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Archaea in coastal marine environments

(archaebacteria/phylogeny/bacterioplankton/molecular ecology)

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ABSTRACT **Archaea (archaebacteria) are a phenotypically diverse group of microorganisms that share a common evolutionary history. There are four general phenotypic groups of archaea: the methanogens, the extreme halophiles, the sulfate-reducing archaea, and the extreme thermophiles. In the marine environment, archaeal habitats are generally limited to shallow or deep-sea anaerobic sediments (free-living and endosymbiotic methanogens), hot springs or deep-sea hydrothermal vents (methanogens, sulfate reducers, and extreme thermophiles), and highly saline land-locked seas (halophiles). This report provides evidence for the widespread occurrence of unusual archaea in oxygenated coastal surface waters of North America. Quantitative estimates indicated that up to 2% of the total ribosomal RNA extracted from coastal bacterioplankton assemblages was archaeal. Archaeal small-subunit ribosomal RNA-encoding DNAs (rDNAs) were cloned from mixed bacterioplankton populations collected at geographically distant sampling sites. Phylogenetic and nucleotide signature analyses of these cloned rDNAs revealed the presence of two lineages of archaea, each sharing the diagnostic signatures and structural features previously established for the domain Archaea. Both of these lineages were found in bacterioplankton populations collected off the east and west coasts of North America. The abundance and distribution of these archaea in oxic coastal surface waters suggests that these microorganisms represent undescribed physiological types of archaea, which reside and compete with aerobic, mesophilic eubacteria in marine coastal environments.**

Application of molecular phylogenetic analyses to ecological questions has recently enhanced the ability of microbial ecologists to assess naturally occurring diversity in mixed microbial assemblages (1–6). In this approach, genes encoding phylogenetically informative macromolecules, derived from extracted nucleic acids of mixed microbial populations, are clonally isolated, sorted, and sequenced. Analysis of the recovered sequences allows inference of the phylogenetic affiliation of individual population constituents. Additionally, this sequence information aids in the design of taxa-specific oligodeoxynucleotide probes (7, 8), for monitoring the spatial and temporal variability of specific groups. This approach has led to the phylogenetic identification of previously uncultured microbes (3–5, 7), as well as estimates of their abundance or distribution (3, 5, 8).

To characterize planktonic versus surface-attached marine bacteria, we cloned and analyzed small-subunit rRNA sequences derived from microbial assemblages occupying these different habitats. Ribosomal RNA genes were amplified (9, 10) from purified, mixed-population nucleic acids (3). The amplified rRNA genes were then cloned, sorted, and sequenced, and compared with a data base of aligned rRNA sequences from well-characterized microorganisms (11). Due to the predominance of eukaryotic nucleic acids in many macroaggregate samples, eubacterial- or archaeal-biased PCR primers were routinely used to exclude the amplification of eukaryotic ribosomal RNA-encoding DNA (rDNA). Surprisingly, archaeal rDNA was detected in many samples. This report describes the detection of two marine archaeal lineages and their preliminary phylogenetic and ecological characterization.

METHODS

Bacterioplankton Collection. Coastal water samples were collected and screened through a 10-μm Nytex mesh prefilter. Bacterioplankton were concentrated from these 10-μm-filtered water samples by using a CH2PR filtration unit (Amicon) fitted with a polysulfone hollow-fiber filter (30-kDa cutoff). Twenty-liter samples were concentrated to a final volume of 100–150 ml. The resulting bacterioplankton concentrates were centrifuged (27,500 × g, 30 min, 4°C), and the cell pellets were stored at −80°C. Bacterial-cell densities in seawater and bacterioplankton concentrates were determined by epifluorescence microscopy of glutaraldehyde-fixed, acridine orange-stained samples (12). Cell recoveries in the concentrates ranged from 78% to 100% of the total cells filtered.

Extraction of Nucleic Acids and PCR Amplification. Cell pellets were lysed, and crude nucleic acids were purified as described (5). Approximately 5–10 μg of this crude nucleic acid preparation was purified by CsCl equilibrium density-gradient centrifugation (100,000 × g, 5–16 hr, 20°C) on a Beckman TL 100 ultracentrifuge using a TLA 100 rotor. Ribosomal DNA was amplified from purified DNA using GeneAMP kit reagents (Perkin–Elmer/Cetus), as recommended by the manufacturer. Reaction mixtures contained 2 mM $MgCl_2$, 10 mM Tris·HCl, pH 8.3/50 mM KCl/200 μM deoxynucleotide triphosphates/2.5 units of *Thermus aquaticus* DNA polymerase/0.2 μM each of oligonucleotide primer/DNA template at 1 ng/μl. Thermal cycling was as follows: denaturation at 95°C for 1.5 min, annealing at 55°C for 1.5 min, and extension at 72°C for 1.5 min for a total of 30 cycles.

The oligonucleotide primer sequences were as follows:

Eubac27F (13):	AGA GTT TGA TCC TGG CTC AG
1492R (13):	GGT TAC CTT GTT ACG ACT T
EukF (10):	AAC CTG GTT GAT CCT GCC AGT
EukR (10):	TGA TCC TTC TGC AGG TTC ACC TAC
Arch21F:	TTC CGG TTG ATC CYG CCG GA
Arch958R:	YCC GGC GTT GAM TCC AAT T

Quantitative Hybridization Experiments. Crude nucleic acids and purified rRNA standards were denatured in 0.5% glutaraldehyde, serially diluted, applied to nylon membranes (Hybond-N; Amersham) with a slot-blotting apparatus, and immobilized by baking in a vacuum at 80°C for 1 hr (14). Membranes were preincubated at 45°C for 0.5 hr in hybrid-

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Abbreviation: rDNA, ribosomal RNA-encoding DNA.

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ization buffer (0.9 M NaCl/50 mM NaH_2PO_4, pH 7.0/5.0 mM Na_2 EDTA/0.5% SDS/10× Denhardt's solution/polyriboadenylic acid at 0.5 mg/ml), followed by the addition of 2×10^7 cpm (specific activity $4–9 \times 10^8$ cpm/μg) of ^{32}P-end-labeled oligonucleotide probe (14). After 12- to 16-hr incubation, the membranes were washed for 30 min at room temperature in 1× SET (150 mM NaCl/20 mM Tris·HCl, pH 7.8/1 mM Na_2 EDTA) containing 0.5% SDS, followed by a 30-min wash at the indicated temperature. Oligodeoxynucleotide probe sequences and wash temperatures were as follows:

Universal probe (8):	ACG GGC GGT GTG TRC	(45°C)
Archaeal probe (14):	GTG CTC CCC CGC CAA TTC CT	(56°C)
Eubacterial probe (14):	GCT GCC TCC CGT AGG AGT	(45°C)
Eukaryote probe (15):	GGG CAT CAC AGA CCT G	(40°C)
Negative control (15):	GTG CCA GCM GCC GCG G	(45°C)

Dried membranes were exposed to preflashed film (Kodak XRP-5) in the presence of an intensifying screen for 1–24 hr at −80°C. Autoradiographic signals were quantified from video images with an interpretive densitometer (Scanalytics, Billerica, MA) with zero-dimensional analysis software. The percentage group-specific rRNA was estimated as the slope of the group-specific probe bound per unit of rRNA, divided by the slope of the universal probe bound per unit of rRNA. Values were background corrected and normalized to the slopes of group-specific probe bound per unit of rRNA for heterologous or homologous rRNA standards, as described by Giovannoni *et al.* (3). The standard homologous and heterologous rRNAs used were as follows: Archaea: *Haloferax volcanii, Desulfurococcus* strain SY, *Sulfolobus solfataricus, Methanobacterium thermoautotrophicum, Methanococcus jannaschii.* Bacteria: *Synechococcus* PCC 6301, *Bacillus megaterium, Escherichia coli, Oceanospirillum linum, Alteromonas macleodii*; Eukarya: *Aequorea victoria, Saccharomyces cerevisiae, Alexandrium fundyense.*

Ribosomal DNA Cloning and Sequencing. Amplified DNA from three to five separate reactions was pooled, phenol/chloroform, 1:1-extracted, chloroform-extracted, ethanol-precipitated, and resuspended in one-tenth vol sterile distilled water (16). The purified, amplified archaeal rDNAs were cloned by using a commercially prepared vector (TA cloning system; Invitrogen, San Diego). Insert-containing clones were identified by agarose gel electrophoresis of small-scale plasmid preparations (16). Denatured, double-stranded plasmid templates were sequenced by the dideoxy nucleotide chain-termination method with Sequenase 2.0 (United States Biochemical) following the manufacturer's recommendations. Universal rRNA-specific sequencing primers (13) and M13 forward and reverse primers (16) were used in sequencing reactions.

Phylogenetic Analyses. Sequences were aligned to a data base of previously determined rRNA sequences obtained from the Ribosomal RNA Database Project (11). GenBank accession numbers for sequences determined in this study are as follows: SBAR 5, M88075; SBAR 1A, M88074; WHAR Q, M88079; WHAR N, M88078; SBAR 12, M88076; and SBAR 16, M88077. Least-squares distance matrix analyses (17) were based on evolutionary distances estimated from similarity values and using the correction of Jukes and Cantor (18). The sequence-editing and distance-analyses software of Olsen (17) was obtained through the Ribosomal RNA Database Project (11). Parsimony "bootstrap" analyses (19, 20) were performed by using PAUP (version 3.0s; D. L. Swofford). The PHYLIP package was used for maximum-likelihood analyses (version 3.4; J. Felsenstein). All analyses were restricted to comparison of 740 highly to moderately conserved sequence positions. Only those positions represented by a known base in all sequences were used in the analyses.

RESULTS

Fig. 1*A* demonstrates the selectivity of the rRNA-specific, archaeal-biased PCR primers. The archaeal-biased primers do not yield amplification products from nucleic acid templates derived from either eubacteria or eukaryotes (Fig. 1*A*, lanes G and H; Fig. 1*B*, lane D). However, DNA from representative archaea (Fig. 1*A*, lanes I–M), as well as DNA extracted from mixed bacterioplankton populations (Fig. 1*B*, lanes F, H, J, and P; Table 1), yielded PCR products of the predicted size (≈950 base pairs) when amplified with the archaeal-biased primers. Positive archaeal rDNA amplifications were obtained with nucleic acids extracted from coastal bacterioplankton populations collected in the Santa Barbara Channel (Fig. 1*B*, lane J), Woods Hole (Fig. 1*B*, lane H), Oregon coastal waters (Table 1), and Santa Monica Basin (Table 1). Archaeal rDNA genes were not detected in bacterioplankton populations from surface waters of the Pacific and Atlantic central ocean gyres (Fig. 1*B*, lanes L and N; Table 1), consistent with previous reports of both "shotgun"

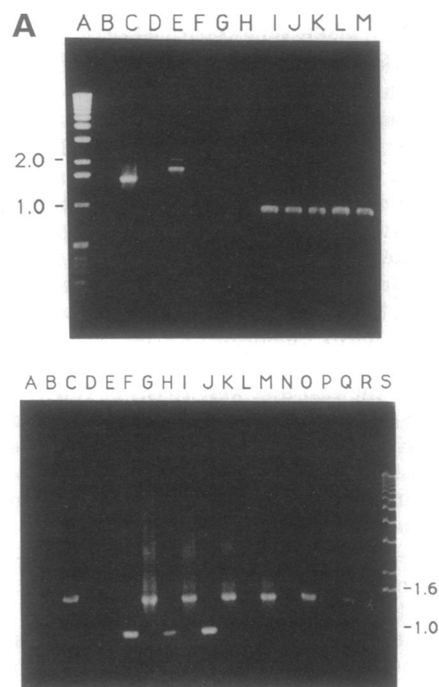

FIG. 1. Amplification of archaeal rDNA from pure cultures and mixed populations using archaeal-biased PCR primers. (*A*) Reactions contained the following domain-specific amplification primers: bacteria-specific, Eubac8F/1492R (lanes B and C); eukarya-specific, EukF/EukR (lanes D and E); archaea-specific, Arch21F/Arch958R (lanes F–M). Reactions contained the following DNA templates: negative control, no DNA (lanes B, D, and F); bacterial DNA, *Sh. putrefaciens* (lanes C and G); eukaryal DNA, *Alexandrium fundyense* (lanes E and H); archaeal DNA, *Methanococcus jannaschii* (lane I); *Methanobacterium thermoautotrophicum*, (lane J); *H. volcanii* (lane K); *Pyrococcus* strain GBD (lane L); *Desulfurococcus* strain SY (lane M). Lane A contains a 1-kilobase ladder (Bethesda Research Laboratories). (*B*) Reactions contained the following domain-specific amplification primers: Eubac8F/1492R (lanes A, C, E, G, I, K, M, O, and Q); Arch21F/Arch958R (lanes B, D, F, H, J, L, N, P, R). Reactions contained DNA extracted from the following organisms or concentrated natural populations: negative control, no DNA (lanes A and B); *Sh. putrefaciens* (lanes C and D); *H. volcanii* (lanes E and F); Woods Hole bacterioplankton (8/13/90) (lanes G and H); Santa Barbara Channel bacterioplankton (10/9/90) (lanes I and J); Central North Pacific bacterioplankton (12/3/88) (lanes K and L); Sargasso Sea bacterioplankton (5/4/87) (lanes M and N); Santa Barbara Channel marine aggregates (10/12/90) (lanes O and P); Santa Barbara Channel marine aggregates (6/4/91) (lanes Q and R). Lane S contains a 1-kilobase ladder.

Table 1. Detection of archaeal rDNA and rRNA in bacterioplankton nucleic acid extracts

Sample site	Date	Amp†	Group-specific rRNA,* %		
			Archaea	Bacteria	Eucarya
Central Pacific	12/3/88	−	ND	98.3	0.4
Woods Hole	8/13/90	+	0.1	67.7	6.8
Santa Barbara	10/9/90	+	—	—	—
Woods Hole	3/9/91	+	ND	70.0	22.9
Santa Monica Basin	3/12/91	+	—	—	—
Santa Barbara	6/4/91	+	2.3	54.5	41.2
Santa Barbara	6/5/91	+	1.0	52.5	46.9
Santa Barbara	6/6/91	+	1.6	64.2	52.6
Oregon coast	7/23/91	+	—	—	—

ND, not detected; —, experiment not done.
*See text for methods.
†DNA that yielded PCR amplified (Amp) products of the predicted size with archaeal-biased primers are indicated by a +.

cloning (5) and archaeal-specific hybridization probe analyses (3).

The relative proportions of eubacterial, eukaryotic, and archaeal rRNA in nucleic acid extracts were estimated to verify the presence of archaea in coastal bacterioplankton populations. Specific rRNAs were quantified by measuring the amount of radiolabeled, group-specific oligonucleotide probe that bound to serial dilutions of mixed-population rRNAs (refs. 3 and 8; Table 1). Archaeal rRNA accounted for as much as 1.0–2.3% of the total rRNA (up to 4% of the total prokaryotic rRNA) in samples taken in June 1991 in the Santa Barbara Channel (Table 1). A smaller but measurable proportion of archaeal rRNA was detected in one Woods Hole bacterioplankton sample.

Archaeal rDNA libraries were prepared from bacterioplankton DNA samples collected from several different sampling sites and times (Woods Hole, 8/13/90; Santa Barbara Channel, 10/9/90, 10/12/90, 6/4/91; Oregon coast, 7/23/91). Partial-sequence analyses of 20 clones from each library revealed the presence of only two major sequence variants in samples from the Santa Barbara Channel, Woods Hole, and Oregon coastal waters (data not shown). The entire sequence of the ≈950-base-pair rDNA insert from six different clones was determined and aligned with a data base of known rRNA sequences (11). Phylogenetic analysis revealed that both groups were phylogenetically distinct from known archaea (Fig. 2). Diagnostic features of archaeal rRNAs (22–25), including a 7-nucleotide bulged loop between the sixth and seventh base pair in the stalk between positions 500–545 (*E. coli* numbering, Fig. 3), and the absence of bulged nucleotides at positions 31 (Table 2), were characteristic of all cloned sequences. All cloned rDNAs contained diagnostic archaeal signature nucleotides or features (25) in 35 out of the 37 relevant positions (Table 2). Secondary-structural models of the cloned rRNAs are consistent with the proposed structure of archaeal rRNA (22, 23) and indicate that the cloned rDNAs were derived from functional rRNA genes. No evidence for chimeric PCR artifacts was observed in the primary or secondary-structural features of the cloned genes.

Phylogenetic analyses using distance, maximum-likelihood, or parsimony analyses consistently placed the cloned rDNAs within one of two groups in the domain Archaea (groups I and II, Fig. 2). Unrestricted similarity values between groups I and II and other characterized archaea ranged from 0.67 to 0.80, well within the range of similarities between known archaea. Distance analyses consistently placed group II within the Euryarchaeota (Fig. 2). Parsimony analyses yielded similar results. Group II fell within the Euryarchaeota in 100 out of 100 "bootstrap" parsimony trees (19, 20), and specifically within the *Thermoplasma*–halophile–*Methanomicrobiales* cluster in 80 out of 100 trees (data not shown). Intradomain signature analysis (25) also indicated a Euryarchaeotal affiliation for group II: 8 out of 12 relevant nucleotide positions in group II sequences were identical with the Euryarchaeotal signature (data not shown).

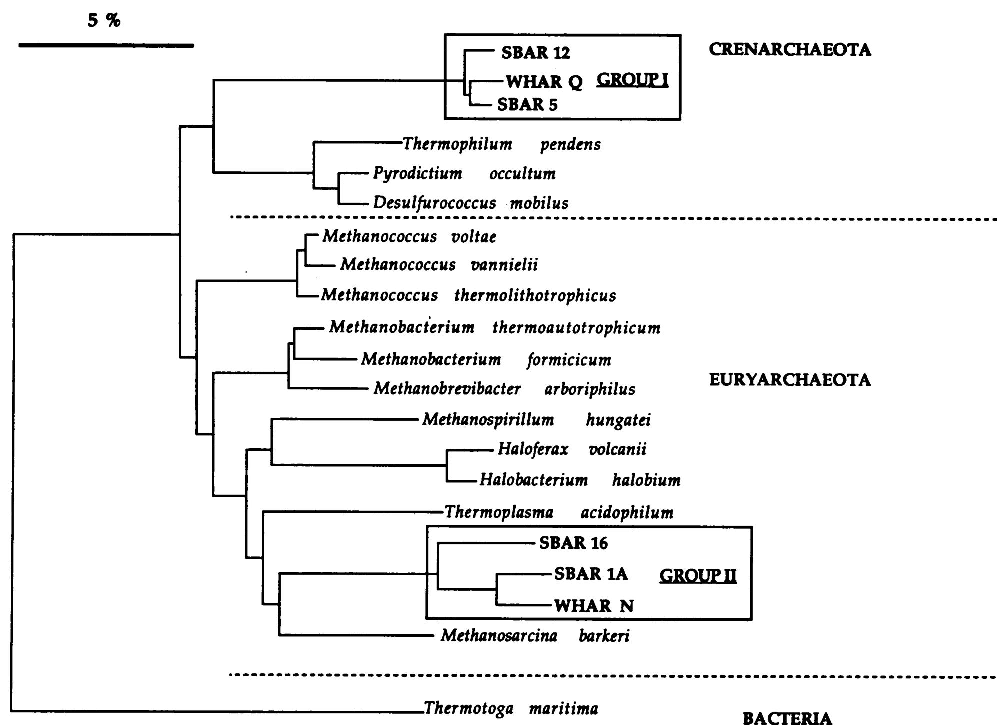

FIG. 2. Phylogenetic analysis of bacterioplankton archaeal rDNAs. Scale bar represents 5 fixed mutations per 100-nucleotide sequence positions. The sample origin for each clone is as follows: SBAR 5, Santa Barbara Channel bacterioplankton, 10/9/90; SBAR 1A, Santa Barbara Channel marine macroaggregate sample, 10/12/90; WHAR Q, WHAR N, Woods Hole bacterioplankton, 8/13/90; SBAR 12, SBAR 16, Santa Barbara Channel bacterioplankton, 6/4/91. Least-squares distance-matrix analyses (17) based on evolutionary distances were estimated from similarity values by using the correction of Jukes and Cantor (18) and transversion-distance analyses (21).

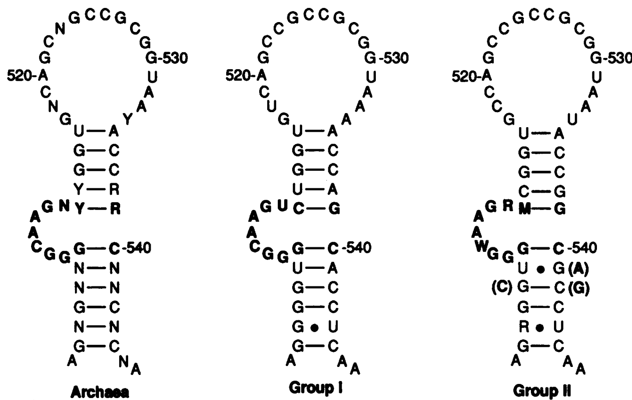

FIG. 3. Conserved archaeal secondary structure in the region between positions 499–546 (*E. coli* numbering). The archaeal consensus secondary structure (25) is compared with that of group I and group II. The 7-nucleotide bulged loop and flanking base pairs are outlined in boldface type. Nucleotides in parentheses were present in only one member of the group.

Phylogenetic placement of group I was more problematic. The large evolutionary distance separating group I from other archaea may indicate that it is a more rapidly evolving, "fast clock" lineage, complicating the phylogenetic analysis (23). Base-compositional differences may also lead to artifacts in analyses, as has been shown for thermophilic lineages that have higher than average G + C ratios in their rDNAs (21, 26). The Crenarchaeota lineage consists entirely of extreme thermophiles, which have relatively high rDNA G + C ratios (≈0.63–0.67; ref. 21). Paradoxically, although the rDNA G + C ratio of group I archaea is low (0.51), this group shares 7 out of 12 relevant signature nucleotides in common with the Crenarchaeota (data not shown; ref. 25). Hence, low G + C content of group I may, in part, account for difficulties encountered in its phylogenetic placement. Transversion analysis, which removes some of the biases associated with base-compositional differences (21), supports this hypothesis (purine content in groups I and II rDNAs is constant, as in other archaea; ref. 21): Transversion-distance analysis most frequently placed group I within the Crenarchaeota, whereas the phylogenetic placement of group II remained unchanged (Fig. 2). In transversion-parsimony bootstrap analyses, group I was specifically affiliated with the Crenarchaeota in 67 out of 100 trees. Exact phylogenetic placement of group I necessarily awaits the acquisition of more extensive small-subunit rRNA sequence data, as well as other phenotypic and genotypic data, from members of this group.

DISCUSSION

The diversity of archaeal rDNA clones recovered from marine habitats was limited, in striking contrast to the phylogenetic diversity found in eubacterial rDNA libraries originating from these and other samples (3, 5). Only two major archaeal lineages were detected in bacterioplankton populations collected from both coasts. Each of these lineages is distinct from any previously cultured archaeal group. Phylogenetic analysis revealed that one lineage, group II, is loosely affiliated with the most physiologically diverse branch of the Euryarchaeota, the *Methanomicrobiales*. The other archaeal lineage identified in this study, group I, shares no close evolutionary relationship with previously cultured Archaea. Nevertheless, it is clear from the secondary structure and nucleotide-signature analysis that group I shares the diagnostic features common to all Archaea (Table 2; refs. 23–25).

Although collected in oxygenated surface waters, the possibility that these recently detected archaea emanate from some other source must be considered. Hyperthermophilic archaea survive oxic conditions well at low temperatures (27) and have been detected in marine surface waters after violent volcanic eruptions, arriving with the advecting hydrothermal plume (28). It seems unlikely that the marine archaeal groups reported here are hyperthermophiles because their rDNA G + C ratios range from 0.51 (group I) to 0.55–0.57 (group II). Most known hyperthermophilic archaea have rDNA G + C contents ranging from 0.60 to 0.69 (21). Potential anaerobic niches, found in submarine hydrocarbon seeps of the Santa Barbara Channel (29) or in marine sediments in general, represent other possible allochthonous sources for these unusual microbes. Although it is possible that these unusual archaeal groups originate from resuspended sediment material, their consistent presence and relative abundance in surface waters render this explanation unlikely.

In the sea, archaeal habitats are thought to be limited to shallow or deep-sea anaerobic sediments (free-living and endosymbiotic methanogens; ref. 30), deep-sea hydrothermal vents (methanogens, sulfate reducers, and extreme thermophiles; refs. 31 and 32), and highly saline land-locked seas (halophiles; ref. 33). The relatively large proportion of archaeal rRNA in coastal bacterioplankton nucleic acid ex-

Table 2. Interdomain signature analysis of group I and group II archaea

Nucleotide position	Base pair or feature				
	Eukarya	Bacteria	Archaea	Group I	Group II
31 bulged base?	No	Yes	No	No	No
33:551	A·U	A·U	Y·R	C·G	C·G
44.1:397	–·A	–·A	U·A	U·A	U·A
47.1 extra base?	Yes	No	Yes	Yes	Yes
52:359	G·C	Y·R	G·C	G·C	G·C
53:358	C·G	A·U	C·G	C·G	C·G (A·U)
113:314	C·G	G·C	C·G	C·G	C·G
121	A	Y	C	C	C
292:308	R·U	G·C	G·C	G·C	G·C
307	Y	Y	G	G	G
335	A	C	C	C	C
338	A	A	G	G	G
339:350	C·G	C·G	G·Y	G·C	R·Y
341:348	U·A	C·G	C·G	C·G	C·G
361	C	R	C	A	C
365	A	U	A	A	A
367	U	U	C	C	C
377:386	Y·R	R·Y	Y·G	U·A	C·G
393	A	A	G	G	G
500:545	U·A	G·C	G·C	G·C	G·C
514:537	G·C	Y·R	G·C	G·C	G·C
549	C	C	U	U	U
558	A	G	Y	U	C(A)
569:881	G·C	Y·R	Y·R	C·G	C·G
585:756	U·A	R·Y	C·G	C·G	C·G
674:716	R·Y	G·A	G·C	G·C	G·C
675:715	U·A	A·A	U·A	U·A	U·A
684:706	G·Y	U·A	G·Y	G·C	G·C
716	Y	A	C	C	C
867	Y	R	Y	C	G(A)
880	U	C	C	C	U
884	G	U	U	U	U
923	A	A	G	G	G
928	A	G	G	G	G
930	G	Y	A	A	A
931	G	C	G	G	G
933	A	G	A	A	A

Relevant signature nucleotides or structural features defining the three domains Eukarya, Bacteria, and Archaea (25) were compared with group I and group II archaea. Nucleotides that were present in only one member of a given group are shown in parentheses.

tracts and the widespread distribution of these microorganisms in spatially and temporally distinct coastal surface waters argue strongly for their planktonic origin. The results indicate that group I and group II archaea are relatively abundant and widely distributed in coastal marine environments. Given their phylogenetic position, group I and group II archaea could represent unusual groups of methanogenic archaea. Methanogens have recently been successfully isolated from plankton samples of oxic marine surface waters (34), and methanogenic activities in plankton samples and fish intestines have been observed as well (34, 35). Although all known free-living and endosymbiotic methanogens originate from anaerobic habitats, transient anoxic microzones (36, 37) or endosymbiotic niches (38) could support anaerobic processes in otherwise oxic environments. A more provocative explanation is that group I and group II represent undescribed mesophilic, aerobic members of the archaea, which reside and compete with eubacterial picoplankton in oxic, marine coastal waters.

The molecular phylogenetic approach described in this study has revealed two as-yet-uncultured, potentially important groups of archaea. Although determination of the phylogenetic affiliation of these groups was possible, it is difficult to predict their phenotypic properties solely on the basis of phylogenetic position. For instance, group II is affiliated with the *Methanomicrobiales*, a group that contains diverse phenotypes, including strict anaerobes, halophiles, and facultatively anaerobic chemoorganotrophs. Characterization of these additional archaeal lineages will require both phenotypic and genotypic characterization. Use of selective antibiotics, in tandem with group-specific, rRNA-targeted hybridization probes for screening purposes, should facilitate cultural isolation of group I and II archaea. It should also be possible to identify archaeal rDNA gene-containing clones in genomic libraries of mixed populations (2, 5). Linkage maps of these clones, which identify flanking genes extending from archaeal rDNA markers, should also help further insight into the nature of these two archaeal groups.

Note. While this paper was under review, the isolation of one archaeal rDNA type with a low G + C ratio, from one Pacific deep-water sample, was reported (39).

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1. Stahl, D. A., Lane, D. J., Olsen, G. J. & Pace, N. R. (1984) *Science* **244**, 409–411.
2. Olsen, G. J., Lane, D. J., Giovannoni, S. J., Pace, N. R. & Stahl, D. A. (1986) *Annu. Rev. Microbiol.* **40**, 337–365.
3. Giovannoni, S. J., Britschgi, T. B., Moyer, C. L. & Field, K. G. (1990) *Nature (London)* **345**, 60–62.
4. Ward, D. M., Weller, R. & Bateson, M. M. (1990) *Nature (London)* **345**, 63–65.
5. Schmidt, T. M., DeLong, E. F. & Pace, N. R. (1991) *J. Bacteriol.* **173**, 4371–4378.
6. Ward, D. M., Bateson, M. M., Weller, R. & Ruff-Roberts, A. L. (1992) *Adv. Microbiol. Ecol.*, in press.
7. Amann, R., Springer, N., Ludwig, W., Gortz, H. D. & Schleifer, W. (1991) *Nature (London)* **351**, 161–164.
8. Stahl, D. A., Flesher, B., Mansfield, H. R. & Montgomery, L. (1988) *Appl. Environ. Microbiol.* **54**, 1079–1084.
9. Sakai, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–494.
10. Medlin, L., Ellwood, H. J., Stickel, S. & Sogin, M. L. (1988) *Gene* **71**, 491–499.
11. Olsen, G. J., Overbeek, R., Larsen, N. & Woese, C. R. (1991) *Nucleic Acids Res.* **19**, 4817.
12. Hobbie, J. E., Daley, R. J. & Jasper, S. (1977) *Appl. Environ. Microbiol.* **33**, 1225–1228.
13. Lane, D. J. (1991) in *Nucleic Acid Techniques in Bacterial Systematics*, eds. Stackebrandt, E. & Goodfellow, M. (Wiley, New York), pp. 115–175.
14. Stahl, D. A. & Amman, R. (1991) in *Nucleic Acid Techniques in Bacterial Systematics*, eds. Stackebrandt, E. & Goodfellow, M. (Wiley, New York), pp. 205–248.
15. Giovannoni, S. J., DeLong, E. F., Olsen, G. J. & Pace, N. R. (1988) *J. Bacteriol.* **170**, 720–726.
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
17. Olsen, G. J. (1988) *Methods Enzymol.* **164**, 793–812.
18. Jukes, T. H. & Cantor, C. R. (1969) in *Evolution of Protein Molecules*, ed. Monro, H. N. (Academic, New York), pp. 21–132.
19. Swofford, D. L. & Olsen, G. J. (1990) in *Molecular Systematics*, eds. Hillis, D. M. & Moritz, C. (Sinauer, Sunderland, MA), pp. 411–501.
20. Felsenstein, J. (1988) *Annu. Rev. Genet.* **22**, 521–565.
21. Woese, C. R., Achenbach, L., Rouviere, P. & Mandelco, L. (1991) *Syst. Appl. Microbiol.* **14**, 364–371.
22. Woese, C. R., Gutell, R., Gupta, R. & Noller, H. F. (1983) *Microbiol. Rev.* **47**, 621–669.
23. Woese, C. R. (1987) *Microbiol. Rev.* **51**, 221–271.
24. Woese, C. R., Kandler, O. & Wheelis, M. L. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4576–4579.
25. Winker, S. & Woese, C. R. (1991) *Syst. Appl. Microbiol.* **14**, 305–310.
26. Weisburg, W. G., Giovannoni, S. J. & Woese, C. R. (1989) *Syst. Appl. Microbiol.* **11**, 128–134.
27. Jannasch, H. W., Wirsen, C. O., Molyneaux, S. J. & Langworthy, T. (1988) *Appl. Environ. Microbiol.* **54**, 1203–1209.
28. Huber, R., Stoffers, P., Cheminee, J. L., Richnow, H. H. & Stetter, K. O. (1990) *Nature (London)* **345**, 179–182.
29. Allen, A. A., Schlueter, R. S. & Mikolaj, P. G. (1970) *Science* **170**, 974–977.
30. Whitman, W. B., Bowen, T. L. & Boone, D. R. (1992) in *The Prokaryotes*, eds. Balows, A., Truper, H. G., Dworkin, M., Harder, W. & Schleifer, K. H. (Springer, Berlin), pp. 719–767.
31. Brock, T. D. (1978) *Thermophilic Microorganisms and Life at High Temperatures* (Springer, Berlin).
32. Stetter, K. O., Fiala, G., Huber, R. & Segerer, A. (1990) *FEMS Microbiol. Rev.* **75**, 117–124.
33. Tindall, B. J. (1992) in *The Prokaryotes*, eds. Balows, A., Truper, H. G., Dworkin, M., Harder, W. & Schleifer, K. H. (Springer, Berlin), pp. 768–808.
34. Cynar, F. J. & Yayanos, A. A. (1991) *Curr. Microbiol.* **23**, 89–96.
35. Oremland, R. S. (1979) *Limnol. Oceanogr.* **24**, 1136–1141.
36. Alldredge, A. A. & Cohen, Y. (1979) *Science* **235**, 689–691.
37. Pearl, H. & Prufert, L. (1987) *Appl. Environ. Microbiol.* **53**, 1078–1087.
38. Vogels, G. D., Hoppe, W. F. & Stumm, C. K. (1980) *Appl. Environ. Microbiol.* **40**, 608–612.
39. Fuhrman, J. A., McCallum, K. & Davis, A. A. (1992) *Nature (London)* **356**, 148–149.