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Vertical Distribution and Phylogenetic Characterization of Marine Planktonic *Archaea* in the Santa Barbara Channel

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Newly described phylogenetic lineages within the domain *Archaea* have recently been found to be significant components of marine picoplankton assemblages. To better understand the ecology of these microorganisms, we investigated the relative abundance, distribution, and phylogenetic composition of *Archaea* in the Santa Barbara Channel. Significant amounts of archaeal rRNA and rDNA (genes coding for rRNA) were detected in all samples analyzed. The relative abundance of archaeal rRNA as measured by quantitative oligonucleotide hybridization experiments was low in surface waters but reached higher values (20 to 30% of prokaryotic rRNA) at depths below 100 m. Probes were developed for the two major groups of marine *Archaea* detected. rRNA originating from the euryarchaeal group (group II) was most abundant in surface waters, whereas rRNA from the crenarchaeal group (group I) dominated at depth. Clone libraries of PCR-amplified archaeal rRNA genes were constructed with samples from 0 and 200 m deep. Screening of libraries by hybridization with specific oligonucleotide probes, as well as subsequent sequencing of the cloned genes, indicated that virtually all archaeal rDNA clones recovered belonged to one of the two groups. The recovery of cloned rDNA sequence types in depth profiles exhibited the same trends as were observed in quantitative rRNA hybridization experiments. One representative of each of 18 distinct restriction fragment length polymorphism types was partially sequenced. Recovered sequences spanned most of the previously reported phylogenetic diversity detected in planktonic crenarchaeal and euryarchaeal groups. Several rDNA sequences appeared to be harbored in archaeal types which are widely distributed in marine coastal waters. In total, data suggest that marine planktonic crenarchaea and euryarchaea of temperate coastal habitats thrive in different zones of the water column. The relative rRNA abundance of the crenarchaeal group suggests that its members constitute a significant fraction of the prokaryotic biomass in subsurface coastal waters.

It is widely accepted that the picoplankton (planktonic organisms with an average diameter of 0.2 to 2.0 μm) is a significant component of marine ecosystems (9, 12). Although these largely prokaryotic assemblages are normally characterized by bulk measurements averaged across the entire assemblage (e.g., total cell counts, radiotracer incorporation), in reality they are composed of different taxonomic groups with potentially different phenotypic properties, physiological activities, and ecological function. The specific composition of marine picoplankton assemblages is becoming better characterized, partly by the use of molecular techniques, in particular by analyses of phylogenetically informative macromolecules such as small-subunit rDNA genes (genes coding for small-subunit rRNA) (14, 16, 29, 30). These studies have revealed that rDNA genes retrieved from naturally occurring planktonic prokaryotes are often different from those of well-characterized, cultivated microorganisms. This general trend is also reflected in analyses of the microbiota of hot springs (35), soils (20, 34), and other microbial habitats. Through the application of molecular phylogenetic techniques, several disparate groups of as yet uncultivated members of the domain *Archaea* have recently been detected in marine plankton (7, 13). The rRNA genes of these microorganisms have been recovered in surface and subsurface waters of North America, in Antarctic coastal waters, and in offshore slope waters (7, 8, 13, 14, 32). The newly described archaeal rRNA sequences belong to one of two groups, a crenarchaeal group (group I) and a euryarchaeal group (group II). Relatives of the marine crenarchaeal group have been found in the guts of abyssal holothurians (22) and in terrestrial soils (34). Recently, a symbiotic association between a related crenarchaeon and a temperate-water marine sponge has also been reported (26). *Archaea* seem to be ubiquitous components of marine prokaryotic assemblages, but little is known about their physiological properties and ecology. To date, they have been identified and characterized solely by their small-subunit rRNA genes, with the exception of a single study which characterized protein-encoding genes linked to an rRNA operon in a planktonic marine crenarchaeon (32).

In aquatic systems, one of the most relevant gradients affecting the distribution of organisms is depth in the water column. It is well known that marine phytoplankton biomass and species distributions vary along vertical profiles, an obvious example being the deep chlorophyll maximum found in many areas of the world's oceans. This distribution probably affects prokaryotic assemblages as well, since phytoplankton distribution appears to be one of the most important biotic variables influencing planktonic bacterial growth (3, 6). The vertical distribution of phototrophic prokaryotes has been studied by microscopy, by flow cytometry, and by high-pressure liquid chromatography with signature pigments (5, 33). The distribution of nonphototrophic prokaryotes, lacking pigments, can be studied by molecular techniques. Evidence for variable distribution of prokaryotic assemblages between the photic and aphotic zones was obtained in total genomic DNA cross-hybridization experiments (19). In more definitive studies, the variable distribution of specific bacterial phylotypes in the water column has been observed with rRNA-targeted probes (15, 17).

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In this study, we examined the distribution and specific composition of the *Archaea* in a coastal marine environment. The relative abundance of archaeal rRNA was quantified in vertical profiles. Oligonucleotide probes were designed, tested, and used to investigate the vertical distribution of the two disparate groups of marine *Archaea*. Clone libraries of archaeal small-subunit rRNA genes were constructed from samples obtained at different depths and screened by a number of methods. Partial sequences were determined for representatives of the crenarchaeal and euryarchaeal groups to obtain a broader description of the phylogenetic heterogeneity contained in the marine *Archaea.*

MATERIALS AND METHODS

Sampling and nucleic acid extraction. Samples were retrieved near the center of the Santa Barbara Channel, approximately 10 miles offshore from the city of Santa Barbara (34°15′N, 119°54′W; sea floor at 522 m). Samples from different depths were taken with Niskin bottles and were stored in dark, insulated containers until filtration in the laboratory several hours after collection. From 4 to 10 liters of seawater was prefiltered through a 1-μm-pore-size fiberglass filter (MSI), and the picoplankton remaining in the filtrate was collected in a 0.2-μm Sterivex filter (Millipore) by using a peristaltic pump. The Sterivex filters were filled with 1.8 ml of lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) and stored at −20°C until nucleic acid extraction was performed. Prokaryotic cell counts and leucine incorporation experiments were performed by standard methods (18, 25).

Nucleic acid extraction began with the addition of lysozyme (1 mg ml^{-1}) to the Sterivex filter unit and incubation at 37°C for 30 min. Then, proteinase K (0.5 mg ml^{-1}) and sodium dodecyl sulfate (SDS) (1%) were added, and the filter was incubated at 55°C for 2 h. The lysate was recovered from the filter and the filter units were rinsed with 1 ml of lysis buffer at 55°C for 10 min. The pooled lysates were then extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1; pH 8) and once with chloroform-isoamyl alcohol (24:1). The aqueous phase was washed with sterile water in a microconcentrater (Centricon 100; Amicon) and reduced to a volume of 100 to 200 μl. In some cases, samples were concentrated by ethanol precipitation. The recovered DNA was quantified by a Hoechst dye fluorescence assay in a fluorometer (Hoefer Scientific Instruments). Extraction yields were between 0.35 and 0.79 μg of DNA per liter of sample. Nucleic acid extracts were stored at −20°C.

Quantitative oligonucleotide hybridization experiments. The quantitative oligonucleotide hybridization experiments were performed as previously described (7) with minor modifications. Nucleic acid extracts were obtained from natural samples and from controls (*Saccharomyces cerevisiae*, *Shewanella putrefaciens*, *Haloferax volcanii*, rRNA extracted from a sponge with an archaeal symbiont, and cloned rDNA inserts from group I and group II *Archaea*). rRNAs were denatured with 0.5% glutaraldehyde–50 mM Na_2HPO_4, (pH 7.2) for 10 min at room temperature. Plasmid templates were denatured in 0.5 N NaOH–1.5 N NaCl. An eightfold serial dilution of each natural sample and a fourfold serial dilution of each control were applied to nylon membranes (Hybond-N; Amersham) with a slot-blotting apparatus and immobilized by UV cross-linking (Stratalinker; Stratagene). Five to six replicates of each membrane were prepared, and each was hybridized with a different oligonucleotide probe. The membranes were preincubated at 45°C for 30 min with 6 ml of hybridization buffer (0.9 M NaCl, 50 mM Na_2HPO_4, 5 mM EDTA, 0.5% SDS, 10× Denhardt's solution, 0.5 mg of polyadenosine ml^{-1}) followed by the addition of 16S rRNA oligonucleotide probes end labeled with ^{32}P. The following probes (27, 31), named according to the Oligonucleotide Probe Database (1) designations, were used in hybridizations: S-*-Univ-1392-a-A-15 (Univ-1392), S-D-Arch-0915-a-A-20 (Arch-915), and S-D-Bact-0338-a-A-18 (Bact-338). After overnight hybridization at 45°C, the membranes were washed for 30 min at room temperature in 1× SET (150 mM NaCl, 1 mM EDTA, 20 mM Tris) containing 1% SDS. They were then transferred to the same buffer preequilibrated to the high-stringency wash temperature (37, 56, and 45°C for Univ-1392, Arch-915, and Bact-338, respectively), washed for an additional 30 min, removed, and dried. Radioactivity was quantified by a radioanalytic gas proportional-counting system (Scanalytics, Bellerica, Mass.).

Probes specific for group I (S-O-Cenar-0554-a-A-20 [GI-554]) and group II (S-O-ArgII-0554-a-A-20 [GII-554]) marine *Archaea* were also designed and tested. Both probes complement positions 573 to 554 of the 16S rRNA (*Escherichia coli* numbering), and their sequences (5′ to 3′) are TTA GGC CCA ATA ATC MTC CT (GI-554) and TTA GGC CCA ATA AAA KCG AC (GII-554). The high-stringency wash temperature used for both probes was 40°C in 1× SET–1% SDS. Probe sequences have been submitted to the OPD (1).

The slope of the probe binding curve (counts per minute of probe bound per unit of rRNA) for each natural sample was determined from four to eight points in the serial dilution and normalized to account for differences in the binding affinity of each probe (16). Correction factors for the archaeal and bacterial probes were calculated by dividing the slope of the universal probe bound by the slope of the group-specific probe bound to the same pure culture control rRNA. The group-specific slope for each natural-sample rRNA was then corrected by multiplying it by the group-specific correction factor calculated from controls. Estimates of percent archaeal rRNA were then calculated by dividing the corrected, group-specific slope by the sum of the corrected *Archaea* and *Bacteria* slopes.

No pure rRNA controls are yet available for archaea belonging to group I and group II, so correction factors normalizing group I and group II probe binding to that of a universal probe cannot yet be determined. Differences in group I and group II probe binding for identical samples are therefore reported as the hybridization signal, which is defined as $[m_{gx}/(m_{gI} + m_{gII})] \times 100$, where m is the slope of the probe binding (counts per minute of probe bound per unit of rRNA) determined from four to eight separate rRNA concentrations in the rRNA dilution series. Subscripts represent the specific probe from which the slope was determined: gI, group I probe; gII, group II probe; gx, either group I or group II probe.

rDNA clone libraries. The 16S rRNA archaeal genes were amplified from natural nucleic acid extracts with the primers S-D-Arch-0021-a-S-20 (21f) and S-D-Arch-0958-a-A-19 (958r) or 20f (TTC CGG TTG ATC CYG CCR G) and Univ-1392. The conditions of the PCR have been described previously (7). The size of the PCR product was checked by agarose gel electrophoresis. The PCR fragments were cloned with a TA Cloning kit (Invitrogen) as previously described (7). Putative positive clones were transferred to a multiwell plate (12 by 8 wells) containing Luria broth and 7% glycerol and stored at −80°C.

For preliminary screening, the rDNA clones were transferred to nylon membranes on Luria broth agar and incubated overnight at 37°C until visible colonies appeared. The colonies were lysed by soaking the membranes in 2× SSC (1× SSC is 150 mM NaCl and 15 mM sodium citrate)–5% SDS for 2 min, heating them in the microwave at maximum setting for 2.5 min, and incubating them with 50 mM Tris–50 mM EDTA–100 mM NaCl–1% (wt/vol) sodium *N*-lauroylsarcosine–250 μg of proteinase K ml^{-1} for 20 min at 37°C (32). Nucleic acids were immobilized on the membranes by UV cross-linking and hybridized with probes Arch-915, GI-554, and GII-554. After being dried, the membranes were exposed to autoradiographic film (Kodak X-Omat) for several hours.

RFLP, sequencing, and phylogenetic analysis. For restriction fragment length polymorphism (RFLP) analysis, plasmids from the clones were extracted by standard techniques (28) and the rDNA inserts were amplified with primers 21f and 958r. The PCR products were subjected to separate enzymatic digestions with *Hae*III and *Rsa*I (2 h at 37°C) and run in a 2.5% low-melting-temperature agarose gel for 2.5 h at 60 to 80 V. Fragments shorter than 50 bp were not considered in analyses. rDNA inserts were partially sequenced by the dideoxynucleotide chain termination method (Sequenase 2.0; U.S. Biochemical) with two internal primers of the 16S rRNA gene (21f and S-*-Univ-0519-a-A-18 [519r; GWA TTA CCG CGG CKG CTG]). Approximately 370 bp of double-stranded sequence was obtained. The entire insert of two clones which were amplified with the primer pair 20f plus Univ-1392r (1,350 bp) was also sequenced (both strands).

Phylogenetic analyses were conducted by using reference sequences and the software (GDE 2.2 and Treetool 1.0) obtained via anonymous ftp from the Ribosomal RNA Database Project (21) and the software package Phylip version 3.5 (11). Distance matrices were calculated with DNAdist by using the Kimura two-parameter model, assuming a transition/transversion ratio of 2.0. Trees were inferred by neighbor-joining analyses. Bootstrap neighbor-joining analysis was conducted on 100 replicates with random taxon addition. Maximum-likelihood analysis (10) was performed with fastDNAml version 1.0 (24), using empirical base frequency, global branch swapping, and bootstrapping options.

Nucleotide sequence accession numbers. Nucleotide sequences have been deposited in the GenBank database under the accession numbers U78195 for SB95-8, U78196 for SB95-16, U78203 for SB95-40, U78204 for SB95-41, U78197 for SB95-53, U78198 for SB95-54, U78199 for SB95-57, U78200 for SB95-59, U78201 for SB95-60, U78202 for SB95-61, U78205 for SB95-71, and U78206 for SB95-72.

RESULTS

Development of specific probes for marine *Archaea*. Oligonucleotide probes specific for two groups of marine planktonic *Archaea* were designed and tested against nucleic acid extracts of cultured *Archaea* and plasmids containing cloned 16S rDNA inserts of marine *Archaea* (Fig. 1). The empirically determined wash temperature used for both probes was 40°C (blots shown in Fig. 1). All templates (except rDNA clone SBAR1A, which has a mismatch in the target region [7, 27]) tested positive with the archaeal probe. All cloned rDNAs identified by sequence analysis as belonging to group I or group II bound to the corresponding group-specific probe (Fig. 1). In addition, rRNA extracted from the sponge *Axinella mexicana*, known to contain relatively high levels of a symbiotic archaeon, *Cenarchaeum symbiosum*, also tested positive with the group I probe.

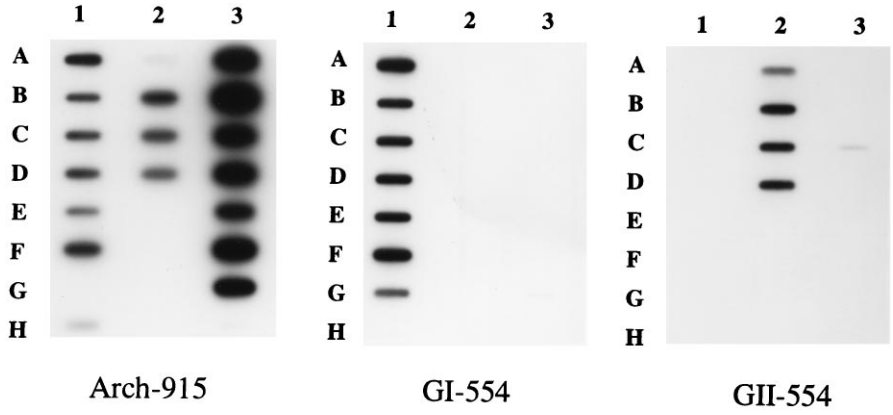

FIG. 1. Hybridization experiments with oligonucleotides which target rRNA of *Archaea*, the crenarchaeal group I, and the euryarchaeal group II. Column 1 contains plasmids with group I rDNA inserts SBAR5, WHARQ, OAR4, OAR7, Fosmid 4B7, and A3 and a nucleic acid extract from sponge tissue containing an archaeal symbiont (26). Cell 1H is empty, except in the Arch-915 blot, where cells 1G and 1H are reversed. Column 2 has plasmids with group II rDNA inserts SBAR1A, WHARN, OAR22, and OARB. Cells 2E to H are empty. Column 3 contains rRNA extracted from the cultured *Archaea Haloferax volcanii*, *Thermoplasma acidophilum*, *Methanobacterium thermoautrophicum*, *Methanococcus jannaschii Pyrococcus* strain GBD, *Desulfurococcus* strain SY, and *Sulfolobus solfataricus*. Cell 3H contains the rRNA of *Saccharomyces cerevisiae* as a negative control.

Additional experiments with these probes gave negative results with nucleic acids from tested members of the domain *Bacteria* or *Eucarya* (data not shown).

Vertical distribution of *Archaea* in the water column. The relative abundance of archaeal rRNA at several depths in the Santa Barbara Channel was estimated by quantitative rRNA hybridization experiments. In one summer profile (Fig. 2A), the percentage of archaeal rRNA was minimal at the surface and increased with depth, reaching about 20% of the total prokaryotic rRNA at 200 m and remaining at this value to a depth of 500 m. In one winter profile (Fig. 2B), the trend was similar, although archaeal rRNA abundance was higher in surface water than in summer (10%), and it appeared to peak at 200 m. The relative rRNA abundance of group I versus group II *Archaea* at different depths and on different dates was also estimated (Fig. 3). Group II rRNA was most abundant at the surface, while group I rRNA dominated at 100 m. Estimates in a profile with five depths sampled (3 December 1994 [results not shown]) corroborated this trend, with equivalent amounts of group I and group II rRNAs at 50 m and a dominance of group I rRNA in the 100- to 400-m layer.

Screening of archaeal rDNA libraries. Samples from 0 m and 200 m (3 December 1994) were used for PCR amplification of archaeal 16S rRNA genes. Positive amplification was obtained for both samples with primer pair 21f and 958r, while primer pair 20f and Univ-1392r yielded amplified rDNA from only the 200-m sample. PCR products were cloned from two different depths with the following primer combinations: 0 m, 21f and 958r; 200 m, 21f and 958r; and 200 m, 20f and Univ-1392r. The clones were transferred to a membrane and hybridized with archaeal, group I, and group II probes (Fig. 4; and Table 1). Virtually all clones detected with the archaeal probe were also detected with one of the two group-specific probes. (Of 575 clones, 4 were positive only with the archaeal probe [positions B3, C4, H5, and H9 in Fig. 4A]. These were sequenced and found to correspond to *Haloferax volcanii*. This result almost certainly represents carryover from positive control DNA [*H. volcanii*] used in the PCR experiments.) No

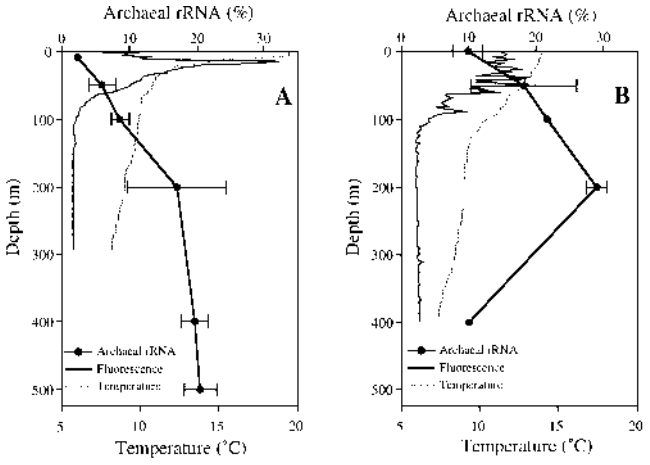

FIG. 2. Percent archaeal rRNA (relative to archaeal rRNA plus bacterial rRNA) in the water column of Santa Barbara Channel in a summer profile (1 July 1993) (A) and a winter profile (3 December 1994) (B), as estimated by quantitative rRNA hybridization experiments. Values shown in panel A are means of three to four separate determinations, with bars representing the standard error. Values shown in panel B are means of two determinations, with bars representing the range (small bars are not visible).

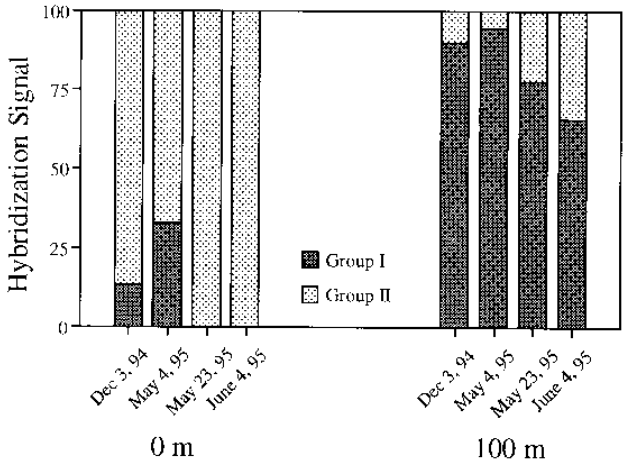

FIG. 3. Hybridization signal derived from group I and group II probes hybridized with rRNA extracted from different depths and sampling dates in the Santa Barbara Channel. The hybridization signal is defined in the text.

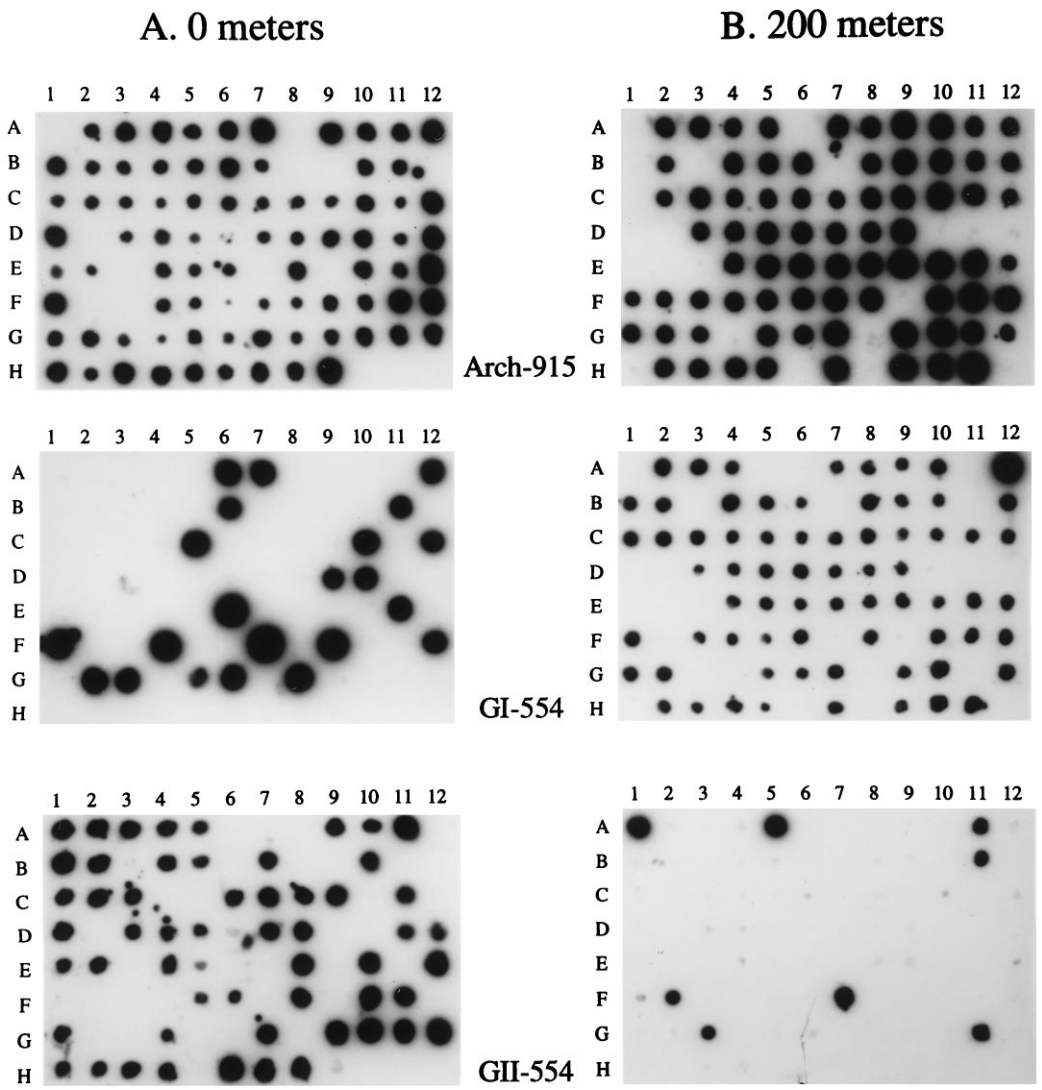

FIG. 4. Screening of the 0-m (21f plus 958r) (A) and 200-m (20f plus Univ-1392r) (B) libraries with archaeal, group I, and group II probes. Archaeal 16S rRNA gene fragments were PCR amplified and cloned. The clones were transferred onto membranes, grown as colonies, lysed, and hybridized with radiolabeled probes. Some clones were inefficiently transferred and did not grow; they do not appear in the blot (A1 in the Arch-915 blot in panel A; A1, B1, and C1 in the Arch-915 blot in panel B). All other blank positions in the Arch-915 blots represent clones without rDNA inserts.

evidence for new archaeal phylotypes, different from group I or group II, were found in the libraries. The majority of clones in the 0-m library belonged to group II (Fig. 4A), while most of the clones in the 200-m libraries belonged to group I (Fig. 4B), indicating the same trend previously detected in quantitative rRNA hybridization experiments (Table 1).

Survey of archaeal phylogenetic variants. Selected clones from the rDNA libraries (0 m, 21f/958r; and 200 m, 20f/Univ-

TABLE 1. Results of screening of 16S rDNA libraries with archaeal, group I, and group II probes

Library		No. of clones in:			% of clones in:		Hybridization signal in[a]:	
Sample	Primers	*Archaea*	Group I	Group II	Group I	Group II	Group I	Group II
0 m	21f plus 958r	110	29	81	26.4	73.6		
200 m	21f plus 958r	276	237	39	85.9	14.1		
200 m	20f plus Univ-1392r	189	175	14	92.6	7.4		
0 m							12.2	87.8
200 m							96.0	4.0

[a] The hybridization signal with group I- and group II-specific oligonucleotide probes hybridized with rRNA extracted from the same samples (0 and 200 m) from which the rRNA gene clones originated. (Hybridization signal data for 0 m also appear in Fig. 3 as the 0-m, 3 December 1994 data point.)

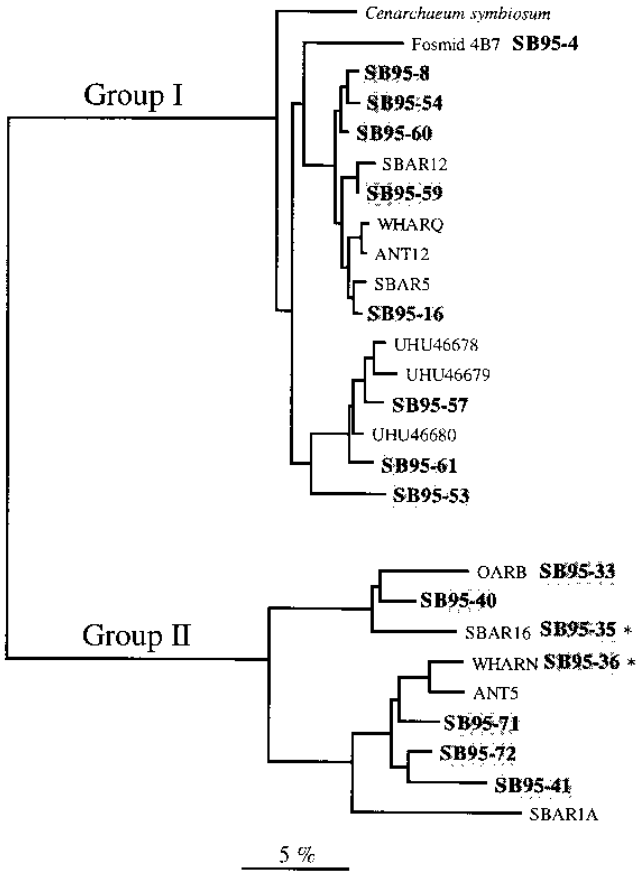

FIG. 5. Phylogenetic analysis of marine *Archaea*, using positions 108 to 513 (*E. coli* numbering). The tree was inferred by DNAdist and neighbor-joining analysis (Phylip version 3.5), using 359 bp. *H. volcanii* was used as the outgroup. The clones sequenced in this study (SB95-) are shaded. Four SB95- clones contained a sequence in the region analyzed identical to that in previously reported clones, and they appear in the same position of the tree. In some cases (designated by an asterisk), they represent a different RFLP pattern, due to sequence variation in another portion of the rRNA gene.

1392r) were sequenced to better describe the phylogenetic diversity of rDNAs from the two marine archaeal groups. RFLP analysis revealed nine different patterns in 40 group I clones and nine different patterns in 40 group II clones. At least one clone representing each pattern was partially sequenced. As expected, all the clones identified with the group I probe clustered with previously reported group I sequences and clones which tested positive with the group II probe clustered with previously reported group II sequences (Fig. 5). For the 16S rRNA region between positions 108 and 513 (*E. coli* numbering), several clones had sequences identical to those of previously reported clones from the Santa Barbara Channel (SBAR16), the Oregon coast (Fosmid 4B7 and OARB), and Atlantic coastal waters off Woods Hole, Mass. (WHARN) (Fig. 5). However, RFLP analysis indicated that some of these clones (indicated with an asterisk in Fig. 5) were not identical, having at least one different restriction site in a region not sequenced. Excluding *Cenarchaeum symbiosum* and Fosmid 4B7, all group I sequences clustered in one of two groups. The cluster containing SBAR5 comprised five RFLP patterns and most of the clones in the libraries. The other group I cluster comprised three RFLP patterns and in addition three rDNA clones recovered from deep-water samples off Hawaii (17a; GenBank database). The euryarchaeal group II contained two main clusters more phylogenetically distant from one another than those within the crenarchaeal group. The first cluster comprised three RFLP patterns but only one new sequence in the region compared, and the second cluster comprised five RFLP patterns and three new sequences.

The entire insert of one group I (SB95-57) and one group II clone (SB95-72) from the 200-m, 20f/Univ-1392r library was sequenced. Phylogenetic analysis of these more extensive sequences confirm previous phylogenetic analyses (Fig. 6). This is particularly relevant for euryarchaeal group II, for which only a few partial sequences have been available to date. Our data support a loose affiliation of group II marine *Archaea* with *Thermoplasma acidophilum* (7).

DISCUSSION

The prevalence of *Archaea* in cold, aerobic marine environments was originally unrecognized, since cultivated members of this domain consisted of methanogens, extreme halophiles, and sulfur-metabolizing thermophiles (37). However, recent molecular phylogenetic surveys indicated that new types of as yet uncultivated *Archaea* are common and widely distributed in nonthermophilic marine habitats (7, 8, 13, 14, 26, 32). The ecological distribution and phylogenetic position of two newly recognized planktonic archaeal groups indicates that they may have phenotypic properties different from those of cultivated *Archaea*. Unfortunately, since none of these organisms have yet been recovered in pure culture, their specific physiological and metabolic traits remain unknown. Estimates of rRNA abundance derived from *Archaea* in marine plankton suggests that they represent considerable biomass and so may contribute significantly to ecological process in the picoplankton. Peculiar biochemical features of known members of *Archaea* (unusual lipids, distinct transcriptional and translational machinery, and unique metabolic capabilities) suggest that marine planktonic *Archaea* may be involved in unique biogeochemical processes. To further understand the significance of *Archaea* in marine plankton, we studied their distribution, abundance, and phylogenetic composition in the Santa Barbara Channel.

Significant amounts of archaeal rRNA were detected in all marine samples studied, taken at different depths and at different times of year (Fig. 2). There was a clear trend of lower relative abundance in surface waters (2 to 10%) than in deep waters (20 to 40%). Maximal relative abundances in temperate deep waters, typically below 100 m deep, are similar to values reported for late-winter coastal Antarctic surface waters (8). Values determined in surface waters of the same environment in previous years (7) generally agree with data presented here, indicating that the presence of *Archaea* and their increase in abundance with depth are regular features of this coastal habitat. Crenarchaeal and euryarchaeal picoplankton had rRNA maxima at different depths in the water column (Fig. 3). The same trend was observed when screening the rDNA libraries prepared from samples at 0 and 200 m (Table 1). Although the two independent approaches used (rRNA hybridization and screening of amplified rDNA genes) are likely to have very different biases and errors, they yielded comparable results. The different distributions of both groups in the plankton is perhaps not unexpected, since they are very disparate phylogenetically (Fig. 6) and their phenotypes may also be very different. Their distinct vertical distribution may also reflect their different ecological roles and biogeochemical activity in marine plankton assemblages.

The doubling times of the prokaryote assemblage in the Santa Barbara Channel, as estimated by leucine incorporation on three dates in summer 1995, were 0.7 to 3.8 days at 0 m and

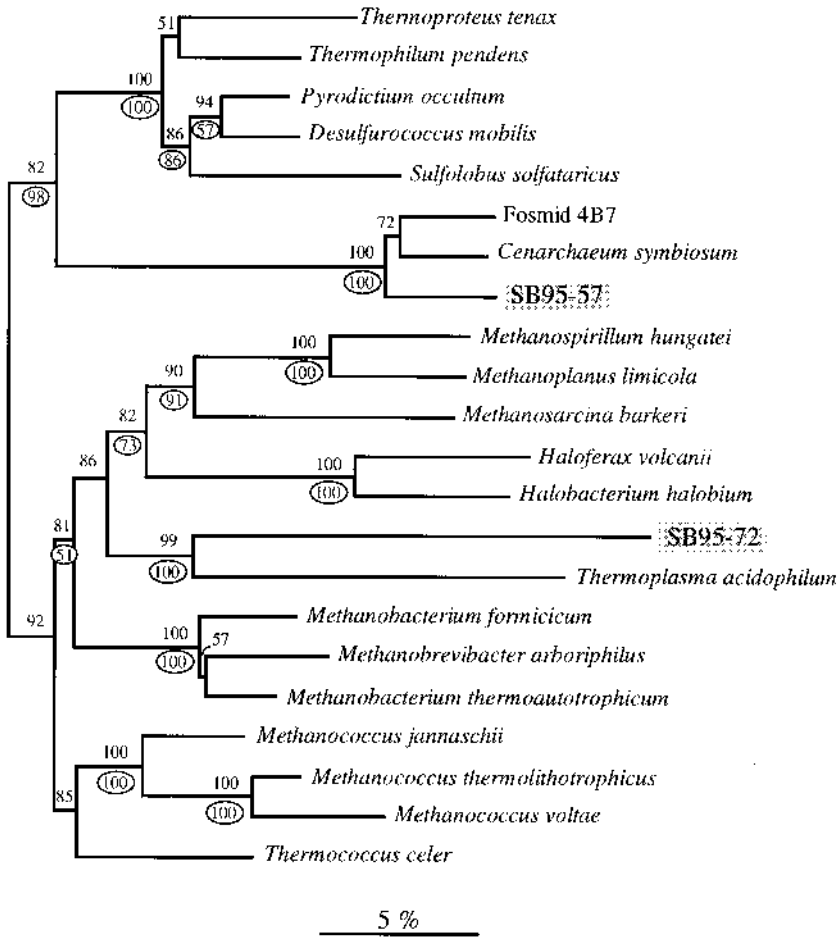

FIG. 6. Phylogenetic analysis relating representatives of cultured, group I, and group II *Archaea*. The tree was inferred by DNAdist and neighbor-joining analysis (Phylip version 3.5), using 1,217 nucleotide positions. *E. coli*, *Choroflexus aurantiacus*, and *Bacillus subtilis* were used as outgroups. The two clones completely sequenced in this study are shaded. Values above the line represent the majority-rule consensus tree for neighbor-joining analysis. Numbers in circles below the line represent bootstrap values obtained in maximum-likelihood analysis on the same data set, using fastDNAml version 1.0.

11.5 to 20.9 days at 100 m. The average cell numbers on those dates were 1.6×10^6 cells ml^{-1} at 0 m and 5.0×10^5 cells ml^{-1} at 100 m. Group II euryarchaeotes were found in the photic zone, where they comprise a small fraction of an active prokaryotic assemblage, whereas group I crenarchaeotes were found below the photic zone, where they represent a larger fraction of a presumably more slowly growing assemblage. A variety of prokaryotic groups have previously been shown to have a vertical stratification in the open ocean. For instance, phototrophic prokaryotes occupy mainly the photic zone, with prochlorophytes (5, 33) sometimes observed at greater depths than cyanobacteria (36). Another example includes as yet uncultivated bacterial phylotypes, such as variants of the SAR11 cluster, which appear to accumulate in the upper photic zone (15), or the SAR406 cluster, which appeared most abundant slightly below the deep chlorophyll maximum (17). It is evident from our results that different archaeal groups also occupy different zones in the water column. As with planktonic photoautotrophs, specific prokaryotic populations accumulate at specific depths in the water column, presumably in response to optimal physical, chemical, or nutritional requirements, as well as biotic interactions including competition and predation.

The data presented reflect the relative rRNA abundance of *Archaea*. Conversion to absolute values, in the absence of other supporting data, is not trivial. However, making the simplifying assumption that all marine prokaryotes (*Bacteria* and *Archaea* at any depth) contain the same cellular content of rRNA, rough estimates can be derived. At 0 m, with typically 2×10^6 prokaryotes ml^{-1} and 5% relative archaeal abundance, there would be around 10^5 *Archaea* ml^{-1}. Similarly, at 100 m, with typically 4×10^5 prokaryotes ml^{-1} and 25% relative archaeal abundance, there would also be around 10^5 *Archaea* ml^{-1}. This might suggest a homogeneous abundance of *Archaea* as a group throughout the water column, with a shift from dominance of group II at the surface to group I at depth, and a change in the total abundance of *Bacteria*, which is much higher at the surface than at depth. Since the inflection point of the archaeal groups occurs at a relatively shallow depth (both groups appeared equally abundant at 50 m in the winter profile), group I members are apparently the most abundant *Archaea* in the marine plankton of the Santa Barbara Channel.

One goal of this study was to identify and obtain sequence information on the diversity of archaeal phylotypes present in the Santa Barbara Channel. The sequences retrieved from our libraries covered most of the phylogenetic variability previously detected in those groups (Fig. 5). The distribution of rRNA gene variability within each cluster parallels similar distributions previously described for *Bacteria*, with many closely re-

lated but distinct rRNA gene sequences recovered from the same habitat (2, 4, 7, 14, 16, 23, 29). As with *Bacteria*, highly similar (sometimes nearly identical) archaeal rDNA sequences have been recovered from distant geographic locales, suggesting a broad distribution of the organisms harboring these genes. The cluster containing SBAR5 and relatives appears to be the largest, comprising many different clones with little sequence variation among them. In contrast, the genetic microheterogeneity of the euryarchaeal group II appears less pronounced, but the evolutionary distance between individual rRNA gene sequences was greater than that seen within crenarchaeal group I. Although some of this rDNA gene variability might be explained by multiple, nonidentical rRNA operons occurring within the same organism, this is unlikely to be the full explanation. For instance, all well-characterized and cultivated crenarchaea contain only one rDNA operon, so that multiple operons seem a less likely explanation for the observed planktonic crenarchaeal rDNA microheterogeneity. In addition, Amann et al. (2) have recently shown that highly similar rDNA genes first detected by PCR cloning studies corresponded to transcribed rRNA sequences expressed in different cell types, as determined by whole-cell hybridization with fluorescent oligonucleotide probes.

It is now known that new groups of *Archaea* are common in marine picoplankton as well as other diverse environments. The bulk of marine planktonic *Archaea* appear to be represented only by two lineages, one dominating at the surface and the other dominating at depth. Marine planktonic crenarchaea (group I) appear particularly abundant below the deep chlorophyll maximum in temperate coastal waters. Marine planktonic euryarchaea seem most prevalent in surface or near-surface waters. Available evidence suggests that these marine *Archaea* are active, abundant, and dynamic components of the picoplankton. Their specific physiology, biogeochemical activities, and ecological roles, however, remain to be determined.

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