water mass abbreviations, *see Table 1*.

Water mass	Abundance (10^5 cells mL^{-1})	Bacteria (% DAPI)	*Crenarchaeota* (% DAPI)	*Euryarchaeota* (% DAPI)
Subsurface (n=16)	2.5±0.2	32±2	17±2	17±2
O_2 minimum (n=7)	0.85±0.12	33±3	26±3	16±2
LSW (n=20)	0.37±0.05	30±2	22±2	17±2
NADW (n=11)	0.22±0.01	32±2	27±2	18±2
DSOW (n=11)	0.25±0.04	27±2	24±2	17±2

other parameters were also averaged for the individual water masses as described and discussed next.

D-Asp versus L-Asp uptake by the bulk prokaryotic community in different water masses—Mean L-Asp uptake rates decreased by two orders of magnitude from 4.5 pmol L^{-1} h^{-1} at 100-m depth to 0.09 pmol L^{-1} h^{-1} in the NADW, increasing again slightly in the DSOW (0.34 pmol L^{-1} h^{-1}; Fig. 4A). In contrast, mean D-Asp uptake rates decreased only from 0.4 pmol L^{-1} h^{-1} at 100-m depth to 0.08 pmol L^{-1} h^{-1} in the NADW (Fig. 4A). Similar to L-Asp uptake, D-Asp uptake rates increased again in the DSOW. The general pattern of D-Asp and L-Asp uptake rates in the different water masses by the bulk prokaryotic community was reflected by the percentage of cells capable of taking up D-Asp versus L-Asp as revealed by microautoradiography (Fig. 4B). The percentage of DAPI-stainable cells taking up L-Asp ranged from 24% at 100-m depth to 3.6% in the NADW, increasing again in the DSOW (Fig. 4B). The fraction of prokaryotic cells taking up D-Asp varied only between 5.3% and 3.8% at 100-m depth and the LSW, respectively (Fig. 4B). The D-Asp : L-Asp uptake ratio for the bulk prokaryotic community increased with depth from 0.1 at 100-m depth to 2.2 in the NADW, decreasing again to 0.7 in the DSOW (Fig. 5). The corresponding ratio of D-Asp : L-Asp active cells obtained by microautoradiography followed the same pattern as the D-Asp : L-Asp uptake ratio, increasing from 0.2 in the subsurface waters to 1.2 in the NADW and decreasing again to 0.6 in the DSOW (Fig. 5).

Because of the lower number of active cells than total prokaryotic abundance in meso- and bathypelagic waters, cell-specific uptake rates on the basis of active cell abundance (as revealed by microautoradiography) were one to two orders of magnitude higher than the specific rates estimated from total prokaryotic abundance. On average, the cell-specific D-Asp uptake rate, considering the number of active and total cells, was 0.14 and 0.004 amol $cell^{-1}$ h^{-1}, respectively. The corresponding averaged cell-specific L-Asp uptake rate of active and total cells was 0.15 and 0.012 amol $cell^{-1}$ h^{-1}, respectively. In subsurface waters (100 m), active cell–specific D-Asp uptake rates ranged from 0.008 to 0.098 amol $cell^{-1}$ h^{-1}, whereas active cell–specific L-Asp uptake rates varied from 0.023 to 0.18 amol $cell^{-1}$ h^{-1}. Active cell–specific uptake rates for D-Asp were significantly lower in subsurface (0.05 ± 0.01 amol $cell^{-1}$ h^{-1}, n = 14) than in meso- and bathypelagic waters (0.16 ± 0.02 amol $cell^{-1}$ h^{-1}, n = 49; Mann–Whitney, $p < 0.0001$). The increase in active

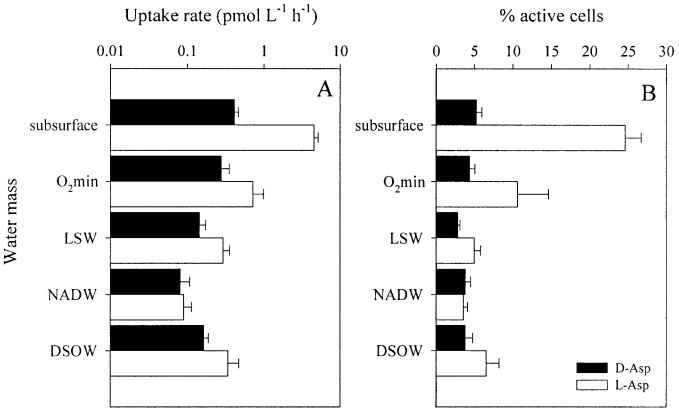

Fig. 4. (A) Uptake rates of D-Asp and L-Asp of the bulk prokaryotic community (pmol L^{-1} h^{-1}) and (B) the percentage of D-Asp and L-Asp active cells in the different water masses of the North Atlantic. Bars represent means ± SE. For water mass abbreviations, *see Table 1*.

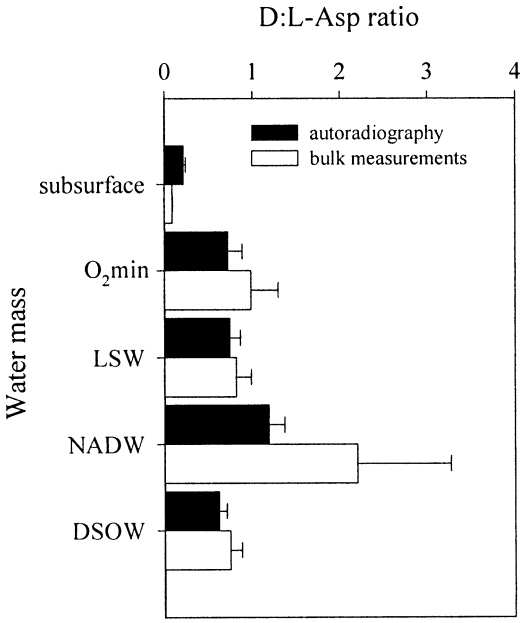

Fig. 5. Comparison of the D-Asp : L-Asp uptake ratio derived from the percentages of active cells obtained by autoradiography and by measurements of D-Asp : L-Asp uptake by the bulk prokaryotic community in the different water masses. Bars represent means ± SE. For water mass abbreviations, *see Table 1*.

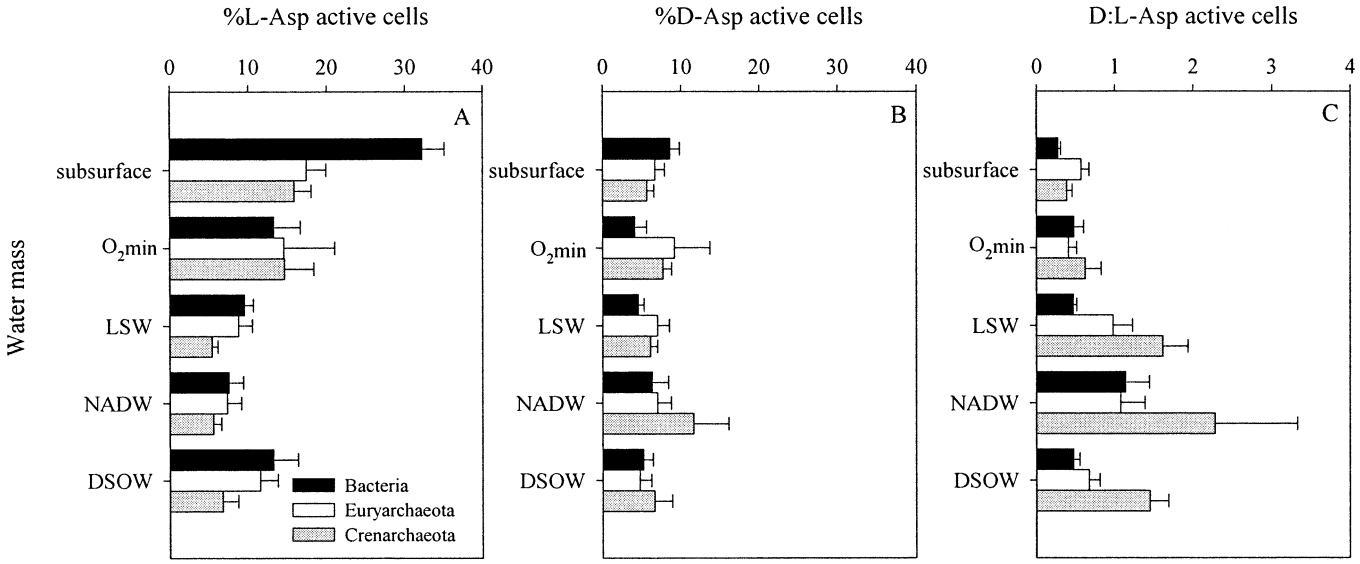

Fig. 6. Percentage of Bacteria, *Euryarchaeota,* and *Crenarchaeota* taking up (A) L-Asp and (B) D-Asp in the different water masses. (C) The ratio of D-Asp : L-Asp active cells for each prokaryotic group is also given. Bars represent means ± SE. For water mass abbreviations, *see Table 1.*

cell–specific uptake rates for L-Asp from surface (0.09 ± 0.01 amol cell^{-1} h^{-1}, $n = 15$) to deeper waters (0.16 ± 0.02 amol cell^{-1} h^{-1}, $n = 54$) was less pronounced (Mann–Whitney, $p = 0.062$) than for D-Asp.

Distribution of D*-Asp and* L*-Asp uptake among Bacteria,* Crenarchaeota, *and* Euryarchaeota *in the different water masses*—Both Archaea (*Crenarchaeota* and *Euryarchaeota*) and Bacteria were taking up D-Asp and L-Asp, although the distribution pattern of Bacteria and Archaea taking up D-Asp versus L-Asp revealed remarkable differences (Fig. 6). The percentage of Bacteria, *Crenarchaeota,* and *Euryarchaeota* taking up L-Asp decreased with depth (Fig. 6A; Table 3). The percentage of Bacteria taking up D-Asp ranged from 9% ± 1% in the subsurface layer to 4% ± 2% in the oxygen-minimum layer (Fig. 6B; Table 3). The percentage of *Euryarchaeota* taking up D-Asp was highest in the oxygen-minimum layer (9% ± 4% of *Euryarchaeota*) and lowest in the DSOW (5% ± 2% of *Euryarchaeota*). The percentage of *Crenarchaeota* taking up D-Asp, however, was highest in the NADW (12% ± 4%; Fig. 6B; Table 3).

Overall, the percentage of cells taking up D-Asp was not significantly different between Bacteria, *Crenarchaeota,* and *Euryarchaeota* (Kruskal–Wallis, $p = 0.332$, $n = 132$) and between the different water masses (Kruskal–Wallis, $p = 0.133$, $n = 132$). However, the percentage of cells utilizing L-Asp and the D-Asp : L-Asp active cells ratio was significantly different between the water masses (Kruskal–Wallis, $p < 0.0001$; $n = 157$ for L-Asp and $n = 123$ for D-Asp : L-Asp) and between the three prokaryotic groups (Kruskal–Wallis, $p < 0.003$; $n = 157$ for L-Asp and $n = 123$ for D-Asp : L-Asp). The fraction of cells actively incorporating L-Asp was significantly higher in the subsurface layer than in the LSW, NADW, and DSOW (Mann–Whitney, $p < 0.001$), although no significant differences were found between LSW, NADW, and DSOW in the percentage of L-Asp active cells. The fraction of cells actively incorporating L-Asp was significantly lower for *Crenarchaeota* than for Bacteria (Mann–Whitney, $p < 0.01$, $n = 120$), and *Crenarchaeota* showed significantly higher ratios in D-Asp : L-Asp active cells than Bacteria (Mann–Whitney, $p < 0.001$, $n = 95$), exhibiting the highest ratios in meso- and bathypelagic waters ranging from 0.7 in the oxygen-minimum layer to 2.2 in the NADW (Fig. 6C).

Table 3. Uptake rates of D-Asp and L-Asp of the bulk prokaryotic community, the contribution of D-Asp AND L-Asp active cells expressed as a percentasge of DAPI-stained cells (% DAPI), and ther percentage of Bacteria (Bact), *Crenarchaeota* (Cren), and *Euryarchaeota* (Eury) taking up D-Asp (% D-Asp) and L-Asp (% L-Asp) in the different water mases of the North Atlantic. Mean ± SE is give. For water mass abbreviations, *see Table 1* legend.

Water mass	D-Asp (pmol L^{-1} h^{-1})	L-Asp (pmol L^{-1} h^{-1})	D-Asp (% DAPI)	L-Asp (% DAPI)	Bact (% D-Asp)	Bact (% L-Asp)	Cren (% D-Asp)	Cren (% L-Asp)	Eury (% D-Asp)	Eury (% L-Asp)
Subsurface (n=16)	0.41±0.06	4.6±0.6	5.3±0.7	24±2	8.6±1.3	32±3	5.6±0.9	16±2	6.7±1.2	17±2
O_2 minimum (n=7)	0.28±0.07	0.7±0.3	4.4±0.7	11±4	4.1±1.5	13±3	7.7±1.1	14±4	9±4	13±6
LSW (n=20)	0.14±0.03	0.29±0.06	2.9±0.3	5.0±0.8	4.6±0.7	9.5±1.1	6.1±0.9	5.4±0.7	6.9±1.6	8.8±1.8
NADW (n=11)	0.08±0.03	0.09±0.02	3.8±0.7	3.6±0.5	6±2	8.3±1.8	12±4	5.6±1.0	6.9±1.8	7.3±1.8
DSOW (n=11)	0.16±0.02	0.34±0.13	3.8±0.9	6.6±1.7	5.2±1.2	13±3	7±2	6.8±1.9	4.8±1.5	12±2

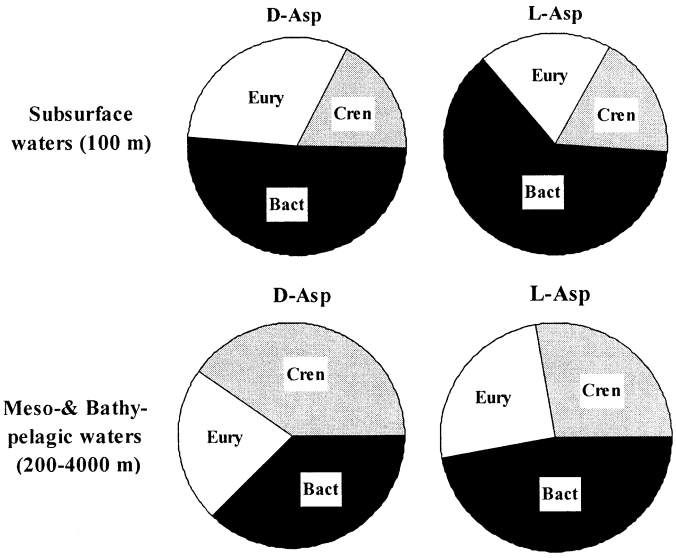

Fig. 7. Contribution of Bacteria (Bact), *Euryarchaeota* (Eury), and *Crenarchaeota* (Cren) to D-Asp and L-Asp uptake in subsurface and meso- and bathypelagic waters of the North Atlantic expressed as percentage of total active cells as determined by microautoradiography. For further explanations, *see text.*

The percentage of Bacteria taking up L-Asp was significantly higher than taking up D-Asp in all the water masses (Mann–Whitney, $p < 0.05$; $n = 29$ for subsurface, $n = 12$ for the oxygen minimum, $n = 23$ for LSW, and $n = 22$ for DSOW) except in the NADW (Mann–Whitney, $p = 0.540$, $n = 19$; Fig. 6; Table 3). The percentage of *Crenarchaeota* taking up L-Asp was not significantly different from that taking up D-Asp (Mann–Whitney, $p > 0.25$) except in subsurface waters, where *Crenarchaeota* taking up L-Asp prevailed over those taking up D-Asp (Mann–Whitney, $p < 0.01$) (Fig. 6; Table 3). A significantly higher percentage of *Euryarchaeota* took up L-Asp in the subsurface and in the DSOW (Mann–Whitney, $p < 0.05$), whereas no significant difference was found in the oxygen minimum, LSW, and NADW (Mann–Whitney, $p > 0.15$).

The contribution of the different prokaryotic groups to D-Asp and L-Asp utilization can be calculated. We first calculated for each prokaryotic group and for D-Asp and L-Asp the number of active cells as a percentage of DAPI-stained cells (i.e., the fraction of DAPI-stained cells that were simultaneously probe- and autoradiography-positive). Then, we normalized the contribution of each group to the number of total active cells for D-Asp and L-Asp separately. The mean contribution of Bacteria, *Crenarchaeota*, and *Euryarchaeota* to D-Asp and L-Asp utilization showed contrasting patterns in subsurface versus meso- and bathypelagic waters (Fig. 7). Bacteria and Archaea (*Crenarchaeota* plus *Euryarchaeota*) equally contributed to D-Asp uptake in the subsurface layer and to L-Asp uptake in the deep waters of the North Atlantic Ocean. In contrast, the archaeal contribution to D-Asp uptake was significantly higher than the corresponding bacterial contribution in meso- and bathypelagic waters (Mann–Whitney, $p < 0.0001$, $n = 76$). The bacterial contribution to L-Asp uptake was significantly higher than the archaeal contribution in the subsurface layer (Mann–Whitney, $p = 0.004$, $n = 28$; Fig. 7).

Discussion

Prokaryotic community composition in the meso- and bathypelagic waters of the North Atlantic—Picoplankton abundance (DAPI-stainable cells) sharply declined from subsurface to mesopelagic waters. Below 1,000-m depth, picoplankton abundance varied over the entire transect by less than twofold. A similar prokaryotic abundance was reported for deep-water picoplankton for the northeast Atlantic (Dufour and Torréton 1996; Patching and Eardly 1997), the Pacific (Nagata et al. 2000), and the northwest Mediterranean Sea (Tanaka and Rassoulzadegan 2004). The numbers of DAPI-stained cells before and after MICRO-CARD-FISH processing were not significantly different (Teira et al. 2004), indicating that the fraction of cells potentially lost during processing was negligible. Application of the recently refined CARD-FISH method and oligonucleotide probes (Teira et al. 2004) allowed us to recover >70% of the DAPI-stained cells. This recovery efficiency is only slightly lower than that obtained with polynucleotide probes (Karner et al. 2001; Church et al. 2003). Previous attempts with oligonucleotide probes in oligotrophic surface waters resulted in an estimated archaeal contribution to the total picoplankton community of ~5% (*see review* by Bouvier and Del Giorgio 2003). Amplification of the fluorescence signal mediated by the HRP and improved permeabilization procedure for Archaea with the use of protease-K instead of lysozyme now offers the opportunity to enumerate specific deep-water prokaryotes because the phylogenetic resolution of oligonucleotide probes is potentially higher than that of polynucleotide probes (Pernthaler et al. 2002).

Our results confirm the generally described pattern of the distribution of *Crenarchaeota* and *Euryarchaeota* with depth. *Crenarchaeota* appeared to be relatively more abundant in meso- and bathypelagic than in subsurface waters (Table 2; Fig. 3). Similar percentages for *Crenarchaeota* were reported by Karner et al. (2001) in the Pacific (~10–39% of DAPI counts), who found a sharp increase in crenarchaeotal abundance between 100- and 150-m depth. Church et al. (2003) and Herndl et al. (2005) report only a moderate increase of *Crenarchaeota* with depth in the Circumpolar Deep Water, and generally, the abundance of this archaeal group is lower in these waters than in the Pacific and in the North Atlantic. We observed a steady increase in the contribution of *Crenarchaeota* to the total picoplankton abundance from the subsurface layers down to ~800-m depth. A crenarchaeotal contribution of >30% of DAPI-stained cells were found from Sta. 11 to 18 (Fig. 3). This increased crenarchaeotal contribution coincided mainly with the presence of low-salinity and low-oxygen (<250 μmol L^{-1}) water masses. Below 900-m depth, a slight decrease in crenarchaeotal abundance was observed, with *Crenarchaeota* contributing between 10% and <30% to the total picoplankton (Fig. 3). The contribution of *Crenarchaeota* increased again in the NADW, characterized by higher salinity and higher oxygen concentrations than in the overlying water masses (Table 1).

The most striking finding was the relatively high euryarchaeotal abundance in these NADW studies compared with previous FISH studies. *Euryarchaeota* were found to contribute only <5–8% to the picoplankton abundance in deep waters of the Pacific and in the Antarctic Circumpolar waters (DeLong et al. 1999; Karner et al. 2001; Church et al. 2003). By contrast, PCR-dependent studies retrieved numerous *Euryarchaeota* sequences from clone libraries in meso- and bathypelagic waters near the Antarctic Polar front (López-García et al. 2001) and in the central Arctic Ocean (Bano et al. 2004). In this study, the contribution of *Euryarchaeota* to total picoplankton averaged 17%. Although higher contributions were frequently found in subsurface waters (~100-m depth), values as high as 35% were also reached in bathypelagic waters (Sta. 18; Fig. 3). The higher contribution of *Euryarchaeota* to the total prokaryotic community reported here than in previous studies might be a result of the improved permeabilization procedure of the archaeal cell wall we applied (*see* Teira et al. 2004). However, it might also reflect to some extent differences in the biogeographic distribution of *Euryarchaeota* in the deep waters of the world's oceans. Several studies point to differences in the biogeographical distribution of Archaea (López-García et al. 2001; Bano et al. 2004). Clearly, more studies on the spatial and temporal dynamics of the main prokaryotic groups and their major representatives are needed before firm conclusions on the biogeographical distribution of Archaea can be drawn.

D-Asp and L-Asp uptake by prokaryotes in the meso- and bathypelagic waters of the North Atlantic—Although there is a bulk of literature on bacterioplankton utilization of L–amino acids and their role as carbon and nitrogen source for bacterial communities, D–amino acid utilization has barely been studied. It has been suggested that the D-/L–amino acid ratio in the DOM pool might be used as an indicator of the diagenetic state of the oceanic DOM (Amon et al. 2001; Nagata et al. 2003). Following this argument, one could expect an increase in the D-/L–amino acid ratio with depth in the oceanic water column as the DOM becomes increasingly refractory. A significant increase in the D-/L–amino acid ratios with depth, however, has not been found in the North Atlantic (Pérez et al. 2003) and the Arctic Ocean (Dittmar et al. 2001). The rather constant D-/L–amino acid ratios with depth have been interpreted as a consequence of a relative increase in the utilization rates of D–amino acids in the ocean's interior (Pérez et al. 2003). Indeed, Pérez et al. (2003) showed that in surface waters, the prokaryotic community takes up D-Asp at rates one to two orders of magnitude lower than L-Asp. In their study, conducted in the Faroe–Shetland Channel of the North Atlantic, the D-Asp : L-Asp uptake ratio increased with depth, reaching a D-Asp : L-Asp uptake ratio of ~1 at 1,000-m depth (Pérez et al. 2003).

Our measurements on the uptake ratio of D-Asp : L-Asp of the bulk prokaryotic community confirm the previous finding that the D-Asp : L-Asp uptake ratio increases with depth (Figs. 4A, 5). The D-Asp : L-Asp uptake ratio derived from the percentages of total active cells obtained by autoradiography also showed the same increasing trend with depth (Figs. 4B, 5). This increase in the D-Asp : L-Asp uptake ratio is mainly caused by the decrease in L-Asp uptake with depth (Figs. 4, 5). No significant differences were found in the active cell–specific D-Asp and L-Asp uptake rates among the different meso- and bathypelagic water masses, whereas D-Asp uptake rates were significantly lower than L-Asp uptake rates in subsurface waters (Table 3). In addition, the active cell–specific D-Asp uptake rate is significantly higher in deep than in subsurface waters by about three times, whereas active cell–specific L-Asp uptake rates increased only by a factor of ~1.8. This depth-related uptake pattern of D-Asp and L-Asp might be interpreted in different ways. It is likely that heterotrophic prokaryotes are preferentially using L–amino acids over D-enantiomeric amino acid species because the demand of L–amino acids for prokaryotic cells is much higher than that of D–amino acids. There is considerable evidence that phytoplankton extracellular release and grazing activity in surface waters contributes to the supply of L–amino acids (Fuhrman 1987) and that heterotrophic prokaryotic plankton are covering a substantial part of their carbon and energy demand via the uptake of L–amino acids in the euphotic layer (Suttle et al. 1991). This supply of readily available L–amino acids is probably considerably lower in meso- and bathypelagic waters. Thus, it might well be that deep-water prokaryotic plankton are less discriminating in their amino acid uptake than surface-water prokaryotes. An alternative explanation might be that meso- and bathypelagic prokaryotes are able to use D-Asp more efficiently than their subsurface counterparts, and this capability could be related to changes in the prokaryotic community composition.

Bacterial and archaeal contribution to D-Asp and L-Asp utilization in meso- and bathypelagic NADW—Pérez et al. (2003) hypothesized that the increase in the D-Asp : L-Asp uptake ratio with depth is related to the presence of specific prokaryotic communities in the mesopelagic realm capable of efficiently using D–amino acids. From their study, it remained unclear, however, which component of the prokaryotic community was actually responsible for the shift in the D-Asp : L-Asp uptake ratio between surface and the lower mesopelagic waters (Pérez et al. 2003).

In a previous study, it was shown that marine Archaea are actively taking up L–amino acids (Ouverney and Fuhrman 2000). To our knowledge, our work represents the first attempt to investigate the uptake potential of D–amino acids by Archaea. Although D–amino acids are important constituents of the peptidoglycan layer in the cell wall of Bacteria, murein has not been found in archaeal cell walls or envelopes (Kandler 1994; Kandler and Konig 1998). Therefore, it has been long believed that Archaea are neither taking up D–amino acids nor do they express amino acid racemases. However, some recent evidence suggests that both are widely distributed among Archaea (Yohda et al. 1996; Nagata et al. 1998, 1999). A high proportion of free D-Asp (>40% of total free Asp) and aspartate racemase activity have been detected in archaeal extracts (Matsumoto et al. 1999; Long et al. 2001). Our results also indicate that the uncultivable, nonextremophilic *Crenarchaeota* and *Euryarchaeota* are actively taking up D-Asp. That Archaea, which lack a peptidoglycan layer, are actively taking up D-Asp challenges the commonly

accepted view that the bacterial cell wall is the main biotic source of D–amino acids in the ocean.

With the use of MICRO-CARD-FISH, we showed that both Bacteria and Archaea were actively taking up D-Asp and L-Asp, although different uptake patterns related to depth and waters masses were obvious for the different groups. The most remarkable difference is the marked decrease in the number of Bacteria using L-Asp from the subsurface waters to the meso- and bathypelagic layers (Fig. 6; Table 3). The proportion of cells using L-Asp in deep waters was significantly lower for *Crenarchaeota* than for Bacteria. The ratio of D-Asp : L-Asp active Bacteria was <0.5 throughout the water column, with the exception of the NADW only (Fig. 6), whereas the corresponding ratio for both groups of Archaea was always >0.5 below the 100-m depth, with the exception of *Euryarchaeota* in the oxygen-minimum layer. Therefore, the change in the ratio D-Asp : L-Asp active cells appears to be determined not simply by changes in the percentage of L-Asp total active cells. Significant differences were found for D-Asp or L-Asp uptake between the three prokaryotic groups. In subsurface waters, all three prokaryotic groups exhibit a higher percentage of L-Asp– than D-Asp–positive cells (Fig. 6; Table 3). In deeper waters, only Bacteria exhibited a higher percentage of L-Asp– than D-Asp–positive cells (Fig. 6; Table 3). Thus, in the deep ocean, Archaea seem to use D–amino acids more efficiently than Bacteria. Overall, 62% of D-Asp uptake in meso- and bathypelagic waters is mediated by Archaea, whereas Bacteria and Archaea equally contribute to L-Asp utilization (Fig. 7).

Our findings suggest that the shift in the D-Asp : L-Asp uptake ratio from surface to deep waters is largely due to Archaea domination in terms of total abundance and abundance of cells taking up D-Asp in the deep North Atlantic. The efficient uptake of D-Asp, especially by the *Crenarchaeota*, in the deep waters suggests a possible previously unrecognized sink of D–amino acids in the ocean's interior.

References

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

———. 2000*b*. The hydrography of the mid-latitude Northeast Atlantic ocean: II, the intermediate water masses. Deep-Sea Res. I **47:** 789–824.

AMANN, R. I., W. LUDWIG, AND K. H. SCHLEIFER. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. **59:** 143–169.

AMON, R. M. W., H.-P. FITZNAR, AND R. BENNER. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine organic matter. Limnol. Oceanogr. **46:** 287–297.

BANO, N., S. RUFFIN, B. RANSOM, AND J. T. HOLLIBAUGH. 2004. Phylogenetic composition of Arctic Ocean archaeal assemblages and comparison with Antarctic assemblages. Appl. Environ. Microbiol. **70:** 781–789.

BOUVIER, T., AND P. A. DEL GIORGIO. 2003. Factors influencing the detection of bacterial cells using fluorescence in situ hybridization (FISH): A quantitative review of published reports. FEMS Microbiol. Ecol. **44:** 3–15.

BROECKER, W. S. 1997. Thermohaline circulation, the Achilles heel of our climate system: Will man-made CO_2 upset the current balance? Science **278:** 1582–1588.

CHURCH, M. J., E. F. DELONG, H. W. DUCKLOW, M. B. KARNER, C. M. PRESTON, AND D. M. KARL. 2003. Abundance and distribution of planktonic *Archaea* and *Bacteria* in the waters west of the Antarctic Peninsula. Limnol. Oceanogr. **48:** 1893–1902.

COTTRELL, M. T., AND D. L. KIRCHMAN. 2000. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl. Environ. Microbiol. **66:** 5116–5122.

———, AND ———. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnol. Oceanogr. **48:** 168–178.

CRUMP, B. C., AND J. A. BAROSS. 2000. Archaeaplankton in the Columbia River, its estuary and the adjacent coastal ocean, USA. FEMS Microbiol. Ecol. **31:** 231–239.

DELONG, E. F., L. T. TAYLOR, T. L. MARSH, AND C. M. PRESTON. 1999. Visualization and enumeration of marine planktonic Archaea and Bacteria by using polyribonucleotide probes and fluorescent in situ hybridization. Appl. Environ. Microbiol. **65:** 5554–5563.

———, K. Y. WU, B. B. PRÉZELIN, AND R. V. M. JOVINE. 1994. High abundance of Archaea in Antarctic marine picoplankton. Nature **371:** 695–697.

DITTMAR, T., H. P. FITZNAR, AND G. KATTNER. 2001. Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D– and L–amino acids. Geochim. Cosmochim. Acta **65:** 4103–4114.

DOBROLYUBOV, S. A., S. S. LAPPO, E. G. MOROZOV, AND A. V. SOKOV. 2003. Water transport through the Charlie–Gibbs fracture zone. Dokl.-Earth Sci. Sect. **391A:** 903–905.

DUFOUR, P. H., AND J.-P. TORRÉTON. 1996. Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical northeastern Atlantic Ocean. Deep-Sea Res. I **43:** 1305–1320.

FUHRMAN, J. 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.

GIOVANNONI, S., AND M. RAPPÉ. 2000. Evolution, diversity, and molecular ecology of marine prokaryotes, p. 47–84. *In* D. L. Kirchman [ed.], Microbial ecology of the oceans. Wiley-Liss.

HANSELL, D. A., AND C. A. CARLSON. 1998. Deep-ocean gradients of dissolved organic carbon. Nature **395:** 443–453.

———, AND H. W. DUCKLOW. 2003. Bacterioplankton distribution and production in the bathypelagic ocean: Directly coupled to particulate organic carbon export? Limnol. Oceanogr. **48:** 150–156.

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.

KANDLER, O. 1994. Cell wall biochemistry in Archaea and its physical implications. J. Biol. Phys. **20:** 165–169.

———, AND H. KONIG. 1998. Cell walls polymers in Archaea (Archaeabacteria). Cell Mol. Life Sci. **54:** 305–308.

KARNER, M. B., E. F. DELONG, AND D. M. KARL. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature **409:** 507–510.

LONG, Z., J. LEE, T. OKAMOTO, AND OTHERS. 2001. Occurrence of D–amino acids and a pyridoxal 5′-phosphate–dependent aspartate racemase in the acidothermophilic archaeon, *Thermoplasma acidophilum*. Biochem. Biophys. Res. Commun. **281:** 317–321.

LÓPEZ-GARCÍA, P., A. LÓPEZ-LÓPEZ, D. MOREIRA, AND F. RODRÍGUEZ-VALERA. 2001. Diversity of free-living prokaryotes

from a deep-sea site at the Antarctic Polar Front. FEMS Microbiol. Ecol. **36:** 193–202.

MASSANA, R., L. T. TAYLOR, A. E. MURRAY, K. Y. WU, W. H. JEFFREY, AND E. F. DELONG. 1998. Vertical distribution and temporal variation of marine planktonic archaea in the Gerlache Strait, Anctartica, during early spring. Limnol. Oceanogr. **43:** 607–617.

MATSUMOTO M., H. HOMMA, Z. LONG, AND OTHERS. 1999. Occurrence of free amino acids and aspartate racemases in hyperthermophilic archaea. J. Bacteriol. **181:** 6560–6563.

MOESENEDER, M. M., C. WINTER, AND G. J. HERNDL. 2001. Horizontal and vertical complexity of attached and free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints. Limnol. Oceanogr. **46:** 95–107.

NAGATA, T., H. FUKUDA, R. FUKUDA, AND I. KOIKE. 2000. Bacterioplankton distribution and production in deep Pacific waters: Large-scale geographic variations and possible coupling with sinking particle fluxes. Limnol. Oceanogr. **45:** 426–435.

———, B. MEON, AND D. L. KIRCHMAN. 2003. Microbial degradation of peptidoglycan in seawater. Limnol. Oceanogr. **48:** 745–754.

NAGATA, Y., T. FUJIWARA, K. KAWAGUCHI-NAGATA, Y. FUKUMORI, AND T. YAMANAKA. 1998. Occurrence of peptidyl D–amino acids in soluble fractions of several eubacteria, archaea and eukaryotes. Biochim. Biophys. Acta **1379:** 76–82.

———, K. TANAKA, T. IIDA, AND OTHERS. 1999. Occurrence of D–amino acids in a few archaea and dehydrogenase activities in hyperthermophile *Pyrobaculum islandicum.* Biochim. Biophys. Acta **1435:** 160–166.

OUVERNEY, C. C., AND J. A. FUHRMAN. 2000. Marine planktonic Archaea take up amino acids. Appl. Environ. Microbiol. **66:** 4829–4833.

PATCHING, J. W., AND D. EARDLY. 1997. Bacterial biomass and activity in the deep waters of the eastern Atlantic—evidence of a barophilic community. Deep-Sea Res. I **44:** 1655–1670.

PÉREZ, M. T., C. PAUSZ, AND G. J. HERNDL. 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., C. M. PRESTON, J. PERNTHALER, E. F. DELONG, AND R. AMANN. 2002. Comparison of fluorescently labeled oligonucleotide and polynucleotide probes for the detection of pelagic marine Bacteria and Archaea. Appl. Environ. Microbiol. **68:**661–667.

SCHLEIFER, K. H., AND O. KANDLER. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. **36:** 407–477.

SUTTLE, C. A., A. M. CHAN, AND J. A. FUHRMAN. 1991. Dissolved free amino acids in the Sargasso Sea: Uptake and respiration rates, turnover times, and concentration. Mar. Ecol. Prog. Ser. **70:** 189–199.

TANAKA, T., AND F. RASSOULZADEGAN. 2004. Vertical and seasonal variations of bacterial abundance and production in the mesopelagic layer of the NW Mediterranean Sea: Bottom-up and top-down controls. Deep-Sea Res. I **51:** 531–544.

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.

WILLIAMS, P. J. LEB. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material, p. 153–200. *In* D. L. Kirchman [ed.], Microbial ecology of the oceans. Wiley-Liss.

YOHDA, M., I. ENDO, Y. ABE, T. OHTA, T. IIDA, T. MARUYAMA, AND Y. KAGAWA. 1996. Gene for aspartate racemase from the sulfur-dependent hyperthermophilic archaeum, *Desulfurococcus* strain SY. J. Biol. Chem. **271:** 22,017–22,021.

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