

Evidence for circumpolar distribution of planktonic Archaea in the Southern Ocean

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ABSTRACT: Surveys using rRNA-targeted probes specific for the 3 domains of life (*Eucarya*, *Archaea*, and *Bacteria*) indicated the presence, and at times high abundance, of archaeal rRNA in a variety of water masses surrounding Antarctica. Hybridization signals of archaeal rRNA contributed significantly to that of total picoplankton rRNA both north and south of the Polar Front in Drake Passage. Late winter surface water populations collected around the South Shetland Islands also yielded relatively high archaeal rRNA hybridization signals, approaching 10% or greater of the total rRNA. Summer samples collected in the western region of the Antarctic Peninsula and at McMurdo Sound in the Ross Sea region of Antarctica yielded lower amounts of archaeal rRNA in the surface waters, and higher levels of archaeal rRNA at depth (150 to 500 m). The hybridization data were compared to biological, chemical, and hydrographic information when possible. In surface waters, archaeal rRNA and chlorophyll *a* varied inversely. The data presented here further supports the hypothesis that planktonic archaea are a common, widespread and likely ecologically important component of Antarctic picoplankton assemblages. The presence of these archaea in circumpolar deep water suggests a conduit for their circumpolar transport around Antarctica via the Antarctic Circumpolar Current, as well as their export to the deep sea, or to intermediate waters of the South Atlantic via mixing at the polar front.

KEY WORDS: Planktonic archaea · SSU rRNA hybridization · Antarctica

INTRODUCTION

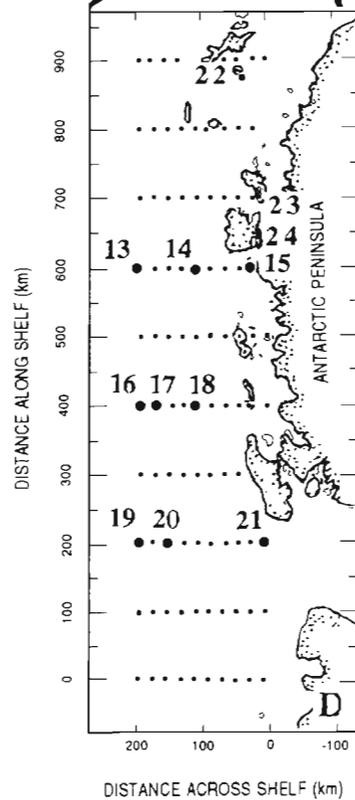
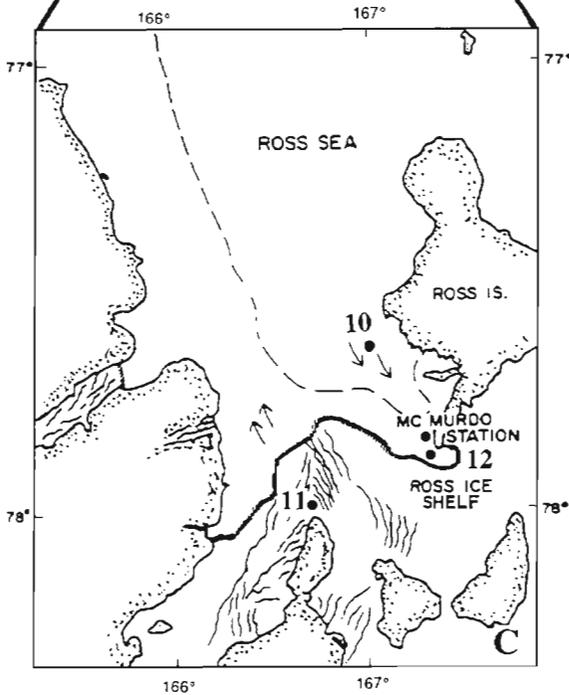
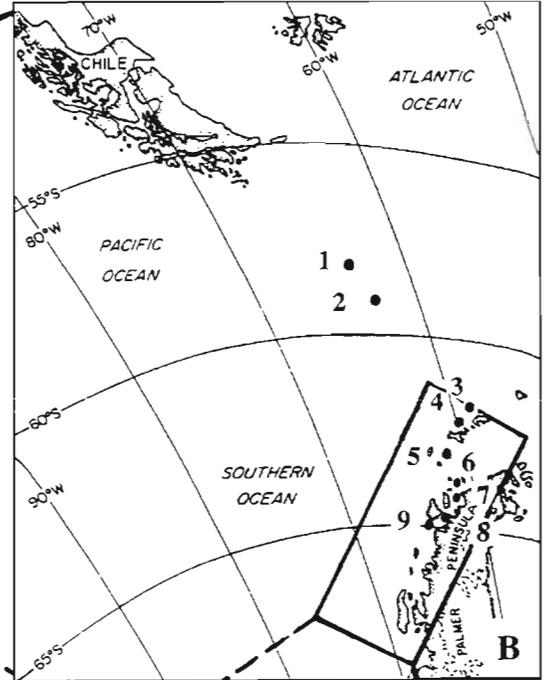
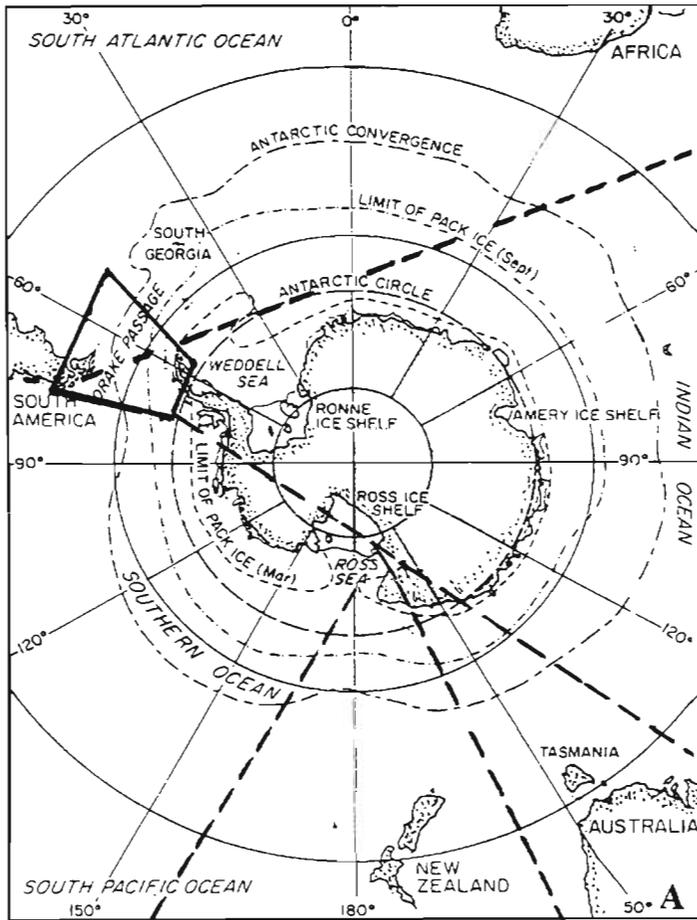
The use of oligonucleotide probes targeted to the SSU rRNA of the 3 domains of life (Woese 1990) has facilitated the exploration of microbial diversity in a variety of environments (for example see Stahl et al. 1988, Giovannoni et al. 1990, DeLong 1992, Raskin et al. 1994, Preston et al. 1996, Fry et al. 1997, Lin et al. 1997, Massana et al. 1997). Furthermore, this approach lends itself to quantification when normalized to a universal probe (Raskin et al. 1994, Zheng et al. 1996) and evaluation of the relative contributions of each domain to the system of interest. This approach along with sequencing of rRNA genes has opened the field of

picoplankton ecology to include the detection of uncultivated prokaryotes, which has been limited to bulk measurements or cultivation based approaches.

Molecular investigations of picoplankton nucleic acid extracts have indicated that the planktonic archaea are indigenous members of temperate coastal oceans (DeLong 1992, Massana et al. 1997) and of deep sea waters (Fuhrman et al. 1992, Fuhrman & Davis 1997). Recently archaeal nucleic acids were detected in high abundance in the nearshore surface waters off Anvers Island, Antarctica (DeLong et al. 1994). Subsequent studies have extended the ecological analyses of the *Archaea* in the Anvers Island region (Murray et al. 1998), and described the distribution of the planktonic archaea in the Gerlache Strait, west of the Antarctic Peninsula (Massana et al. 1998).

The presence of planktonic archaea in both temperate and Antarctic waters suggests a potential for global

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distribution in marine plankton and a biological capability for existing in -1.5°C polar waters. The majority of the planktonic archaeal rRNA sequences form a closely related monophyletic clade in the Crenarchaeota division of the archaea referred to as marine Group I. Recently, other phylogenetic relatives of the marine Group I archaea have been detected in a variety of environments such as in deep sea holothurians (McInerney, et al. 1995), lake sediments (Hershberger, et al. 1996, MacGregor et al. 1997, Schleper et al. 1997), deep sea vents (Moyer et al. in press) and terrestrial soils (Ueda et al. 1995, Bintrim et al. 1997). The other group of planktonic archaea which appear to be less abundant in the plankton, the marine Group II, are affiliated with the euryarchaeota, and have a higher degree of phylogenetic diversity among sequenced representatives. Recent studies have reported sequences phylogenetically related to the marine Group II from marsh sediments (Munson et al. 1997) and from a hydrothermal vent microbial mat system (Moyer et al. in press). However these 'vent' samples and the Group I vent sequences referred to above were convectively mixed with low temperature water (seawater sampled was 15 to 30°C), and contained bacterial types closely related to known psychrophiles, so the source of Group I and Group II archaea detected in these microbial samples might be initially from the surrounding seawater habitat. The physiological lifestyles of the planktonic archaea remain unknown, although the widespread distribution of mesophilic and psychrophilic archaea implies the potential for a variety of metabolic and ecological roles.

The Antarctic marine environment provides a unique study site since the Antarctic Circumpolar Current (ACC) has isolated the continent and surrounding waters, and is contiguous around the continental margins of Antarctica. Local flora and fauna display high endemism, resulting from this isolation (Dayton 1990). This environment is suitable as a testing ground for examining the habitat range and distribution of the planktonic archaea. The physical oceanography of the Southern Ocean has several well-defined water masses, fronts, and frontal zones (for detailed descriptions see Sievers & Nowlin 1984, Hofmann et al. 1996) with substantial mesoscale structure and flow (Hofmann et al. 1996) as well as intense advective features

(Huntley & Niiler 1995). As a consequence, the distribution of Antarctic plankton has been reported to be strongly influenced by hydrographic properties (Vincent 1988, Stein & Heywood 1994, Huntley & Niiler 1995).

To investigate the geographic distribution of the planktonic archaea we surveyed the relative abundance of *Archaea* in picoplankton samples collected from Drake Passage, the Antarctic Peninsula, and McMurdo Sound using SSU rRNA oligonucleotide probes. We postulate that if the *Archaea* are present in Antarctic surface water and Circumpolar Deep Water, dispersal will be facilitated by the ACC leading to large scale circumpolar distribution.

METHODS

Picoplankton collection and hydrographic data acquisition. Sample collections were undertaken in 4 different Antarctic field seasons and locations. From September 15–19, 1995 surface seawater was sampled at 7 stns in the Antarctic Peninsula region between King George and Anvers Island (Fig. 1B). Samples were collected by bucket off the aft deck of the RV 'Polar Duke' (20 to 25 l per sample). Seawater samples were processed on board using a method previously described (Murray et al. 1998) in which seawater was filtered through an in-line $1.0\ \mu\text{m}$ glass fiber filter (Micron Separations Inc.) then through $0.22\ \mu\text{m}$ filter units (Sterivex, Millipore). Lysis buffer (1.8 ml, 40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) was immediately added to each filter unit. The units were sealed and stored at -70°C until subsequently processed for nucleic acid contents. Bacterioplankton abundance was determined by DAPI staining and epifluorescence microscopy (Porter & Feig 1980). Chlorophyll *a* concentrations (Parsons et al. 1984) were determined for each seawater sample as previously described (Murray et al. 1998). Thermosalinograph data were recorded continuously throughout the cruise track; sea surface temperature and salinity were also recorded at each station and sample depth.

Picoplankton samples were collected from hydrocasts at 3 stations on each of the 200, 400, and 600 LTER grid lines (total of 9 stations), in Discovery Bay at Deception

Fig. 1. (A) Map of the Antarctica, Southern Ocean, neighboring oceans, seasonal pack ice extent and location of the Antarctic convergence. (B) Drake Passage sampling sites, Stn 1 ($58^{\circ} 09.76' \text{ S}$, $63^{\circ} 38.36' \text{ W}$) north of the polar front and Stn 2 ($59^{\circ} 16.05' \text{ S}$, $63^{\circ} 12.88' \text{ W}$) south of the polar front. Stns 3 to 9 sampled west of the Antarctic Peninsula (Ant. Pen.) near the South Shetland Islands. (C) McMurdo Sound sample locations, Stn 10 at the ice edge, Stn 11 at the Bratina Island Ice Crack, and Stn 12, 3 miles west of McMurdo Station. The arrows indicate surface current direction and the dashed line indicates the ice edge in January; map modified from Hodson et al. (1981). (D) Location of LTER grid lines, and locations sampled west of the Antarctic Peninsula. Grid lines 200, 400, and 600 (Stns 13 to 21) are designated by distance along and across the shelf. Deception Island (Stn 22) and Palmer sampling stations PALB and PALE (Stns 23 and 24) located in the nearshore region of Anvers Island were also sampled

Island and at Palmer sampling stations PALB and PALE between December 25, 1994 and February 5, 1995 (Fig. 1d). Typically 3 depths were sampled on each hydrocast: surface, midwater (35 to 120 m) at the base of the mixed layer or at the temperature minimum, and 500 m when the bottom depth exceeded 500 m (Stn 400.120 was sampled at 330 m, Deception Island at 150 m, PALB at 60 m, and PALE at 145 m). These samples (4 to 36 l per sample) were processed by filtration through a 202 μm mesh nitex screen followed by vacuum filtration onto 0.2 μm Supor-200 filters (47 mm dia., Gelman Sciences). Filters were folded in half and stored frozen until transport to Santa Barbara, CA, USA, on dry ice for nucleic acid extraction and hybridization experiments. CTD, chlorophyll, nutrient, and bacterial production data were available for these stations from the Palmer LTER data archive on the WWW site (<http://www.ices.ucsb.edu/lter>).

Samples from McMurdo Sound (25 and 250 m) were collected 3 miles west of McMurdo Station, at the Bratina Island ice crack (25 and 250 m) and at the ice edge (25 m) 20 miles north of McMurdo Stn by niskin bottle cast in December 1995 (Fig. 1C). In all cases, between 5 and 30 l of seawater was filtered directly onto Sterivex filters using a peristaltic pump. These samples were frozen without the addition of lysis buffer and transported on dry ice to Santa Barbara for nucleic acid extraction.

Hydrocasts in Drake Passage were conducted at stations on either side of the Polar Front in transit to Palmer Stn on July 29, 1996 on the RV 'Polar Duke' (see Fig. 1B for station locations). We identified the position of the ship in relation to the polar front by monitoring thermosalinograph data, and examining data from a crossing of Drake Passage a week earlier. Twenty to 30 l of seawater was collected in Go-Flo bottles (10 l capacity, General Oceanics) at depths of 5, 50, 150, 350, and 500 m at the North Stn, and at depths of 5, 50, 100, 250, and 500 m at the South Stn. Samples were processed by filtration through a GF/A glass fiber filter (Whatman) then through a Sterivex filter using a peristaltic pump. Lysis buffer was added to each filter unit, the samples were sealed and frozen at -70°C until nucleic acid extraction. Hydrographic measurements (depth, temperature, salinity, oxygen saturation, *in situ* fluorescence) were recorded with both casts (Seabird SBE-19 profiler).

Nucleic acid extraction. Nucleic acid extraction has been previously described (Massana et al. 1997, Murray et al. 1998). In brief, lysozyme (1 mg ml^{-1}) was added to the Sterivex filter units containing 1.8 ml lysis buffer, or to 47 mm filters which were broken up when frozen in 15 ml conical tubes containing 5 ml lysis buffer. Samples were incubated at 37°C for 30 min then 1% SDS and freshly prepared proteinase K

(0.5 mg ml^{-1}) were added and incubated at 55°C for 2 h. Crude lysates were extracted with phenol:chloroform:isoamylalcohol (25:24:1; pH 8.0), then with chloroform:isoamylalcohol (24:1). The aqueous phase containing nucleic acids was concentrated and washed with 2 ml TE (pH 8.0) in microconcentrators (Centricon 100, Amicon), resulting in a final volume of 100 to 200 μl . DNA concentrations were quantified for all extracts with the Hoechst dye assay (modified from method of Paul & Myers 1982) on a fluorometer (TKO, Hoeffer Scientific).

rRNA oligonucleotide hybridization. The method recently described by Massana et al. (1997) was used to characterize the relative amounts of eucaryal, bacterial and archaeal rRNA in Antarctic picoplankton assemblages. Nucleic acid samples and rRNA controls were applied to nylon membranes (Hybond-N, Amersham) using a slot blotting apparatus. Each blot had 3 to 4 samples in a 6- to 8-fold dilution series starting with 50 to 200 ng DNA in the initial dilution and rRNA controls for each domain in 4-fold dilution series. Ribosomal RNAs from *Saccharomyces cerevisiae*, *Escherichia coli* or *Shewanella putrefaciens*, *Haloferax volcanii*, and *Cenarchaeum symbiosum*, and rDNAs from an archaeal GII plasmid, OAR22 were used as controls for all experiments. Nucleic acids and rRNAs were denatured in 0.5% glutaraldehyde/50 mM Na_2PO_4 ; the plasmid DNA was denatured by boiling for 10 min. Four to 5 replicate blots were prepared for each set of samples. Nucleic acids were UV-crosslinked to the membranes ($120000\text{ }\mu\text{joules cm}^{-2}$; Stratalinker, Strategene). Membranes prehybridized for 30 min at 45°C in hybridization buffer (5 to 10 ml; 0.9 M NaCl, 50 mM Na_2PO_4 , 5 mM EDTA, 0.5% SDS, $10\times$ Denhardt's solution, 0.5 mg ml^{-1} polyadenosine), then SSU rRNA probes endlabeled with ^{32}P were added. Table 1 lists the oligonucleotide probes used in these hybridization experiments. Hybridizations ran $>8\text{ h}$, then blots were washed for 30 min at room temperature and then for 30 min at the high stringency wash temperature for each probe (Massana et al. 1997) in pre-warmed wash buffer $1\times$ SET/1% SDS ($1\times$ SET: 150 mM NaCl, 20 mM Tris-HCl pH 7.8, 2 mM $\text{Na}_2\text{-EDTA}$). Membrane hybridization signals were quantified with a radioanalytic gas proportional counter (Ambis).

Hybridization signal (HS) of each domain was calculated by (1) determining amount of domain specific probe bound per unit picoplankton rRNA, (2) slopes were multiplied by a probe specific correction factor (universal probe bound per unit control rRNA \times domain-specific probe bound per unit control rRNA $^{-1}$), (3) corrected slopes were divided by the amount of universal probe bound per unit picoplankton rRNA, and (4) hybridization signals of *Archaea*, *Bacteria* and

Eucarya were expressed as percentages of the universal probe. In all cases the sum of the *Archaea*, *Bacteria* and *Eucarya* probe hybridization signals added up to between 75 and 125% of the universal probe HS. This variability may be due to quantitative differences in rRNA composition of the samples, as well as systematic errors associated with the methodology. The Group I and Group II archaeal hybridization signals were calculated in the same manner, except that the slopes were multiplied by a group-specific probe correction factor (archaeal probe bound per unit control rRNA \times group-specific probe bound per unit control rRNA⁻¹) then divided by the amount of archaeal probe bound per unit picoplankton rRNA.

RESULTS

rRNA hybridization studies provided us with the ability to survey a large assortment of picoplankton samples (56 different samples) in order to assess the relative abundance of archaeal rRNA in these picoplankton assemblages. The data presented here are based on the relative binding of Domain or group specific oligonucleotide probes to bulk nucleic acids extracted from mixed microbial populations. It should be noted that any methodology applied to complex, heterogeneous samples has inherent limitations and biases. For example, it will always be difficult to accurately assess lysis and extraction efficiency of uncultivated microorganisms having unknown genome sizes and cell wall compositions. Also, since the hybridization results are relative, absolute increases or decreases in any group remain uncertain. In addition, methodological variations in the collection and storage of some of our samples could have had some influence on the results. Nevertheless, the relative quantities and patterns of archaeal rRNA distribution we report here are consistent with previous reports from other locales, using a variety of different methods (DeLong 1992, Fuhrman et al. 1992, Fuhrman & Davis 1997, Murray et al. 1998).

We used the universal 1392 probe to normalize our hybridization results in this study, which appears to overestimate the archaeal HS. A report by Zheng et al. (1996) indicated a potential bias using this probe resulting in overestimating the amount of archaeal signal in mixed microbial assemblage samples. We investigated this potential bias by conducting numerous experiments ($n = 96$; Massana et al. 1998 and $n > 100$; Murray et al. 1998). Using our experimental protocol with Antarctic samples, we empirically determined that the universal 1392 probe overestimates the archaeal rRNA hybridization signal by a factor of approximately 2.0 in comparison to the universal 1390 probe. The ratio of the archaeal HS₁₃₉₂:HS₁₃₉₀ = 1.95 ± 0.5 , $n = 128$.

rRNA composition of Antarctic Peninsula picoplankton assemblages in austral late winter and summer

Picoplankton assemblages from late winter Antarctic Peninsula surface waters contained 7 to 21% archaeal rRNA (Ant. Pen. samples, Table 2). The majority of this archaeal signal was attributed to Group I crenarchaeota (averaging 81.6% of the archaeal HS). Bacteria rRNA dominated most picoplankton samples (averaging 68.5% for this data set). Total prokaryote counts ranged between 0.9 and 1.6×10^8 cells l⁻¹ in the Ant. Pen. samples. Eukaryote rRNA, ranging from 12 to 29%, was moderately low and correlated with the low chlorophyll *a* concentrations (ranging from 0.1 to 0.23 $\mu\text{g l}^{-1}$ in September) detected in this region during the late winter.

rRNA analysis of picoplankton samples from near-shore stations PALB and PALE in the austral summer (Table 2) indicated an extremely low archaeal rRNA signal in surface waters, in comparison to data collected in late winter-early spring Ant. Pen. samples. The relative proportion of archaea at PALB and PALE did increase with water column depth. This observation corroborates with findings from another study where average archaeal HS values were reported to be

Table 1. Oligonucleotide probe database (OPD) codes and probe sequences used in this study. Probes used in analysis of Antarctic Peninsula surface samples (AP), and the samples collected on the LTER grid (LTER), at McMurdo Sound (MS) and in Drake Passage (DP) are indicated

ODP code	Sequence (5'-3')	Wash temp. (°C)	Source	AP	LTER	MS	DP
S-*Univ-1392-a-A-15	ACGGGCGGTGTGTRC	37	Pace et al. (1986)	+	+	+	+
S-D-Euca-1209-a-A-16	GGGCATCACAGACCTG	37	Giovannoni et al. (1988)	+	+	+	+
S-D-Arch-0915-a-A-20	GTGCTCCCCCGCCAATTCCT	56	Amann et al. (1990b)	+	+	+	+
S-D-Bact-0338-a-A-18	GCTGCCTCCCGTAGGAGT	45	Amann et al. (1990a)	+	+	+	+
S-O-Cenar-0554-a-A-20	TTAGGCCCAATAATCMTCCCT	40	Massana et al. (1997)	+			+
S-O-ArGII-0554-a-A-20	TTAGGCCCAATAAAKCGAG	40	Massana et al. (1997)	+			+

Table 2. Relative abundance and distribution of picoplankton rRNA in waters of the Antarctic Peninsula and McMurdo Sound

Location	Date	SW fraction	rRNA HS ₁₃₉₂ (%)		
			Archaea ^a	Bacteria	Eucarya
Austral winter					
Ant. Pen. 3	15 Sep 95	<1.0 µm	11.5	77.6	28.6
Ant. Pen. 4	16 Sep 95	<1.0 µm	14.6	85.7	12.8
Ant. Pen. 5	17 Sep 95	<1.0 µm	16.4	47.4	11.9
Ant. Pen. 6	18 Sep 95	<1.0 µm	17.2	96.8	14.9
Ant. Pen. 7	19 Sep 95	<1.0 µm	20.8	64.6	19.1
Ant. Pen. 8	19 Sep 95	<1.0 µm	12.7	60.1	23.5
Ant. Pen. 9	19 Sep 95	<1.0 µm	6.9	70.4	21.1
PALB surface	13 Sep 96	<1.6 µm	34	56.4	22.6
PALB 50 m	13 Sep 96	<1.6 µm	35.6	57.8	16.2
Austral summer					
PALB surface	Jan 95	Whole water	0.4	35.5	87
PALB 60 m	Jan 95	Whole water	3.8	69	41.8
PALE surface	Jan 95	Whole water	0.4	19.2	84.9
PALE 35 m	Jan 95	Whole water	1.3	20.6	82
PALE 145 m	Jan 95	Whole water	12.8	41.9	56.7
Deception 5m	24 Dec 94	Whole water	0.2	28.4	71.7
Deception 50 m	24 Dec 94	Whole water	0.6	28.5	79.5
Deception 150 m	24 Dec 94	Whole water	7.8	55.4	44.7
Bratina Crack 25 m	9 Dec 95	Whole water	0.9	84.1	10.2
Bratina Crack 250 m	14 Dec 95	Whole water	9.6	36.7	31.7

^aHS₁₃₉₂ may overestimate actual HS values by approximately 2-fold. See 'Results', Massana et al. (1998) and Murray et al. (1998)

2.3 ± 2.4 for the austral summer (Murray et al. 1998). It appears that regardless of size fraction investigated (whole water in this study, and <1.6 µm fraction in the Murray et al. 1998 study) the archaeal rRNA constitutes a minimal proportion of the picoplankton rRNA in austral summer surface waters. Size fractionation experiments conducted during late winter in the Gerlache Strait (Massana et al. 1998) indicated that the relative percent of archaea increased 2- to 5-fold between the whole water and the 0.2 to 0.8 µm size fraction. This suggests that there are differences in relative abundance hybridization data depending on the size fraction collected. For Antarctic summer surface samples this difference was small, between 0.4 and approx. 2.3%. Samples from Whaler's Bay, Deception Island, displayed a similar pattern of extremely low archaeal HS in the surface waters, increasing to 7.8% at 150 m.

Spring-summer samples collected at LTER grid stations also showed relatively low archaeal rRNA HS in surface waters averaging 1.0 (SD 1.4, n = 8, Fig. 2). Subsurface samples collected at depths between 75 and 150 m (below the chlorophyll max.) also indicated low archaeal hybridization signals at 6 out of 8 stations (average 0.9; SD 0.5, n = 6). Significant variation in the archaeal HS was found at the mid-water depths on the 200 grid line (60 to 120 m), where values of 7.6 and 10.8 were much higher than the average for the other samples. In most of the deep profiles (to 300 or 500 m),

archaeal rRNA hybridization signals were the highest values detected in each profile, averaging 8.0 (SD 3.5, n = 9).

Evaluation of water column hydrographic, chemical and biological data corresponding to these casts suggests the following general trends. (1) Density profiles indicated a mixed layer depths between 8 and 35 m; in all cases the archaeal HS was very low in the mixed layer. The mid-water samples were collected at the temperature minimum, which generally corresponded to the pycnocline (Fig. 3A). No consistent trends between the mid-water archaeal HS and depth, or intensity of the pycnocline was observed. (2) Depths of the chlorophyll *a* maximum (>0.5 µg l⁻¹) were near 50 m at outer and mid-line stations, and at 100 m at inshore stations on the 400 and 200 lines (Fig. 3B). Archaeal rRNA hybridization signals were never over 4% in any case where the chlorophyll concentration was >0.5 µg l⁻¹. Correlation analysis suggested that there was a significant negative relationship between chlorophyll *a* concentration and archaeal rRNA HS ($r = -0.39$, $p < 0.05$, $n = 25$), and that there was a positive relationship between eucaryl rRNA and chlorophyll concentration ($r = 0.46$, $p < 0.02$, $n = 25$). The 200.000 stn had a high chlorophyll *a* concentration above 115 m averaging 4.2 µg l⁻¹, which is typical of that station. The archaeal HS was moderately high (12%) below the chlorophyll maximum at the same station. (3) Bacterial production measured by leucine incorpo-

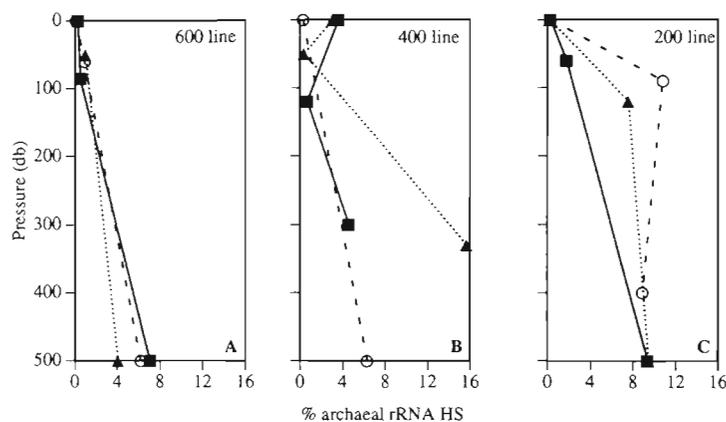


Fig. 2. Profiles of archaeal rRNA HS₁₃₉₂ at LTER stations collected in austral summer 1994-95. Profiles collected on the same grid-line are plotted together. (■—■) Offshore Stns 600.200, 400.200, and 200.200. (O---O) Mid-line Stns 600.120, 400.180, 200.160. (▲---▲) Inshore Stns 600.040, 400.120, and 200.000. (HS₁₃₉₂ may overestimate actual HS values by approximately 2-fold. See results, Massana et al. 1998, and Murray et al. 1998)

ration (data not shown) indicated that the majority of the activity on the 600 line was in the upper 50 m, where the corresponding archaeal HS was minimal. (4) Nitrate concentrations on the 600 grid line (data not shown) were generally high with values in the upper 50 m between 26 and 30 μM increasing to 28–33 μM below 75 m. Outer and mid stations on the 200 grid line were similar with nitrate concentrations of 24 μM in the upper 50 m increasing to 33 μM below 100 m. The 200.000 stn surface waters (upper 100 m) were lower in nitrate (19 to 23 μM) in correspondence with the high phytoplankton abundances found there (as indicated by chlorophyll *a* concentrations).

Presence of archaeal rRNA in McMurdo Sound

Archaeal rRNA was detected in all (7) samples collected in the McMurdo Sound region. Samples collected offshore of Bratina Island showed higher rRNA abundances at depth than in the surface waters (Table 2), which is similar to the trends seen in surface waters on the other side of the continent. Other samples collected off Bratina Island (500 m), in McMurdo Sound (25 m and 250 m), as well as at the ice edge (25 m) all indicated the presence of archaea, though these samples were not quantified.

Archaeal rRNA distribution in water masses north and south of the Polar Front

Sampling at the frontal boundary of Subantarctic and Antarctic waters in Drake Passage (Fig. 4A) indicated high abundance of archaeal rRNA throughout the water column. Although the hydrographic characters were completely different on either side of the Polar Front (PF), archaeal HS showed similar patterns with depth. Subsurface maxima in both archaeal rRNA profiles were found at the thermocline on either side of the PF (100 and 150 m, Figs. 4B,C). Hybridization analyses with the Group I and Group II archaeal probes suggested that crenarchaeal Group I archaea accounted for an average of 93.4% of the archaeal signal, while the Group II HS was only 2.9% of the archaeal HS for the Drake Passage samples. Surface eucaryal rRNA constituted 20 and 50% of the hybridization signal in the <1.6 μm sea-

water fraction north and south of the PF respectively. The eukaryote rRNA dropped off in both casts to <5% below 150 m. Fluorescence maxima at 15 m on the north side of the front and at 75 m on the south side did not correspond to the eukaryote rRNA signal.

Further evidence of the discrete differences in water mass characteristics is clear upon comparison of the temperature-salinity (T-S) diagrams from both casts (Fig. 5A). The T-S space occupied by these data are consistent with those reported for Antarctic Surface, Subantarctic Surface, and Circumpolar Deep Water masses (ASW, SASW, and CDW respectively) described by Whitworth (1980). The nearly isohaline salinity pro-

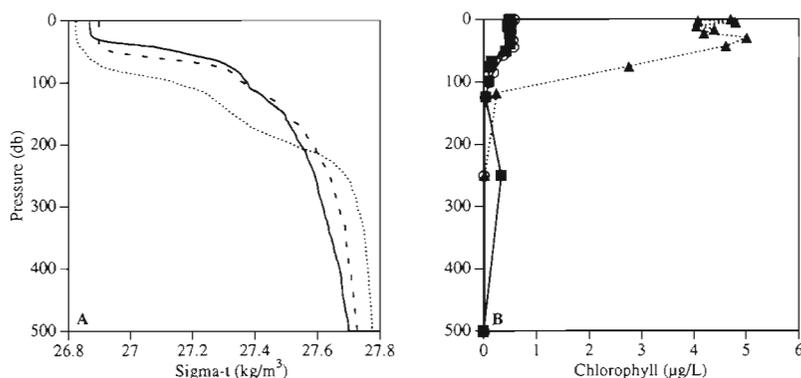


Fig. 3. (A) Sigma-t profiles on the LTER grid 200 line. Samples were collected at the surface, mid-water sample depths of 60, 90, and 120 m for Stns 200.200, 200.160, and 200.000 respectively, and at 500 m. Solid line corresponds to the cast at Stn 200.200; dashed line corresponds to Stn 200.160, and dotted line corresponds to Stn 200.000. (B) Chlorophyll *a* concentrations at the same sampling stations as in (A). Symbols and lines as in Fig. 2

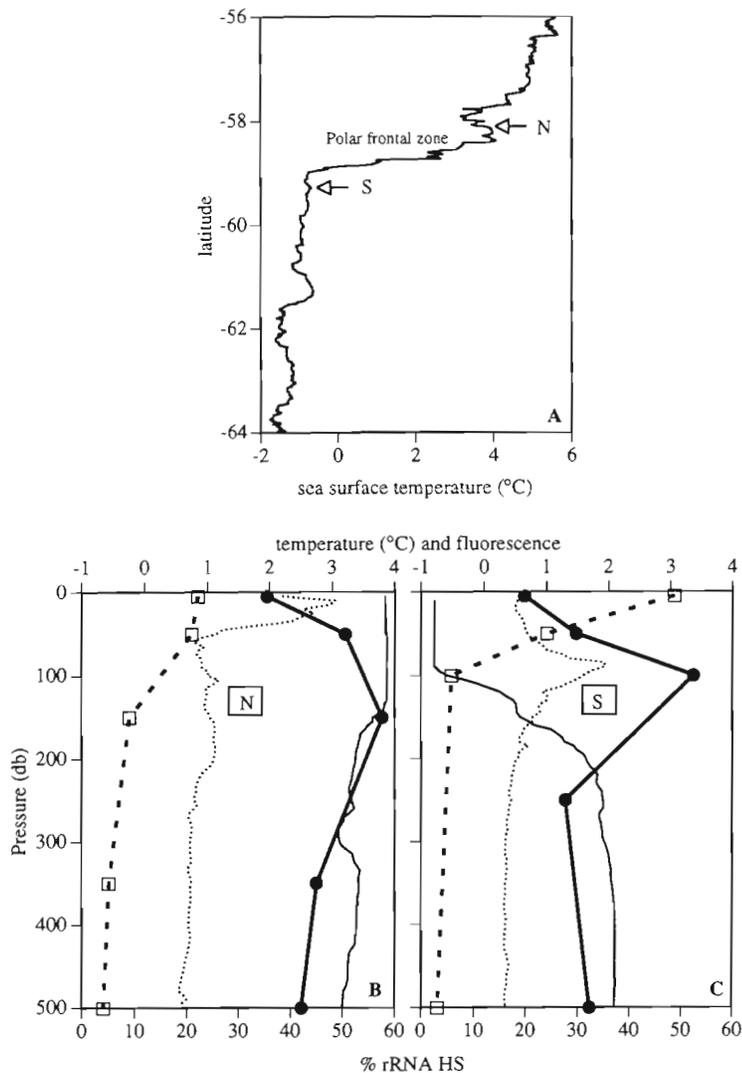


Fig. 4. Drake Passage hydrocasts were performed at stns north (N) and south (S) of the polar front (A). Distribution of archaeal (●) and eucaryal (□) rRNA HS₁₃₉₂ N (B) and S (C) of the polar front. Temperature profiles shown with solid line, *in situ* fluorescence (arbitrary units) shown with dotted line. (HS₁₃₉₂ may overestimate actual HS values by approximately 2-fold. See results, Massana et al. (1998), and Murray et al. (1998))

file north of the PF (Fig. 5B) is indicative of SASW above 300 m. Below this an inflection in the salinity profile at 300 m indicates the upper region of Antarctic Intermediate Water, which is a mixture of ASW, and SASW characterized by a salinity minimum, densities (σ_t) of 27.25 to 27.35 kg m⁻³, cold waters, and nutrients representative of the ASW (Sievers & Nowlin 1984). South of the PF, the salinity profile clearly shows the differences between ASW (mixed isohaline layer in the upper 100 m) and CDW (T > 1.0°C, Fig. 4C; salinity maxima, Fig. 5B) described by Sievers & Nowlin (1984). Oxygen profiles (data not shown) also corroborate with these observations. The ASW was saturated with oxy-

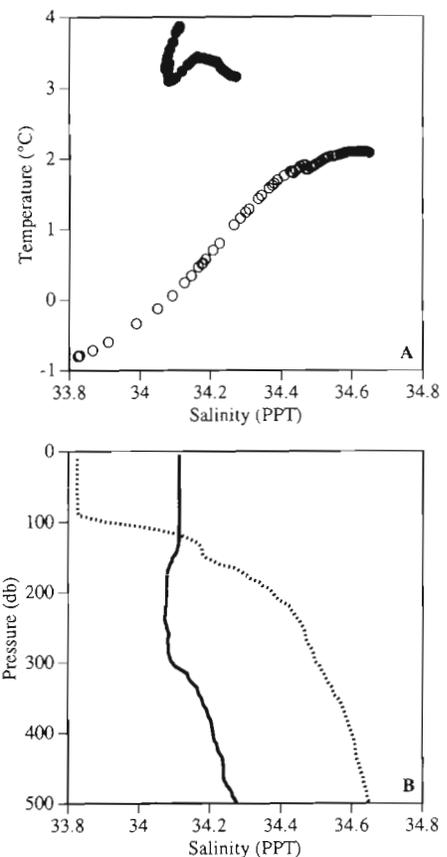


Fig. 5. Hydrographic characteristics of Drake Passage water masses north and south of the Polar Front July 29, 1996. (A) T-S diagram of (●) north and (○) south profiles. (B) Salinity profiles in Drake Passage where the solid line displays salinity of the north cast and dotted line corresponds to the south cast

gen (360 $\mu\text{mol kg}^{-1}$) below which the underlying CDW displayed a characteristic O₂ minimum (333 $\mu\text{mol kg}^{-1}$ at 250 m). The SASW was lower in oxygen (315 $\mu\text{mol kg}^{-1}$), having a weak subsurface maxima at 285 m (324 $\mu\text{mol kg}^{-1}$).

DISCUSSION

SSU rRNA targeted oligonucleotide hybridization studies have enabled us to assess the relative contribution of *Archaea*, *Bacteria* and *Eucarya* in Antarctic marine picoplankton assemblages. This approach is particularly suitable for studies of the planktonic archaea, since they appear to be comprised predominantly of only 2 divergent phylogenetic groups. The Antarctic archaeal planktonic assemblage is predominantly comprised of the Group I phylotypes as shown

in this study and others (DeLong et al. 1998, Massana et al. 1998, Murray et al. 1998). Hence, despite the fact that we have used probes directed at the domain level for the majority of this work, our study has focused on the distribution of a phylogenetically restricted group of organisms.

In total, our findings indicate that the planktonic archaea may have circumpolar distribution in Antarctic waters. This extends previous observations of planktonic archaea off Palmer station (DeLong et al. 1994, Murray et al. 1998) and in the Gerlache Strait (Massana et al. 1998), suggesting that the archaea are resident organisms of nearshore Antarctic waters. The data presented here indicate that archaeal distribution extends into the Antarctic surface, Subantarctic surface and Circumpolar Deep waters of the Southern Ocean (including those of the Ross Sea).

Transport by the circumpolar current, the largest known current system in the world's ocean (Vincent 1988), is likely an efficient mechanism of dispersal, and maintenance of widely distributed plankton assemblages. There is also potential for transport of planktonic archaea to the deep sea in areas of deep water formation or to Atlantic and Pacific Ocean basins via intermediate water masses. Smaller mesoscale advective features have been shown to be important in plankton transport and population dispersal as indicated by Lagrangian drifter experiments originating in the Gerlache Strait (Huntley & Niler 1995). Although physical transport by currents may be an effective means of dispersal, the relative abundances and distributions of picoplankton in different water masses might be representative of those water masses, as reported by Holm-Hansen et al. (1997) in a study in which differences in phytoplankton distribution and abundance could be correlated with different water masses.

The presence of planktonic archaea in McMurdo Sound demonstrates the extensive regional and probably global distribution of these planktonic organisms. The waters of eastern McMurdo Sound originate from the Ross Sea, where the conditions are biologically productive in comparison to the waters in the western sound (sampled at the Bratina Ice Crack), where the water is advected from under the Ross Ice Shelf (Hodson et al. 1981). Depending on sampling location in McMurdo Sound (eastern vs western sound), microbial productivity (Fuhrman & Azam 1980), and uptake of DOM (Hodson et al. 1981) have been reported to be typical of temperate and deep sea environments respectively. At the time of sample collection there were *Phaeocystis* blooms in the surface waters of the Bratina Island ice crack (DeVries pers. comm.) with corresponding low archaeal rRNA. At 250 m the archaeal HS was significant, implying that archaeal

populations might be sustained under the Ross Ice Shelf, the likely source of deep water at this location.

Collectively, our data strongly suggests that the Group I archaea are common, widely distributed Antarctic picoplankton constituents. Rather than showing a uniform distribution, it appears that the planktonic archaea vary temporally and spatially in Antarctic waters. A decrease in the archaeal signal between late winter and summer in the surface waters was evident in the Antarctic Peninsula picoplankton samples. This observation is in agreement with a more comprehensive study of temporal variation at stations PALB and PALN (Murray et al. 1998). Archaeal rRNA signal increased with depth in austral summer profiles examined from the LTER grid, at Whaler's Bay and at McMurdo Sound. Whether these observations are a result of the relative nature of these data (i.e. a result of relative increases in eukaryal or bacterial biomass) or whether archaea increase in absolute cell number requires further testing.

The observation of subsurface maxima in Antarctic archaeal rRNA distribution directly below the thermocline (Drake Passage, this study; Palmer Basin, Murray et al. 1998; several profiles in the Gerlache Strait, Massana et al. 1998) provides relevant information regarding their ecology, and suggests that their abundance and biological activity is variable in different strata in the water column. This location in the water column is an active region of particulate organic matter remineralization (Libes 1992). The chemical environment at this location in the water column is rich in inorganic nutrients, and from a biological perspective, there may be less competition for resources (at least between primary and secondary producers) than in the photic zone. There could also be specific biotic interactions between other planktonic bacteria and the archaea. Other studies (Gordon & Giovannoni 1996) have reported on the distribution of an uncultivated bacteria, SAR406, which also peaks below the chlorophyll max. Some marine nitrifying bacteria also have a well characterized maxima below the thermocline (Ward 1984).

This report has provided more extensive information on the spatial and temporal distribution of planktonic archaea in the Southern Ocean. The significance of their ubiquity and abundance, contribution to total microbial activity, remineralization capabilities, and potential as food resources for predator populations still remain speculative, although their dominance in certain Antarctic oceanic habitats suggests that they might play a significant role in each of these ecological functions. Survival in the pelagic environment requires adaptive capabilities that confer resistance to viral attack, growth rates sufficient to balance removal by grazing, and the ability to survive nutrient and light

variability encountered in seasonal cycles. The strong seasonal fluctuation of planktonic archaeal distribution in Antarctic surface waters indicates susceptibility to at least one of these environmental variables. Their high abundance in the Southern Ocean, as well as in sub-surface temperate waters, confirms that planktonic archaea have adapted well to a planktonic niche which is globally distributed, and suggests that these microorganisms influence the biology and chemistry of this habitat significantly.

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