

Stratified Communities of Active Archaea in Deep Marine Subsurface Sediments

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Archaeal 16S rRNA was extracted from samples of deep marine subsurface sediments from Peru Margin site 1227, Ocean Drilling Program leg 201. The amounts of archaeal 16S rRNA in each extract were quantified by serial dilution and reverse transcription (RT)-PCR. The results indicated a 1,000-fold variation in rRNA content with depth in the sediment, with the highest concentrations found near the sediment surface and in the sulfate-methane transition zone (SMTZ). The phylogenetic composition of the active archaeal population revealed by cloning and sequencing of RT-PCR products changed with depth. Several phylotypes affiliated with marine benthic group B (MBGB) dominated clone libraries from the upper part of the SMTZ and were detected only in this layer. Members of the miscellaneous crenarchaeotal group (MCG) dominated clone libraries from the other layers. These results demonstrate that archaeal communities change in activity and community composition over short distances in geochemically distinct zones of deep seafloor sediments and that these changes are traceable in the rRNA pool. It was shown for the first time that members of both the MCG and MBGB *Archaea* are more active in the SMTZ than in layers above and below. This indicates that they benefit either directly or indirectly from the anaerobic oxidation of methane. They also appear to be ecophysiologically flexible, as they have been retrieved from a wide range of marine sediments of various geochemical properties.

Results obtained through the Deep Sea Drilling Project and Ocean Drilling Program (ODP) have shown that marine subsurface sediment constitutes one of the largest and most widespread reservoirs of biomass on Earth and that the activities of subsurface prokaryotes have profound implications for the global carbon cycle (9, 10, 11, 29, 37, 38, 55). Numerous studies have documented diverse prokaryotic communities in subsurface sediments by extraction and analysis of genomic DNA material (17, 35, 41, 45), but since most of the obtained phylotypes belong to previously unknown and uncultivated groups, the physiological properties of the subsurface organisms remain unknown. DNA may persist in intact but inactive cells as well as in the environment as extracellular material after cell death (8, 34). Since DNA-based gene surveys do not distinguish between living and dead populations, it has remained unclear whether the detected phylotypes reflect metabolically active organisms. This problem has led to the “paleome” concept, wherein deep-subsurface sediments and marine sedimentary rocks are viewed as repositories for inactive cells and ancient DNA (16, 18), and to ensuing debate about this interpretation of microbial community DNA signatures (14). In contrast to genomic DNA, ribosomes are being continuously turned over in cells and the concentration of rRNA is correlated to the growth rate (23, 24, 25, 28, 30). Thus, by analyzing rRNA rather than genomic DNA in sediment samples, it is

possible to specifically target the active part of the microbial community and to more directly correlate biogeochemical gradients with the activity of specific phylogenetic groups (32, 33, 52, 53). This could be of particular value in subsurface sediments where a large proportion of the cells may be inactive.

Between one-third and one-tenth of the prokaryotic cells in subsurface sediment samples from ODP site 1227 at the Peru Margin contain sufficient 16S rRNA for staining with catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH). Active *Archaea* were not detected with this approach, but the presence and distribution of archaeal genes for 16S rRNA was documented through quantitative PCR amplification (42). In another study, archaeal genes for 16S rRNA were amplified and characterized from four different depths in the sediment column at site 1227 (19). The detected phylotypes were affiliated with a number of uncultured groups of *Archaea*, and their physiological status and ecological significance could not be determined. Recently, a combination of intact phospholipid (IPL) extraction, FISH, and rRNA analysis demonstrated that *Archaea* dominate the active prokaryotic community in subsurface sulfate-methane transition zones (SMTZ) from several different ODP sites on the Peru Margin, including site 1227, where up to 98% of the detected cells hybridized with an *Archaea*-specific oligonucleotide probe (3). However, depth-related changes of the active archaeal community, for example, between zones of net methanogenesis and methane consumption, were not addressed.

In the present study, the active archaeal communities were characterized throughout the upper 50 m of sediment at Peru Margin site 1227 by extraction and analysis of rRNA. By selecting for metabolically active organisms, changes in archaeal

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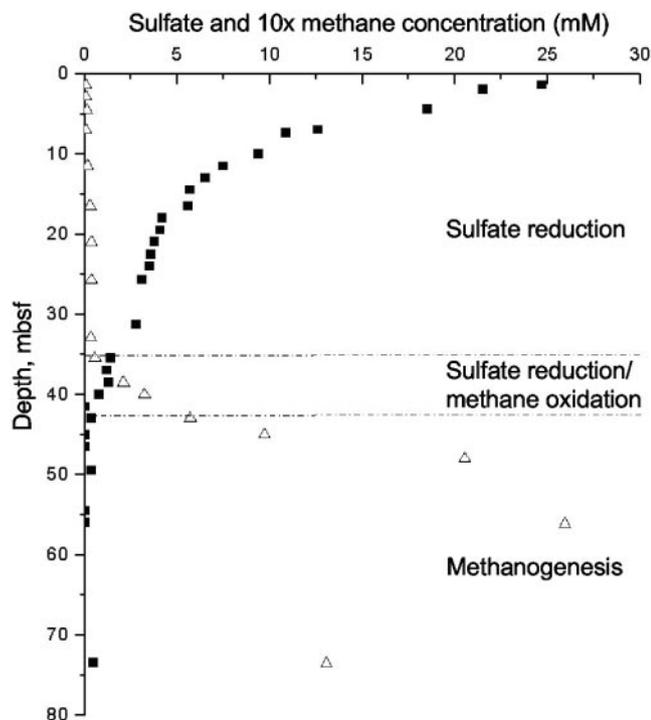


FIG. 1. Concentration profiles of sulfate (black squares) and methane (open triangles) at site 1227 (redrawn from reference 17).

activity and community composition were shown to be related to the strong geochemical gradients that exist at this site.

MATERIALS AND METHODS

Study site and sampling. ODP site 1227 is located on the Peru continental shelf within the upwelling zone in 427 m of water (44). The sediment column is characterized by strong geochemical gradients in concentrations of dissolved sulfate and dissolved methane (Fig. 1) (44). The concentration of dissolved sulfate declines rapidly in the upper 15 m of sediment and more slowly below this level, down to about 41 m below the seafloor (mbsf), where sulfate is depleted. Methane accumulates to several millimolar concentrations below the sulfate-containing zone and is consumed in the layers between 35 and 41 mbsf, presumably with sulfate serving as the electron acceptor. The sediment can thus be divided into three zones, each characterized by different overall methane/sulfate dynamics: an upper zone from 0 to 35 mbsf, where the sulfate concentration decreases gradually with depth and where methane is present only in trace amounts; a transition zone from 35 to 41 mbsf, where sulfate is depleted by microbially mediated reactions with methane; and a deep methanogenic zone below 41 mbsf. Samples were collected during ODP leg 201 in 2001 under strict contamination controls, as have been described elsewhere (15).

Extraction of RNA. RNA was extracted from samples of boreholes 1227A (2H-2, 7.35 m; 3H-5, 21.35 m; 5H-3, 37.35 m; 5H-5, 40.35 m; 6H-2, 45.35 m; 6H-5, 49.85 m) and 1227D (1H-5, 6.55 m; 4H-6, 34.25 m; 5H-2, 37.75 m). About 4 ml of sediment was retrieved from the center of each sample core with sterile spatulas and immediately mixed with 10 ml of a 1:1 mixture of extraction buffer (250 mM sodium acetate, 5 mM EDTA, 2% sodium dodecyl sulfate [pH 5]) and phenol (pH 5). The mixture was distributed into 2-ml bead-beating tubes containing 0.5 g of 0.1-mm zirconium beads (BioSpec Products, Inc., Bartlesville, OK). The tubes were homogenized on a FastPrep FP120 homogenizer (Qbiogene, Inc., Carlsbad, CA) for 30 s at level 6.5 and subsequently centrifuged at $10,000 \times g$ for 5 min at 4°C. The aqueous phases were transferred to new 1.5-ml centrifuge tubes and kept on ice. The phenol phases, containing sediment and beads, were subjected to two more rounds of homogenization, centrifugation, and transfer of the water phases, each time with 300 μ l of new extraction buffer. The combined aqueous phases from the three rounds of homogenization were extracted three times by vortexing and centrifugation with equal volumes of phenol, phenol-chloroform, and finally chloroform. Extracted RNA was precip-

itated for 2 h at -20°C with 0.5 volumes of 7.5 M ammonium acetate and 1 volume of isopropanol. The extract was spun down, washed with 80% ethanol, air dried, and finally resuspended 86 μ l of water. To digest trace amounts of DNA in the sample, 10 μ l of $10\times$ DNase buffer and 4 μ l of DNase (Fisher) was added and the sample was digested for 30 min at 37°C . The sample was finally purified with the RNeasy minikit (QIAGEN Inc.) according to the manufacturer's instructions. Parallel blind extractions, in which the entire extraction procedure was performed without addition of sample, were consistently performed, and in case the control yielded a visible reverse transcription (RT)-PCR product, the extraction was redone.

RNA quantification. Each extraction product was serially diluted between 1 and 2,187 times in a $3\times$ dilution series. Template (2 μ l) from each dilution was reverse transcribed and amplified in 50- μ l reaction mixtures with the archaeal primers ARC8f (5'-TCCGGTTGATCTGCC-3') and either ARC915r (5'-GTGCTCCCCGCCAATTCCT-3') or ARC518r (5'-GGT(G/A/T)TTACCGCGG C(G/T)GCTG-3') using the One-Step RT-PCR kit (QIAGEN Inc.). The RT-PCR program consisted of 30 min at 50°C and 15 min at 95°C , followed by 35 cycles of 30 s at 92°C , 30 s at 58°C , and 4 min at 72°C . The final elongation step was increased to 10 min. To test for the presence of trace amounts of DNA in the RNA extracts, the extracts were used as templates in PCRs. No PCR products were observed. Upon amplification, the entire volume of each RT-PCR product was loaded onto a 1% low-melting-point agarose gel, run at 70 V for 40 min, and stained with SYBR gold (Bio-Rad). DNA on the gels was visualized with a Dark Reader transilluminator (Clare Chemicals, Dolores, CO), and the smallest amount of template from which a visible amount of DNA could be obtained was recorded. This served as a relative measure of the amount of RNA in the extraction product. Coextraction of unknown PCR inhibitors hindered PCR amplification of undiluted template amounts larger than 2 μ l. DNA bands were excised and extracted from the gel with the QIAEXII gel extraction kit, according to the manufacturer's instructions.

Phylogenetic analysis. The excised RT-PCR products were cloned as described previously (45). Sequence data were obtained from the sequencing facility at the Marine Biological Laboratory (Woods Hole, MA). Sequences of more than 98% similarity were regarded as identical phylotypes. The obtained phylotypes were BLAST analyzed against the GenBank 16S rRNA database (1). The sequences were aligned with representative members of the *Euryarchaeota* and *Crenarchaeota* with the CLUSTAL W program, followed by manual alignment. Bootstrapping of the data set, calculations of Jukes-Cantor distance matrices, and tree construction by neighbor joining or maximum parsimony was performed with the PHYLIP software package (12). The sequence data set was screened for potential chimeric structures by using the CHECK_CHIMERA application at the Ribosomal Database Project website and by constructing phylogenetic trees from three different fragments of the sequence alignment (bp 1 to 250, bp 251 to 500, and bp 501 to the end of the sequence). Sequences found to be of potential chimeric origin either through the screening or due to inconsistent positions in the phylogenetic trees were excluded from this study.

Diversity analysis. The total number of phylotypes in each clone library was estimated by calculating S_{Chao1} estimators (5, 6):

$$S_{\text{Chao1}} = S_{\text{obs}} + \frac{F_1^2}{2(F_2 + 1)} + \frac{F_1 \cdot F_2}{2(F_2 + 1)^2}$$

where S_{obs} is the number of different phylotypes detected in a clone library and F_1 and F_2 are the numbers of phylotypes encountered one and two times, respectively. The estimated number of phylotypes depends on the number of phylotypes detected and is prone to underestimation in insufficiently sampled datasets. To evaluate the stability of the obtained phylotype richness estimates, S_{Chao1} estimators were calculated for randomly sampled subsets, including one-fourth, one-half, or three-fourths of the clone libraries (22).

Nucleotide sequence accession numbers. The phylotypes detected in this study are available from the GenBank nucleotide database, at the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov>), under accession numbers DQ301959 to DQ302007.

RESULTS

RNA quantification. RNA was successfully extracted, reverse transcribed, and amplified from seven of the nine samples (Fig. 2a). The amounts of archaeal 16S rRNA extracted from the sediment samples were highly dependent on depth in the sediment column. The concentration decreased with depth down to 34 mbsf, where no rRNA could be amplified, indicat-

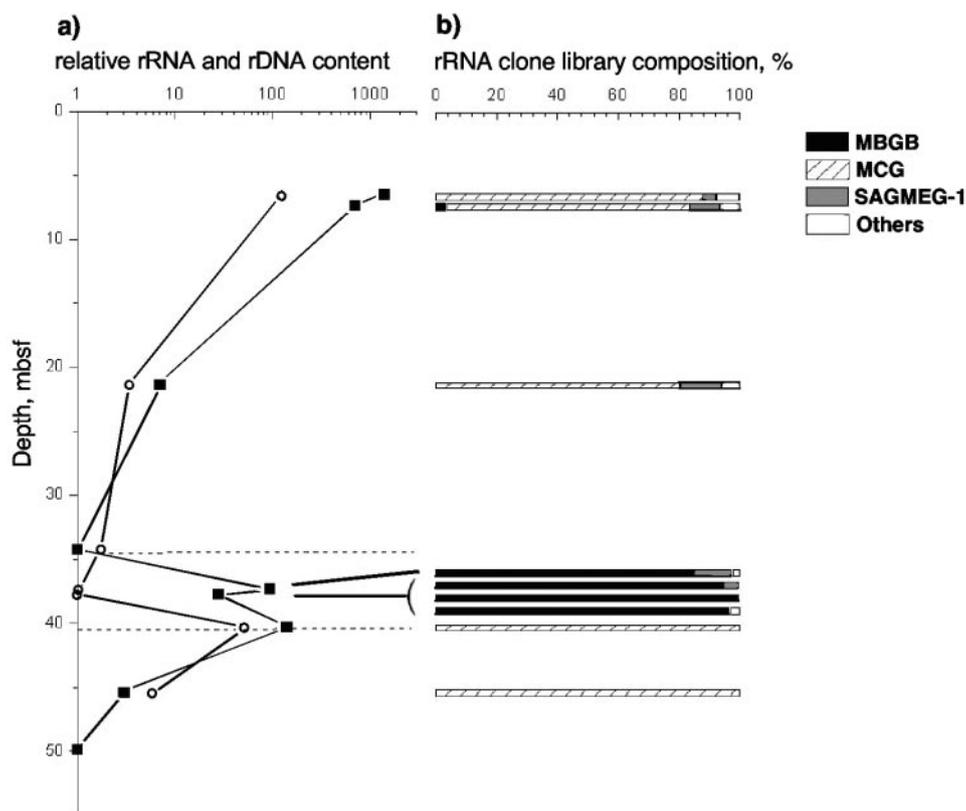


FIG. 2. Results of 16S rRNA extraction and analysis. (a) Relative amounts of 16S rRNA in extracts from different depths (black squares). A value of 1 corresponds to the detection limit. The data are compared with the amounts of archaeal genes for 16S rRNA extracted from similar depths in the sediment and quantified by Q-PCR (open circles) (redrawn from reference 42). These numbers were normalized such that the amount of DNA found at 37.75 mbsf corresponds to a value of 1. The SMTZ is indicated in the figure by the stippled lines. (b) Phylogenetic composition of RT-PCR clone libraries from each depth. The sample from 37.75 mbsf was extracted twice; the second extract was amplified with two primer sets, yielding three independent clone libraries. Groups shown include MBGB (54), MCG (17), and SAGMEG-1 (51).

ing that the concentration was at least 1,000-fold lower than at the surface. The amount of 16S rRNA then increased through the SMTZ and finally decreased again at greater depths. Also shown in Fig. 2a are relative abundances of *Archaea* detected in a previous study by quantitative PCR (Q-PCR) for 16S rRNA genes at site 1227 (42). A general trend of decreasing numbers of *Archaea* with depth and a local maximum at the SMTZ was also found with the Q-PCR approach (42). However, the relative differences between layers were smaller by about an order of magnitude, and the increased rRNA concentrations at 37 mbsf were not registered in the Q-PCR analysis.

Phylogenetic profiling. Clone libraries were constructed from the reverse-transcribed and PCR-amplified archaeal rRNA. The distribution of clones between major phylogenetic groups is summarized in Fig. 2b. Neighbor-joining trees outlining the phylogenetic positions of the detected phylotypes and close relatives are shown in Fig. 3 and Fig. 4. The phylogenetic profile of the active archaeal community changed substantially with depth. In the sediment above the transition layer, the clone libraries were dominated by phylotypes belonging to the miscellaneous crenarchaeotic group (MCG) (17, 50). Smaller numbers of clones were affiliated with South African gold mine euryarchaeotic group 1 (SAGMEG-1) (51) or other groups of uncultured *Archaea*, including the VAL III (Valkea

Kontinen) group (20), the terrestrial miscellaneous euryarchaeotic group (TMEG) (51), marine benthic group D (MBGD) (54), and a cluster of SAGMEG-1 and -2 relatives (51). In the samples from 37.35 and 37.75 mbsf, both located within the SMTZ, the clone libraries were dominated by members of marine benthic group B (MBGB) (54), a deeply rooted sister lineage to the *Crenarchaeota* that is synonymous with the deep-sea archaeal group (DSAG) (50). Samples from the bottom of the transition zone and below yielded exclusively phylotypes of MCG *Archaea*. The MCG-MBGB switch in the active archaeal community of the SMTZ was tested and reproduced twice. A second extraction of the sediment from 37.75 mbsf, RNA quantification, and sequencing of clones obtained with the same primers as before (ARC8f and ARC915r) confirmed the original results. To further evaluate the robustness of the results, a second clone library from the material of this second extraction was constructed with an alternative reverse PCR primer, ARC518r. In general, the same phylotypes were detected whether reverse primer ARC915r or ARC518r was used, but the relative frequencies of some phylotypes in the clone libraries varied depending on the primer used (Fig. 3). A new archaeal group, the ancient archaeal group (AAG), was detected with the alternative reverse primer. This group is deeply rooted within the *Euryarchaeota* and has previously been found at a deep-sea hydrothermal vent (49). Published

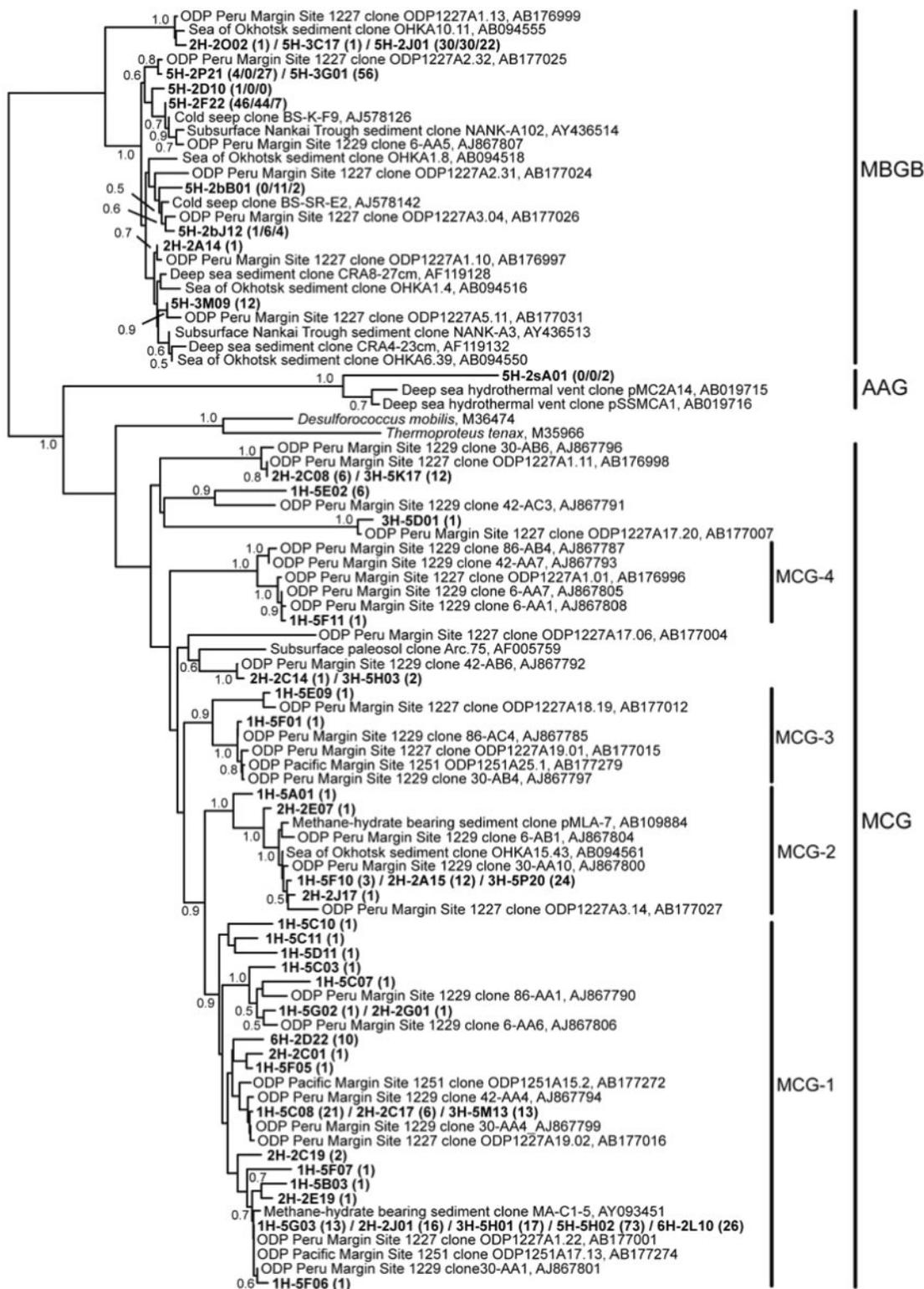


FIG. 3. Neighbor-joining tree showing phylogenetic positions of the crenarchaeotal phylotypes retrieved during this study. Phylotype names refer to sample 1H-5 (6.55 mbsf), 2H-2 (7.35 mbsf), 3H-5 (21.35 mbsf), 5H-2 (37.75 mbsf), 5H-3 (37.35 mbsf), 5H-5 (40.35 mbsf), or 6H-2 (45.35 mbsf). Some phylotypes were retrieved from several layers, as indicated. Numbers in parentheses indicates the number of times found in clone libraries. The three numbers given at each phylotype from sample 5H-2 refer to the first extraction, second extraction, and second extraction using an alternative reverse primer for PCR. Bootstrap values equal to or larger than 0.5 are indicated. The values were determined using 1,000 replicates.

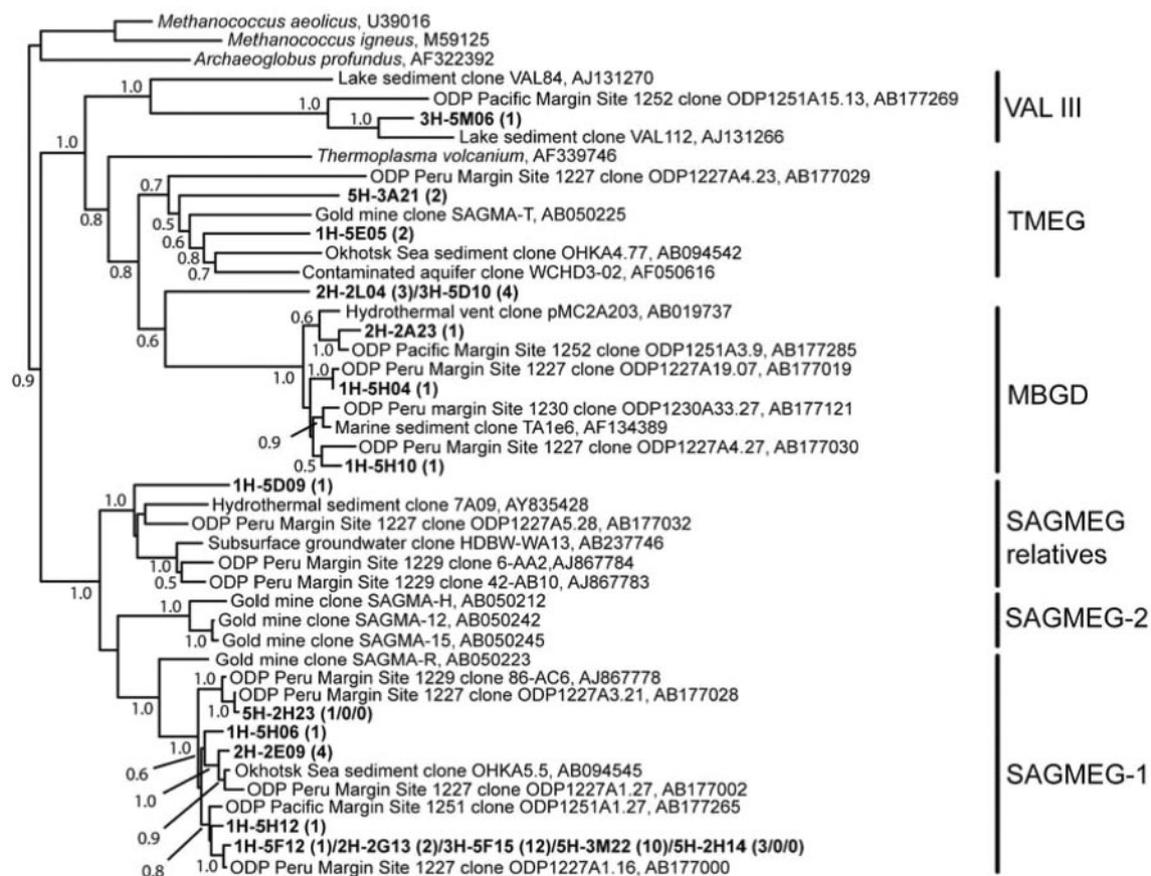


FIG. 4. Neighbor-joining tree indicating the phylogenetic positions of the euryarchaeotal phylotypes found during this study.

sequences of AAG *Archaea* show several mismatches in the target region of primer ARC915r, suggesting that the group was not detected previously due to primer mismatches.

Most of the phylotypes detected in this study were closely related or nearly identical to phylotypes found during 16S rRNA gene surveys from Peru Margin sites, as well as other subsurface marine sediments (19, 38). Several well-supported subclusters (bootstrap values 0.9 to 1.0) were identified within the MCG (Fig. 3). One of these, MCG-1, contained all phylotypes detected in and below the SMTZ. Phylotypes related to known methanogens or putative anaerobic methane oxidizers of the ANME-1 and -2 groups (4, 26, 36) were not detected in any sediment horizon.

Richness analysis. The total numbers of phylotypes were estimated to be 246, 26, and 10 in clone libraries from samples 1H-5 (6.55 mbsf), 2H-2 (7.35 mbsf), and 3H-5 (21.35 mbsf), respectively. To test the stability of these numbers, similar calculations were performed with randomly selected subsets of one-fourth, one-half, and three-fourths of the data. The results are shown in Fig. 5. No plateau was reached with sample 1H-5, suggesting that the total diversity in this clone library may be higher than the estimated 246 phylotypes (22). In contrast, estimated phylotype numbers were stable when 50% or more of the clone libraries from sample 2H-2 or 3H-5 were used, suggesting that phylotype richness had reached saturation in

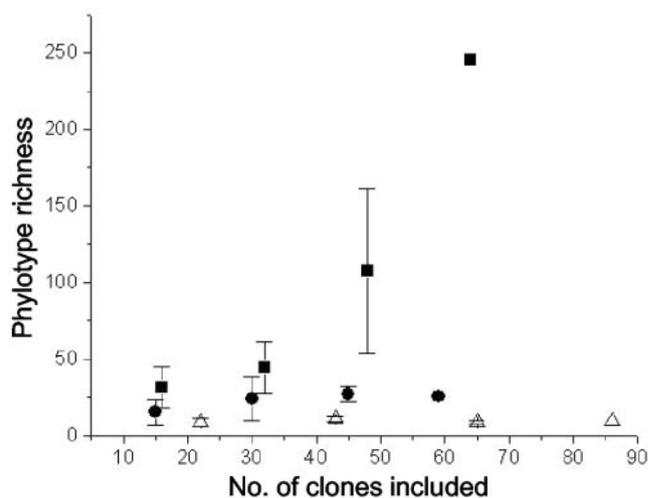


FIG. 5. S_{Chao1} estimates of total numbers of phylotypes in clone libraries from cores 1H-5, from 6.55 mbsf (squares); 2H-2, from 7.35 mbsf (circles); and 3H-5, from 21.35 mbsf (triangles). Estimators were calculated from randomly sampled subsets as well as from the entire data set from each sample (22). Error bars indicate standard deviations in richness estimates for four resamplings.

these samples. The number of different phylotypes in the clone libraries thus declined rapidly with depth above the SMTZ.

DISCUSSION

Archaeal communities in subsurface sediments. The archaeal communities in deep subsurface sediments consist largely of unknown groups of organisms and are only distantly related to cultured strains. With the exception of marine group I (13, 21, 27, 40), organisms belonging to the groups in Table 1 have been detected solely in the form of 16S rRNA gene phylotypes. Some of the groups, including MBGB, MCG, and SAGMEG-1 and -2, appear to be ubiquitous in subsurface environments, while other groups have been detected more sporadically. Since cells may be transported into and within sediments by porewater flow, and since organisms deposited on the sediment surface may become buried in deeper sediment layers during ongoing sedimentation, the distribution of gene phylotypes may or may not be correlated to metabolic activity in the sediments. As a consequence, it has not been clear whether subsurface phylotypes reflect living and actively metabolizing organisms. The finding of RT-PCR-amplifiable rRNA affiliated with MBGB, MBGD, MCG, TMEG, and SAGMEG shows that members of these groups are active in the subsurface sediment at site 1227. As is clear from this and other studies (Table 1), archaeal communities vary considerably from site to site; future research will undoubtedly demonstrate activity of further groups of organisms within subsurface sediments.

MCG. Members of the MCG have been found frequently in 16S rRNA gene surveys of both marine and terrestrial environments, and they appear to be widespread in both surface and subsurface systems (2, 7, 17, 26). The group dominated 16S rRNA gene clone libraries from ODP Peru Margin sites 1227 and 1229, ash layers of Okhotsk Sea sediments, and some sediment layers at the Nankai Trough (Table 1). The majority of the MCG-related rRNA phylotypes found by RT-PCR were closely related or even identical to phylotypes obtained by PCR amplification of genomic DNA extracted during a previously published study of the sediment at site 1227 (19). Others, e.g., the frequently recovered phylotype represented by clone 6H-2D22 within MCG-1, did not have closely related counterparts in rRNA gene clone libraries and are good candidates for highly active *Archaea* that are not dominant in DNA surveys (Fig. 3).

MBGB. Members of the MBGB were originally found in deep-sea sediments and at hydrothermal vent sites (49, 54), and the group is now detected in a growing number of benthic marine environments, including Atlantic deep-sea sediments, marine mud volcanoes, marine carbonate crusts from the Black Sea, organic-poor deep subsurface sediments of the central oceanic basins, and organic-rich methane- or methane-hydrate-containing sediments near continental margins (3, 26, 38, 45, 54). The group was recently reported to dominate clone libraries obtained from methane-hydrate-containing Peru Margin and Pacific Rim sediments, while being rare in clone libraries from nonhydrate sediments, including site 1227 (19). The RT-PCR results presented here demonstrate that the group may be abundant in certain layers of nonhydrate sediments.

TABLE 1. Frequencies of uncultured archaeal lineages in clone libraries of genes for 16S rRNA from marine subsurface sediments

Phylotype group (reference)	Frequency (%) of uncultured archaeal lineage at indicated location, depth range (mbsf) (source or reference)									
	Peru Margin site 1227 ^a 6.55–49.85 (this study)	Peru Margin site 1227, 0–50 (19)	Peru Margin site 12300–278.3 (19)	Equatorial Pacific site 1225, 1.5–7.8 (19)	Peru Margin site 1229, 6.7–86.7 (38)	Peru Basin site 1231, 1.5–43 (45)	Mediterranean sapropel ^b 0.13–3.68 (7)	Okhotsk Sea ^c 1–58 (17)	Nankai Trough, 165–298 (41)	Nankai Trough, 4.15 (35)
MBGB (54)	>85 (at mid-MSTZ)	0–9	>70 (above 50 mbsf)	44.4 (at 1.5 mbsf)	4 (at 6.7 mbsf)	100 (at 9 mbsf)	2.1	77 (in pelagic clay)	86 (at 165 mbsf)	44.1
MCG (13)		0–3		15–37		50 (at 1.8 mbsf)	4.2			14.7
MBGA (54)				14.8–85						2.9
MBGD (54)	0–4	0–3	0–4		4 (at 6.7 mbsf)			6 (in pelagic clay)		17.7
MCG (17)	>80 (except at mid-MSTZ)	9–80			88–100		83.3	71 (in ash layers)	14–100	20.6
SAGMEG-1, -2 (51)	0–14 (at MSTZ)	3–80			4–7	11 (at 1.8 mbsf)	8.3	12 (in ash layers)	71 (at 298 mbsf)	
TMEG (51)		0–3				37 (at 1.8 mbsf)	2.1			
Other ^d	0–4	0–4				41 (at 43 mbsf)				

^a Clone libraries were constructed from 16S rRNA rather than from genes for 16S rRNA.

^b Alternating sediment and sapropel layers; values are averages for all layers.

^c Alternating layers of pelagic clay and volcanic ash.

^d Includes the VAL III group (20), AAG (49), uncultured chimney group II (43), the Peru Basin group (45), and unidentified *Archaea*.

SAGMEG. As the name implies, members of SAGMEG-1 and -2 were originally found in South African gold mines, but they have subsequently been detected numerous times in studies of subsurface and hydrothermal systems. Together with members of the MCG, SAGMEG-related phylotypes dominated clone libraries constructed from 16S rRNA genes from site 1227 (19). While less abundant in the rRNA libraries from the same site, the detection of the group demonstrates an *in situ* activity.

Activity of archaeal groups with depth in the sediment. The amount of rRNA and the RNA/DNA ratio in actively metabolizing cells may differ between species and strains, reflecting species- or strain-specific responses to environmental stimuli, as well as differences in rRNA gene copy number (31, 46). Furthermore, phylotype frequency in clone libraries of PCR products may be biased by selection during extraction and amplification (39, 47, 48). Consequently, the relative frequencies among different phylogenetic groups in the RT-PCR clone libraries presented here do not necessarily translate into relative levels of metabolic activity. On the other hand, vertical variations in the concentration of rRNA within a specific group or phylotype may provide a measure of its activity at different depths. The rRNA profiles permit evaluation of such vertical variations in activity of two of the detected phylogenetic groups, MCG and MBGB.

A downwardly decreasing richness in 16S rRNA phylotypes affiliated with the MCG group was observed in the clone libraries. The decreasing amount of template rRNA with depth may have diluted rare phylotypes to extinction, resulting in a lower diversity in the deep clone libraries. However, since only one phylotype was detected in sample 5H-5 from 40.35 mbsf, where the rRNA concentration was relatively high, the decreasing phylotype richness probably reflects a lower diversity in the active MCG community rather than a dilution effect. The MCG *Archaea* constituted more than 80% of all archaeal clone libraries from samples taken from throughout the upper 35 m, and the decreasing amount of rRNA with depth therefore strongly suggests a decreasing activity level of this group. MCG phylotypes were only sporadically detected at the top of the SMTZ, where the large amount of MBGB-related rRNA overshadowed other groups of organisms. Below the MBGB-dominated layer, two related MCG phylotypes accounted for the activity peak in the deepest part of the SMTZ. The phylotype represented by clone 6H-2L10 was detected in clone libraries from throughout the sediment column (Fig. 3), suggesting either that it is not directly involved in methane oxidation or that it is physiologically flexible.

Members of the MBGB had a striking affinity for a narrow sediment horizon at the center of the SMTZ, where they completely dominated the clone libraries. This strongly indicates an increased activity of the group in this specific layer. The increased activity of MBGB at 37.35 and 37.75 mbsf was not reflected in Q-PCR data obtained previously from the same sediment depth at site 1227 (42). This apparent discrepancy between RNA and DNA abundance could be a result of a higher rRNA/DNA ratio in the active MBGB than in the active MCG organisms.

The vertical rRNA profile indicates that the activity of two groups of *Crenarchaeota* is higher in the SMTZ than in the sediment above and below. High activity of *Archaea* in the

SMTZ is consistent with another study of site 1227, in which *Archaea* were quantified by FISH counts and extraction of IPL. Of total cells in sediment samples from the SMTZ at site 1227, 98% hybridized with *Archaea*-specific oligonucleotide probes, and between 31 and 48% of extracted IPL in the SMTZ was of archaeal origin (3). Carbon isotopic data obtained from individual cells and from the IPL extracted from the SMTZ at site 1227 indicated that photosynthetically produced organic carbon, not methane, was the primary carbon source for the dominant archaeal community (3). If MBGB and/or MCG *Archaea* play any role in methane oxidation in the SMTZ, they do not assimilate methane-derived carbon and must be limited to a strictly dissimilatory methane metabolism (3). Both MCG and MBGB *Archaea* show a conspicuously cosmopolitan occurrence pattern in a wide spectrum of sediments (Table 1) and vents, indicating considerable ecological flexibility.

The correspondence between the vertical zonation of dissolved chemicals and active archaeal communities indicates a strong coupling between environmental chemistry and community composition in deep seafloor sediments, with community activities and composition both responding to and mediating the geochemical gradients. Often, the microbial communities of subsurface SMTZs have been evaluated based on sediment from a single depth horizon within the SMTZ (38). The results presented here indicate that considerable stratification of the microbial community may occur within the SMTZ or similar geochemically well-defined sediment horizons. Therefore, detailed habitat studies should include multiple depth horizons within every specific, geochemically defined sediment layer in order to track unforeseen, steep gradients in microbial community composition and activity.

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REFERENCES

1. Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-blast: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
2. Barns, S. M., R. E. Fundyga, M. W. Jeffries, and N. R. Pace. 1994. Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. *Proc. Natl. Acad. Sci. USA* **91**:1609–1613.
3. Biddle, J. F., J. S. Lipp, M. A. Lever, K. G. Lloyd, K. B. Sorensen, R. Anderson, H. F. Fredricks, M. Elvert, T. J. Kelly, D. P. Schrag, M. L. Sogin, J. E. Brenchley, A. Teske, C. H. House, and K.-U. Hinrichs. 2006. Heterotrophic *Archaea* dominate sedimentary subsurface ecosystems off Peru. *Proc. Natl. Acad. Sci. USA* **103**:3846–3851.
4. Boetius, A., K. Ravensschlag, C. J. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B. B. Jørgensen, U. Witte, and O. Pfannkuche. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**:623–626.
5. Chao, A. 1984. Non-parametric estimation of the number of classes in a population. *Scand. J. Statist.* **11**:265–270.
6. Chao, A. 1987. Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* **43**:783–791.
7. Coolen, M. J. L., H. Cypionka, A. M. Sass, H. Sass, and J. Overmann. 2002. Ongoing modification of Mediterranean sapropels mediated by prokaryotes. *Science* **296**:2407–2410.

8. Coolen, M. J. L., and J. Overmann. 1998. Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment. *Appl. Environ. Microbiol.* **64**:4513–4521.
9. D'Hondt, S., B. B. Jørgensen, D. J. Miller, A. Batzke, R. Blake, B. A. Cragg, H. Cypionka, G. R. Dickens, T. Ferdelman, K. U. Hinrichs, N. G. Holm, R. Mitterer, A. Spivack, G. Z. Wang, B. Bekins, B. Engelen, K. Ford, G. Gettemy, S. D. Rutherford, H. Sass, C. G. Skilbeck, I. W. Aiello, G. Guerin, C. H. House, F. Inagaki, P. Meister, T. Naehr, S. Niituma, R. J. Parkes, A. Schippers, D. C. Smith, A. Teske, J. Wiegand, C. N. Padilla, and J. L. S. Acosta. 2004. Distributions of microbial activities in deep seafloor sediments. *Science* **306**:2216–2221.
10. D'Hondt, S. L., S. Rutherford, and A. J. Spivack. 2002. Metabolic activity of subsurface life in deep-sea sediments. *Science* **295**:2067–2070.
11. D'Hondt, S. L., B. B. Jørgensen, and D. J. Miller. 2003. Proceedings of the Ocean Drilling Program, initial reports 201. Ocean Drilling Program, Texas A&M University, College Station, Tex. [CD-ROM.]
12. Felsenstein, J. 1989. PHYLIP—phylogeny inference package (version 3.2). *Cladistics* **5**:164–166.
13. Fuhrman, J. A., K. McCallum, and A. A. Davis. 1992. Novel archaeobacterial group from marine plankton. *Nature* **356**:148–149.
14. Hoehler, T. M. 2005. Cretaceous Park? A commentary on microbial paleomics. *Astrobiology* **5**:95–99.
15. House, C., B. Cragg, and A. Teske. 2003. Drilling contamination tests on ODP leg 201 using chemical and particulate tracers. Proceedings of the Ocean Drilling Program, initial reports 201. Ocean Drilling Program, Texas A&M University, College Station, Tex. [CD-ROM.]
16. Inagaki, F., K. Takai, T. Komatsu, T. Kanamatsu, K. Fujioka, and K. Horikoshi. 2001. Archaeology of Archaea: geomicrobiological record of Pleistocene thermal events concealed in a deep-sea seafloor environment. *Extremophiles* **5**:385–392.
17. Inagaki, F., M. Suzuki, K. Takai, H. Oida, T. Sakamoto, K. Aoki, K. H. Nealson, and K. Horikoshi. 2003. Microbial communities associated with geological horizons in coastal seafloor sediments from the sea of Ohkotsk. *Appl. Environ. Microbiol.* **69**:7224–7235.
18. Inagaki, F., H. Okada, A. I. Tsapin, and K. H. Nealson. 2005. Microbial survival—the paleome: a sedimentary genetic record of past microbial communities. *Astrobiology* **5**:141–153.
19. Inagaki, F., T. Nunoura, S. Nakagawa, A. Teske, M. Lever, A. Lauer, M. Suzuki, K. Takai, M. Delwiche, F. S. Colwell, K. H. Nealson, K. Horikoshi, S. D'Hondt, and B. B. Jørgensen. 2006. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. USA* **103**:2815–2820.
20. Jurgens, G., F. O. Glöckner, R. Amann, A. Saano, L. Montonen, M. Likolammi, and U. Munster. 2000. Identification of novel Archaea in bacterioplankton of a boreal forest lake by phylogenetic analysis and fluorescent in situ hybridization. *FEMS Microbiol. Ecol.* **34**:45–56.
21. Karner, M. B., E. F. DeLong, and D. M. Karl. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **409**:507–510.
22. Kemp, P. F., and J. Y. Aller. 2004. Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us. *FEMS Microbiol. Ecol.* **47**:161–177.
23. Kemp, P. F., S. Lee, and J. Laroche. 1993. Estimating the growth rate of slowly growing marine bacteria from RNA content. *Appl. Environ. Microbiol.* **59**:2594–2601.
24. Kerkhof, L., and P. Kemp. 1999. Small ribosomal RNA content in marine Proteobacteria during non-steady-state growth. *FEMS Microbiol. Ecol.* **30**:253–260.
25. Kerkhof, L., and B. B. Ward. 1993. Comparison of nucleic acid hybridization and fluorometry for measurement of the relationship between RNA/DNA ratio and growth rate in a marine bacterium. *Appl. Environ. Microbiol.* **59**:1303–1309.
26. Knittel, K., T. Lösekann, A. Boetius, R. Kort, and R. Amann. 2005. Diversity and distribution of methanotrophic Archaea at cold seeps. *Appl. Environ. Microbiol.* **71**:467–479.
27. Könneke, M., A. E. Bernhard, J. R. de la Torre, C. B. Walker, J. B. Waterbury, and D. A. Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**:543–546.
28. Kramer, J. G., and F. L. Singleton. 1993. Measurement of rRNA variation in natural communities of microorganisms on the southeastern U.S. continental shelf. *Appl. Environ. Microbiol.* **59**:2430–2436.
29. Kvenvolden, K. A. 1993. Gas hydrates—geological perspective and global change. *Rev. Geophys.* **31**:173–187.
30. Lee, S. H., and P. F. Kemp. 1994. Single cell RNA content of natural marine planktonic bacteria measured by hybridization with multiple 16S ribosomal RNA targeted fluorescent probes. *Limnol. Oceanogr.* **39**:869–879.
31. Lepp, P. W., and T. M. Schmidt. 1998. Nucleic acid content of *Synechococcus* spp. during growth in continuous light and light/dark cycles. *Arch. Microbiol.* **170**:201–207.
32. Mengoni, A., E. Tatti, F. Decorosi, C. Viti, M. Bazzicalupo, and L. Giovannetti. 2005. Comparison of 16S rRNA and 16S rDNA T-RFLP approaches to study bacterial communities in soil microcosms treated with chromate as perturbing agent. *Microbiol. Ecol.* **50**:375–384.
33. Moeseneder, M. M., J. M. Arrieta, and G. J. Herndl. 2005. A comparison of DNA- and RNA-based clone libraries from the same marine bacterioplankton community. *FEMS Microbiol. Ecol.* **51**:341–352.
34. Naviaux, R. K., B. Good, J. D. McPherson, D. L. Steffen, D. Markusic, B. Ransom, and J. Corbeil. 2005. Sand DNA—a genetic library of life at the water's edge. *Mar. Ecol. Prog. Ser.* **301**:9–22.
35. Newberry, C. J., G. Webster, B. A. Cragg, R. J. Parkes, A. J. Weightman, and J. C. Fry. 2004. Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough. *Ocean Drilling Program leg 190. Environ. Microbiol.* **6**:274–287.
36. Orphan, V., C. House, K. Hinrichs, K. McKeegan, and E. DeLong. 2001. Methane-consuming Archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science* **293**:484–487.
37. Parkes, R., B. Cragg, and P. Wellsbury. 2000. Recent studies on bacterial populations and processes in seafloor sediments: a review. *Hydrogeol. J.* **8**:11–28.
38. Parkes, R. J., G. Webster, B. A. Cragg, A. J. Weightman, C. J. Newberry, T. G. Ferdelman, J. Kallmeyer, B. B. Jørgensen, I. W. Aiello, and J. C. Fry. 2005. Deep sea-floor prokaryotes stimulated at interfaces over geological time. *Nature* **436**:390–394.
39. Polz, M. F., and C. M. Cavanaugh. 1998. Bias in template-to-product ratios in multitemplate PCR. *Appl. Environ. Microbiol.* **64**:3724–3730.
40. Preston, C. M., K. Y. Wu, T. F. Molinski, and E. F. DeLong. 1996. A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. *Proc. Natl. Acad. Sci. USA* **93**:6241–6246.
41. Reed, D., Y. Fujita, M. Delwiche, D. Blackwelder, P. Sheridan, T. Uchida, and F. Colwell. 2002. Microbial communities from methane hydrate-bearing deep marine sediments in a forearc basin. *Appl. Environ. Microbiol.* **68**:3759–3770.
42. Schippers, A., L. Neretin, J. Kallmeyer, T. G. Ferdelman, B. A. Cragg, R. J. Parkes, and B. B. Jørgensen. 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. *Nature* **433**:861–864.
43. Schrenk, M. O., D. S. Kelly, J. R. Delaney, and J. A. Baross. 2003. Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney. *Appl. Environ. Microbiol.* **69**:3580–3592.
44. Shipboard Scientific Party. 2003. Site 1231, p. 1–64. *In* S. L. D'Hondt, B. B. Jørgensen, D. J. Miller, et al. (ed.), Proceedings of the Ocean Drilling Program, initial reports 201. Ocean Drilling Program, Texas A&M University, College Station, Tex. [CD-ROM.]
45. Sørensen, K. B., A. Lauer, and A. Teske. 2004. Archaeal phylotypes in a metal-rich and low-activity deep subsurface sediment of the Peru Basin, ODP leg 201, site 1231. *Geobiology* **2**:151–161.
46. Stevenson, B. S., and T. M. Schmidt. 2004. Life history implications of rRNA gene copy number in *Escherichia coli*. *Appl. Environ. Microbiol.* **70**:6670–6677.
47. Suzuki, M., M. S. Rappe, and S. J. Giovannoni. 1998. Kinetic bias in estimates of coastal picoplankton community structure obtained by measurements of small-subunit rRNA gene PCR amplicon length heterogeneity. *Appl. Environ. Microbiol.* **64**:4522–4529.
48. Suzuki, M. T., and S. J. Giovannoni. 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl. Environ. Microbiol.* **62**:625–630.
49. Takai, K., and K. Horikoshi. 1999. Genetic diversity of Archaea in deep sea hydrothermal vent environments. *Genetics* **152**:1285–1297.
50. Takai, K., T. Komatsu, F. Inagaki, and K. Horikoshi. 2001. Distribution of Archaea in a black smoker chimney structure. *Appl. Environ. Microbiol.* **67**:3618–3629.
51. Takai, K., D. P. Moser, M. DeFlaun, T. C. Onstott, and J. K. Fredrickson. 2001. Archaeal diversity in waters from deep South African gold mines. *Appl. Environ. Microbiol.* **67**:5750–5760.
52. Teske, A., C. Wawer, G. Muyzer, and N. Ramsing. 1996. Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. *Appl. Environ. Microbiol.* **62**:1405–1415.
53. Treude, T., K. Knittel, M. Blumenberg, R. Seifert, and A. Boetius. 2005. Subsurface microbial methanotrophic mats in the Black Sea. *Appl. Environ. Microbiol.* **71**:6375–6378.
54. Vetriani, C., H. W. Jannasch, B. J. MacGregor, D. A. Stahl, and A. L. Reysenbach. 1999. Population structure and phylogenetic characterization of marine benthic archaea in deep-sea sediments. *Appl. Environ. Microbiol.* **65**:4375–4384.
55. Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. USA* **95**:6578–6583.