

Methanogenic and Methanotrophic Archaeal Diversity in Northern Gulf of Mexico Sediments

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Abstract

Archaeal small subunit (SSU) ribosomal Ribonucleic acid (rRNA) gene diversity was surveyed in sediment samples obtained at 50-, 100-, 150-, 300-, 400-, and 600-centimeter depths below the sea floor in core MD02-2571_c2 located above a gas chimney at a water depth of 647 meters in the West Mississippi Canyon area. The distribution of methanogenic and methanotrophic archaea was compared to pore-water chemical profiles indicative of methane and sulfate consumption. SSU rRNA sequences corresponding to the methane-oxidizing archaeal (MOA) groups ANME-1 and ANME-2 were recovered at 100 centimeters below the sediment surface concomitant with a localized increase in dissolved methane concentration and again at 300 and 400 centimeters below the sediment surface in close proximity to the sulfate-methane interface (SMI). Methanogen-related SSU rRNA sequences spanning the genus *Methanosarcinales* were recovered at 50, 100, 300, 400, and 600 centimeters below the sea floor. The distribution of SSU rRNA sequences associated with additional archaeal orders, *Thermoplasmatales* and *Crenarchaeota*, appeared to vary inversely with MOA groups across sediment horizons. These results are consistent with a broad distribution and complex community structure of methane cycling archaea across multiple sediment horizons in samples associated with core MD02-2571_c2.

Introduction

In July 2002, the research vessel (RV) *Marion Dufresne* obtained the box core MD02-2571_c2 from the West Mississippi Canyon area in the Gulf of Mexico as part of the International Marine Past Global Changes Study (IMAGES) VIII and Paleooceanography of the Atlantic and Geochemistry (PAGE) 127 research programs. One of the goals of this coring effort was to characterize the microbial ecology associated with gas hydrates and deep zones of active anaerobic methane oxidation (AOM) in continental margin sediments. Previous studies in the Gulf of Mexico using lipid biomarker (Zhang and others, 2002) or molecular phylogenetic approaches (Lanoil and others, 2001) have identified microbial groups associated with gas hydrates and superficial sediments from several subsurface environments, including but not limited to Green Canyon, Mississippi Canyon, Atwater Valley, and the edge of the Sigsbee Escarpment. The latter two locations were found to contain assemblages of bacteria and archaea physically associated with gas hydrates, including members of the methane-oxidizing archaeal groups ANME-1 and ANME-2 (Lanoil and others, 2001).

These observations suggest that microbial activity may play an important role in the formation and stability of marine gas hydrates. While these studies provide a useful foundation for identifying the microbial community structure physically associated with gas hydrates in the Gulf of Mexico, ecological and evolutionary questions relating to the distribution, relatedness, and activity of community members within and beyond the gas hydrate stability zone as a function of sediment depth and geochemical profile remain. Specifically, little is known about the disposition of methane-oxidizing archaea associated with subsurface AOM in sediment intervals below 15–20 centimeters (cm). This report summarizes archaeal diversity data determined for MD02-2571_c2, spanning a zone

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of active AOM at 300 cm. The analysis is based on SSU rRNA sequence analysis in conjunction with pore-water sulfate and methane concentration measurements (Ussler and Paull, this volume, chapter 8).

Methods

Between 25 and 50 grams (g) of sediment was subsampled from MD02-2571_c2 at 25-cm intervals to correspond directly in depth with pore-water samples collected approximately 2 hours after recovery of the core. Samples were collected serially from top to bottom only in regions of intact core that could be cut away with a spatula using sterile techniques. This approach excluded the most superficial layers of the core down to 25 cm. In order to avoid cross contamination with adjacent layers and the sidewalls of the box core, samples were collected from a central and internal portion of each interval by using a clean spatula. The last 50 cm of core was excluded from analysis because of the likelihood of contamination with superficial layers when the box core first penetrated the sea floor. In addition to the 25-cm intervals along the 1,038-cm length of the entire core, 15 g of sediment were subsampled at 2-cm intervals between 100 and 400 cm for subsequent vertical mapping of the microbial community structure across the SMI. Sediment samples were immediately frozen at -80 degrees Celsius ($^{\circ}\text{C}$) and thawed only for subsequent shore-based deoxyribonucleic acid (DNA) extraction. Sediment pore-water samples were collected and analyzed according to methods described in an accompanying chapter by Ussler and Paull (this volume, chapter 8). For DNA extraction, 0.5-g sediment was removed from the central portion of selected intervals by using sterile techniques and processed using a Fast soil prep kit (MoBio, San Diego, CA) following the manufacturer's protocol. Final elution volumes varied between 30 and 50 micro-liter (μL) TE buffer (Tris-EDTA buffer [trihydroxymethylaminomethane ethylenediaminetetraacetic acid]) (10 millimoles (mM) Tris, 1 mM EDTA, pH 7.5).

Archaeal SSU rRNA sequences were amplified by the Polymerase Chain Reaction (PCR) from sediment extracts by using archaeal-specific primers (A20_F 5' TTCCGGT-TGATCCYGCCRG and A958_R 5' YCCGGCGTTGAMTC-CAATT). Amplification reaction mixtures contained 1 μL template DNA, 41.5 μL 1X buffer, 1 μL each 10 micrometer (μM) forward and reverse primer, 2.5 units TaqPlus Precision polymerase (Stratagene, La Jolla, CA), 5 μL 10 mM stock dNTP (deoxyribonucleotide triphosphate) mixture in a total reaction volume of 50 μL . Amplifications were carried out using the following profile: 94 $^{\circ}\text{C}$ /3 minutes, X36 cycles 94 $^{\circ}\text{C}$ /40 seconds, 55 $^{\circ}\text{C}$ /1.5 minutes, and 72 $^{\circ}\text{C}$ /2 minutes, followed by a final extension at 72 $^{\circ}\text{C}$ /10 minutes.

SSU rRNA were visualized on 1-percent agarose gels in 1XTBE buffer (50 mM Tris, 50 mM boric acid, 1 mM EDTA, pH 8.3) and purified directly using Qiaquick PCR

purification kit (Qiagen, Valencia, CA). Purified SSU rRNA amplicons were cloned into pCR4-TOPO vector by using a TOPO TA cloning kit for sequencing (Invitrogen, Carlsbad, CA) and transformed by chemical transformation into TOP10 one-shot cells according to the manufacturer's instructions. Transformants were transferred to 96-well plates containing 180 μL Lb_{kan50} and 7-percent glycerol and stored at -80 $^{\circ}\text{C}$. Plasmid DNA was purified from glycerol stocks by using the Montage Plasmid Miniprep₉₆ kit (Millipore, Bedford, MA) following the manufacturer's protocol and stored at -20 $^{\circ}\text{C}$. Plasmid insert sequence data were collected on an ABI Prism 3100 DNA sequencer (Applied Biosystems, Inc., Foster, CA) by using Big DyeTM chemistry (PE Biosystems, Foster, CA) according to manufacturer's instructions. Plasmids were sequenced bidirectionally with M13F and M13R primers. Sequences were edited manually from traces by using Sequencher software V4.1.2 (Gene Codes Corporation, Ann Arbor, MI).

Representative euryarchaeal SSU rRNA ribotypes were selected for phylogenetic analysis. SSU rRNA sequence data were compiled with ARB software (<http://www.arb-home.de>) and aligned with sequences from the Genbank database by using the FastAligner program. Aligned sequences were visually inspected for conservation of secondary structure features and manually edited when necessary. SSU rRNA trees were based on comparison of 675 nucleotides. The SSU rRNA phylogenetic tree was generated using distance and parsimony methods implemented in PAUP* version 4.0b10 (Swofford, 2000). SSU rRNA sequence distances were estimated using the Kimura two-parameter model with the evolutionary rate adjusted according to a gamma distribution ($\alpha = 0.5$). Bootstrapping for distance and parsimony was accomplished with 1,000 replicates per tree by using heuristic search methods.

Results

A total of 68 euryarchaeal and 87 crenarchaeal SSU rRNA sequences were obtained from sediment intervals 50, 100, 150, 300, 400, and 600 cm below the sea floor in box core MD02-2571_c2. Euryarchaeal sequences corresponding to Thermoplasmatales, methanogens spanning the genus Methanosarcinales, and the MOA groups ANME-1 and ANME-2, in addition to crenarchaeal sequences corresponding to various group I subdivisions were found to partition phylogenetically by depth (fig. 1B). For comparison, pore-water sulfate and methane concentration data are listed in relation to archaeal SSU rRNA sequence recovery by depth interval (fig. 1). Consistent with a methanotrophic lifestyle, ANME ribotypes were recovered from the 300- and 400-cm intervals in close proximity to the chemically defined SMI. ANME ribotypes also were recovered from the 100-cm interval in sediments containing relatively high sulfate (~ 25 mM) and methane (~ 27 μM) concentrations (fig. 1A). Methanogen-

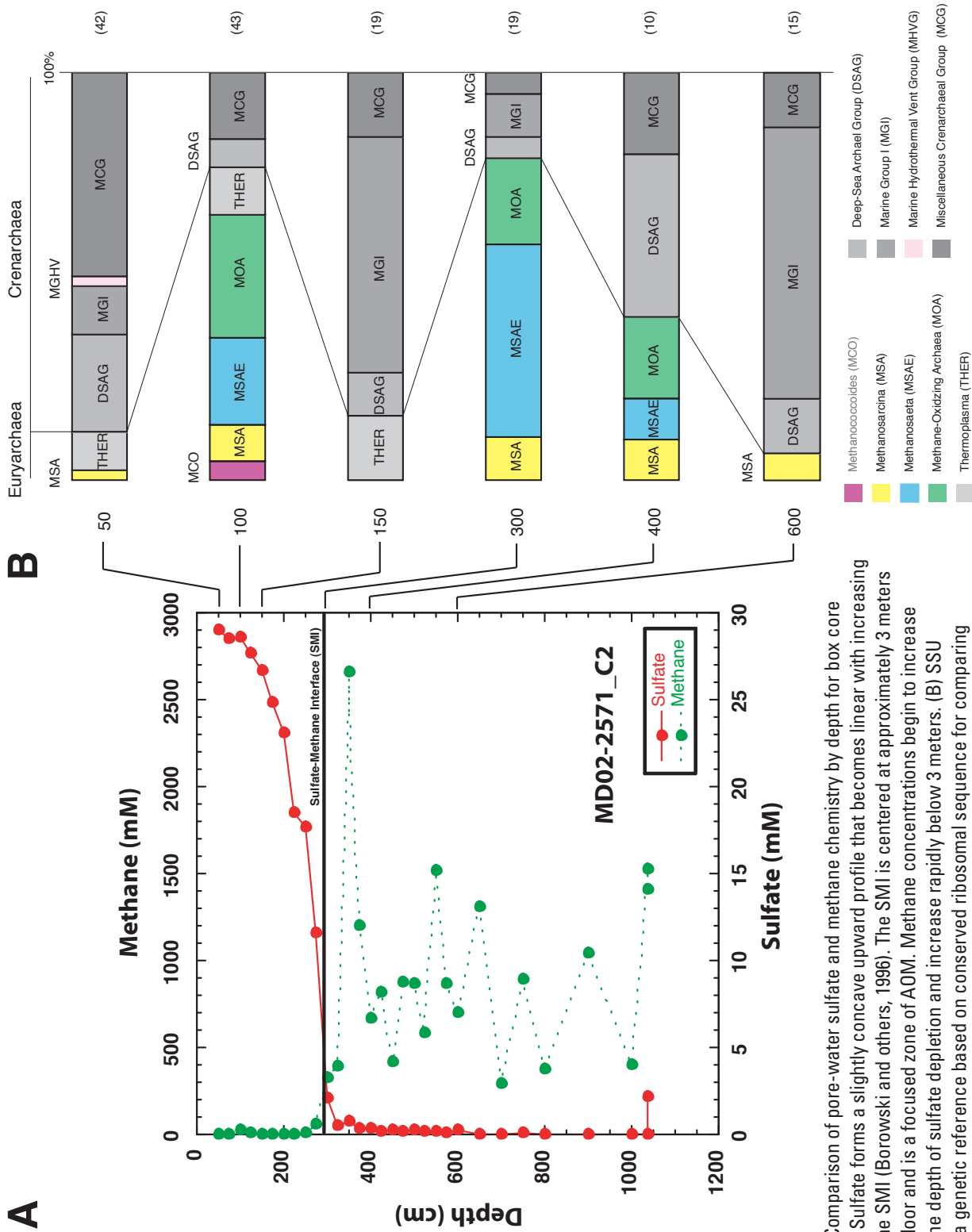


Figure 1. (A) Comparison of pore-water sulfate and methane chemistry by depth for box core MD02-2571_c2. Sulfate forms a slightly concave upward profile that becomes linear with increasing depth toward the SMI (Borowski and others, 1996). The SMI is centered at approximately 3 meters below the sea floor and is a focused zone of AOM. Methane concentrations begin to increase slightly above the depth of sulfate depletion and increase rapidly below 3 meters. (B) SSU rRNA provides a genetic reference based on conserved ribosomal sequence for comparing phylogenetic relations among archaeal groups. Sequences are grouped into major subdivisions with the Euryarchaeota and Crenarchaeota and represented as a percentage of total recovered sequences. Numbers in parentheses to the right of each sampling interval contain the total number of sequenced archaeal SSU rRNA clones for that interval. Methane-oxidizing archaeal groups are highlighted in green.

related sequences affiliated with the order Methanosarcinales were recovered from all sampling intervals with the exception of 150 cm. Phylogenetic affiliation of methanogenic and methanotropic archaeal SSU rRNA sequences recovered in this study are represented in figure 2. SSU rRNA sequences spanning the order Thermoplasmatales were recovered from the 50- and 150-cm intervals. Crenarchaeal SSU rRNA sequences were recovered from all sampling intervals. Overall, the abundance of thermoplasma and crenarchaeal ribotypes diminished in the presence of MOA with SSU rRNA sequences associated with the order Thermoplasmatales disappearing in samples from below 150 cm.

Discussion

Archaeal Diversity Associated with MD02-2571_c2

Previous phylogenetic studies from a wide range of geological settings (Hinrichs and others, 1999; Boetius and others, 2000; Orphan, Hinrichs, and others, 2001; Orphan and others, 2002; Teske and others, 2002) and coupled fluorescent *in situ* hybridization and isotopic analysis (FISH-SIMS) (Orphan, House, and others, 2001) have identified the MOA groups ANME-1 and ANME-2 as mediators of anaerobic methane oxidation (AOM). The molecular and phylogenetic evidence described in this study indicates the presence of ANME groups in close proximity to the chemically determined SMI of MD02-2571_c2. The identification of ANME-1 near the SMI of MD02-2571_c2 is consistent with a methanotrophic lifestyle and provides a glimpse at MOA community composition in the high flux, methane-hydrate rich sediments of the Gulf of Mexico. However, the identification of ANME ribotypes at 100 cm in the absence of a SMI suggests that MOA groups may have the potential to utilize alternative metabolic subsystems to derive cellular energy and carbon. Likewise, the presence of methanogen-related SSU rRNA sequences well above the SMI of MD02-2571_c2 suggests that high levels of primary productivity at the SMI coupled to high rates of fluid advection and(or) diffusion could fuel hydrogen-based alternatives to the methanogenic lifestyle at higher elevations in the substrata. MOA groups living in syntrophic association with methanogens could harness a local methane cycle far removed from the chemically defined SMI. The punctuated increase in dissolved methane concentration at 100 cm and the presence of a diverse assemblage of methanogen and MOA ribotypes appears to be consistent with this latter hypothesis. However, it remains formally possible, given the limited sample number and sensitivity of amplification-based approaches used in this study, that MOA ribotypes recovered from the 100-cm horizon could represent the decomposing remains of dead cells, a remnant population of metabolically inactive cells, or a founder population recently transported upward by fluid advection.

Future deep-core chemotaxonomic studies, including lipid biomarker analysis, in the Gulf of Mexico and beyond will be required to ascertain the generality of this phenomenon. Due to the limited number of SSU rRNA sequences analyzed in this study, the observed patterns of vertical stratification should be considered a preliminary finding. A more comprehensive treatment of the data, including an expanded sequence set across additional sampling intervals and concomitant statistical analysis, will be required to more accurately describe the anatomy of this core.

Geochemical studies indicate that AOM occurs at an interface between upward-rising methane and downward-diffusing sulfate termed the SMI (Barnes and Goldberg, 1976; Reeburgh, 1976; Whiticar and others, 1986; Borowski and others, 1996; Reeburgh, 1996; Joye and others, 1999; Martens and others, 1999). AOM produces linear sulfate gradients that reflect the rate of methane transport by fluid advection and(or) diffusion toward the surface with steeper gradients exhibiting higher flux (Borowski and others, 1996). At present, little is known about the vertical thickness of the SMI, although current models suggest a sharp boundary over several meters. Given the well-defined linear pore-water chemistry profile available for MD02-2571_c2, future studies aimed at higher resolution mapping of microbial diversity have the potential

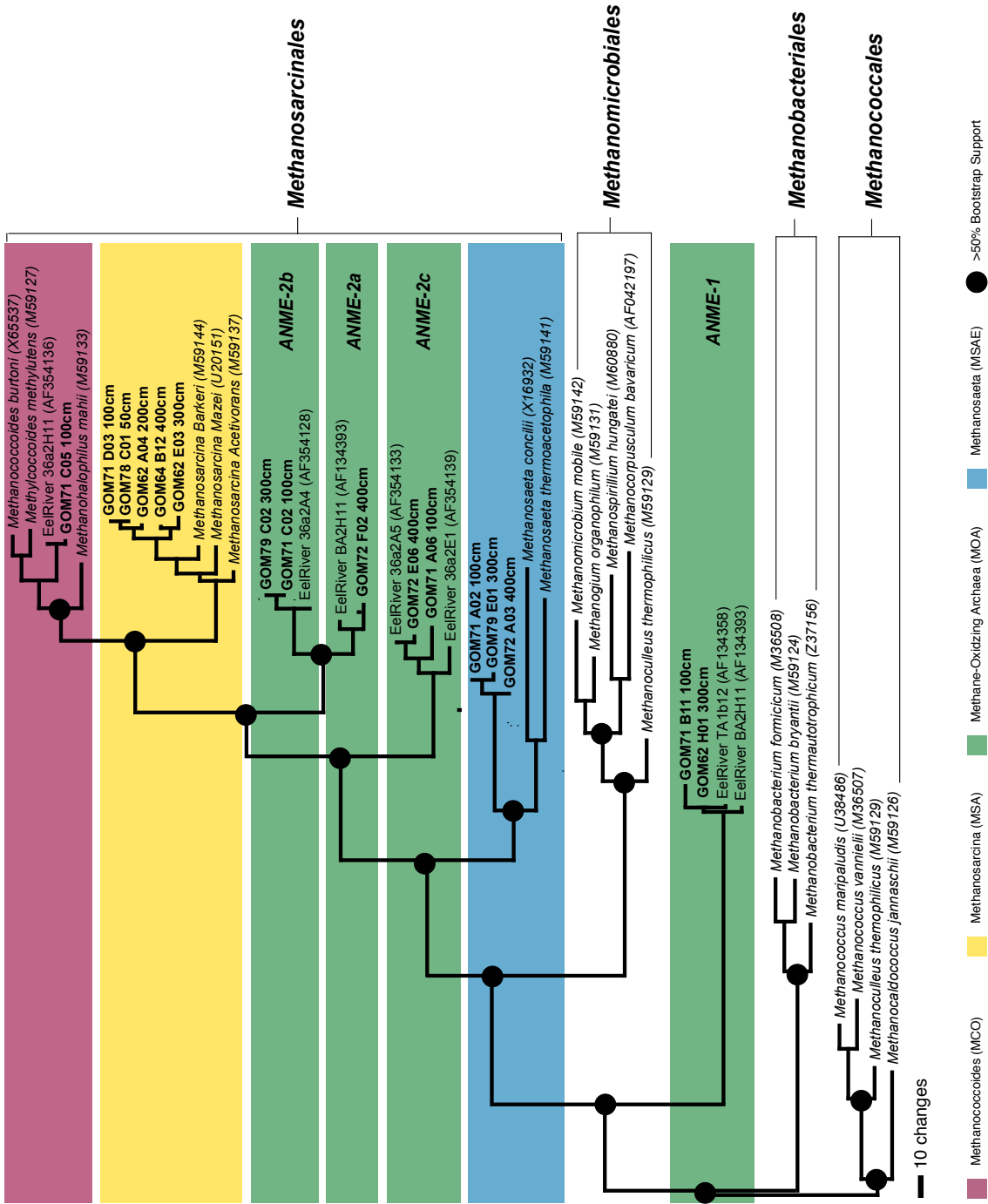


Figure 2. Phylogenetic tree comparing representative methanogen and methanotroph SSU rRNA sequences recovered from box core MD02 2571_c2. Bootstrap values are based on 1,000 replicates (neighbor-joining and parsimony). Scale bar represents 10-percent estimated sequence divergence. Sequences recovered in this study begin with GOM (Gulf of Mexico) followed by a clone number and the sampling interval. Methane-oxidizing archaeal groups are highlighted in green. Genbank accession numbers follow clone names when available.

to more closely bracket the chemically determined SMI with both qualitative and quantitative genetic information.

Conclusions

Archaeal SSU rRNA sequences obtained from 50-, 100-, 150-, 300-, 400-, and 600-cm intervals of MD02-2571_c2 suggest the following:

1. The anaerobic methanotrophic groups ANME-1 and ANME-2, identified at 300 and 400 cm, likely mediate AOM in MD02-2571_c2 sediments.
2. In addition to ANME-1 and ANME-2 groups, methanogen-related sequences most similar to *Methanosarcinales* spp. were identified throughout the core, suggesting a potential role for methanogenesis at this site. SSU rRNA sequence analysis suggests that methanogenic archaeal groups exhibit a vertical distribution spanning the sediment matrix 3 m above and at least 1 meter below the SMI.
3. More comprehensive analyses of MD02-2571_c2 intervals and clone libraries will be required to accurately determine archaeal community structure across the SMI. Future work using quantitative PCR approaches could provide more accurate analyses of microbes and microbial processes that coincide with pore-water chemical profiles to better define the dynamic biogeochemistry in the Gulf of Mexico subsea-floor system.

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