Molecular survey of methane-cycling archaea in methane-soaked subsurface sediments (Guaymas Basin, Gulf of California)

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Introduction

The Guaymas Basin is a young marginal rift basin in the Gulf of California characterized by active seafloor spreading, rapid deposition of organic-rich sediments, and steep geothermal gradients where sedimentary organic material of photosynthetic origin turns into hydrocarbons, potential microbial substrates. Methane is abundant in the Guaymas Basin hydrothermal sediments ranging, on average, from 0.5 to ~20 mM. The deep subsurface of Guaymas Basin was probed in eight drilling sites during IODP Expedition 385 (Fig. 1) by DNA extraction and PCR amplification of the key gene in methanogenesis and methane-oxidation, methyl coenzyme M reductase subunit α (*mcrA*).

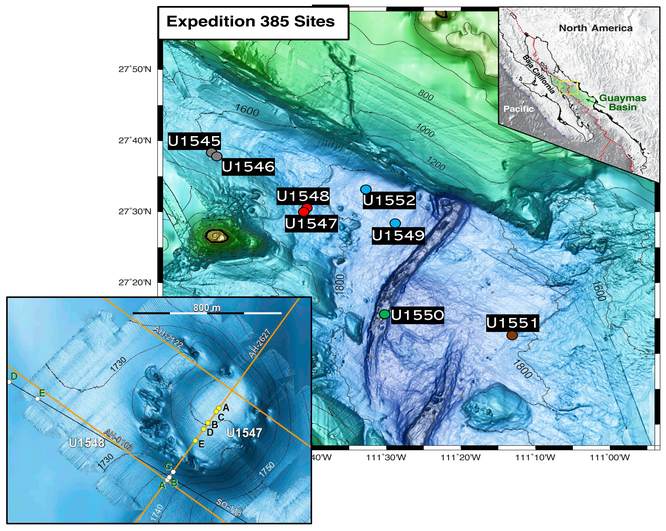

Figure 1: Guaymas Basin drilling site map with detailed view of the Ringvent site

Methods

DNA Extraction: Subsurface sediment samples were selected from eight drilling sites on the flanking regions off- and in the northern axial trough of Guaymas Basin to explore the diversity, depth range and in-situ temperature range of methane-cycling archaea in the Guaymas Basin subsurface. DNA was extracted from near-surface (0.7-2.1 mbsf), intermediate (15.4-36.8 mbsf) and deeper depths (74.2-101.9 mbsf) using commercial DNA extraction kits (FastDNA™ SPIN Kit, MP Biomedical) and was PCR amplified using the general mcrIDR primer pair that targets the mcrA gene and the ANME-1 primer pair that targets the ANME-1-related archaeal groups (Lever & Teske, 2015).

Phylogeny: We constructed a minimum-evolution phylogeny using the five most abundant ANME-1 *mcrA* gene sequences from 7/8 drilling sites (Fig. 2). *mcrA* genes from methanogenic lineages were placed into a separate phylogeny (Fig. 3). Reference sequences from NCBI GenBank were included to add phylogenetic context to the tree and allow for cluster labeling. Additionally, site specific trees were inferred using all *mcrA* gene sequences with >100 clones (supplemental material provided).

Results

Three of the eight sites (1548B, 1547B, 1550B) yielded the *mcrA* marker gene related to previously cultured methanogenic genera and families, such as *Methanosaetaceae, Methanosarcinaceae, Methanomicrobiaceae,* and *Methanocaldococcaceae* (Fig. 3). Close relatives of the extreme hyperthermophile *Methanocaldococcus bathoardescens* (from the Juan de Fuca spreading center) constituted the most abundant phylotypes in Ringvent sites 1547 and 1548. Uncultured *mcrA* groups included *mcrA* group e (Hallam et al., 2003) and a new uncultured *mcrA* lineage unrelated to any known methanogenic lineage. The methane-sulfate interfaces, which extended into intermediate depths (15.4-36.8 mbsf), yielded numerous ANME-1 sequences from all seven sites included in the trees. Great diversity is observed within the ANME-1 lineage with sixteen distinct and well supported phylogenetic clusters (Fig. 2). Numerous ANME-1 phylotypes were closely related to reference sequences from the Napoli mud volcano (characterized as “a window to the subsurface”), as well as sequences previously collected from Guaymas Basin (Holler et al., 2011, Biddle et al., 2012) and Gulf of Mexico subsurface sediments (Fig. 2). A distinct cluster of ANME-1 phylotypes branched with reference sequences from hot, surficial sediments collected by DSV Alvin, and a distinct cluster (No. 13) of phylotypes from the terrestrially influenced site 1551 (Fig. 2).

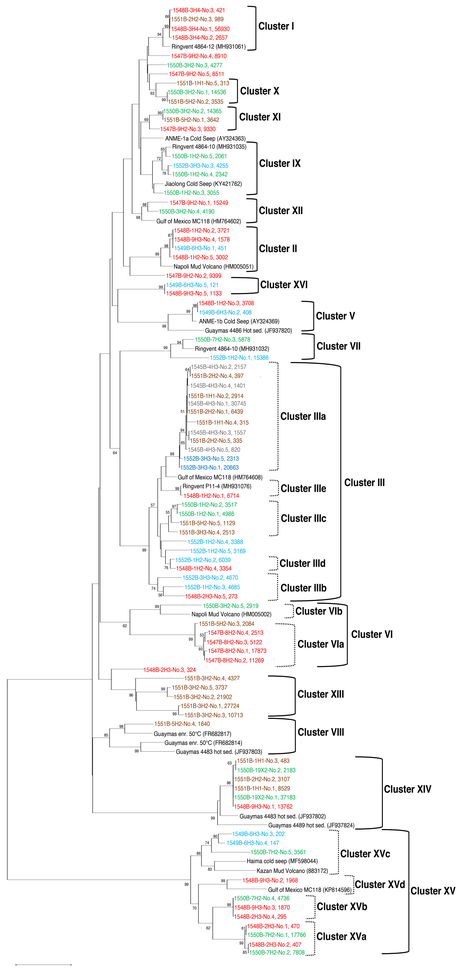

Figure 2: General ANME-1 phylogeny, based on partial *mcrA* gene sequences.
Sequences color coded by drilling site from Fig. 1

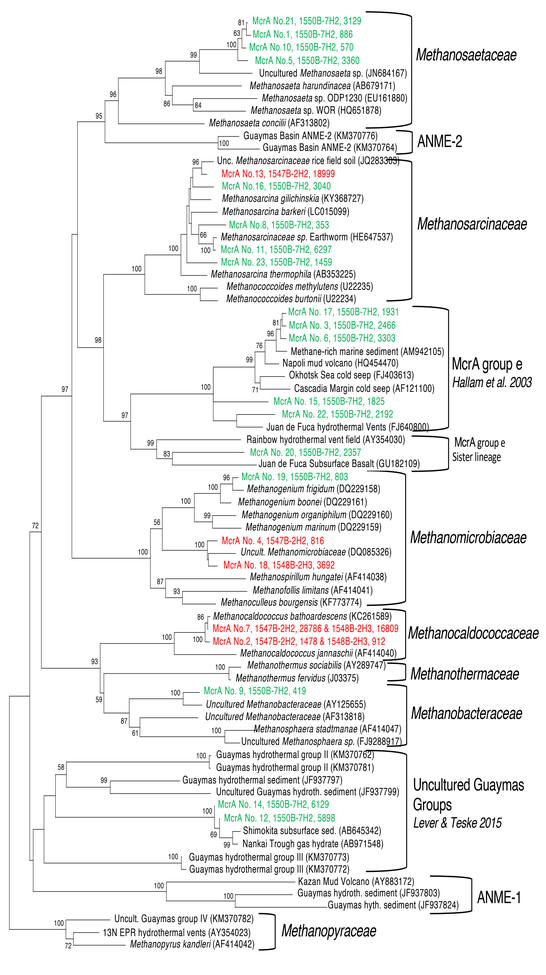

Figure 3: Methanogen phylogeny, based on partial *mcrA* gene sequences
Sequences color coded by drilling site from Fig. 1

Discussion

A mix of hydrothermal and biogenic methane is abundant in Guaymas Basin. The ANME-1 methane oxidizers are widespread within and around methane-sulfate interfaces. Methanogen populations are relatively sparse, as they were only observed in three of the seven sites. Ringvent sites (47 & 48) are characterized by a high influx of methane from the volcanic sill and sustain a diverse community of methane-cyclers. Ringvent sites are characterized by steeper thermal gradients relative to other Guaymas sites (Fig. 4). These sites are home to several thermophilic ANME-1 and methanogen lineages.

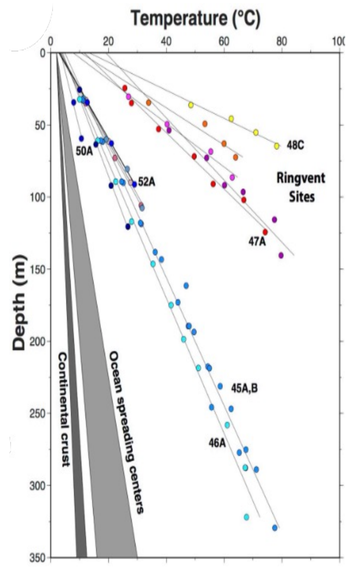

Figure 4: Temperature profiles of all Guaymas drilling sites (derived from Neumann et al., 2023)

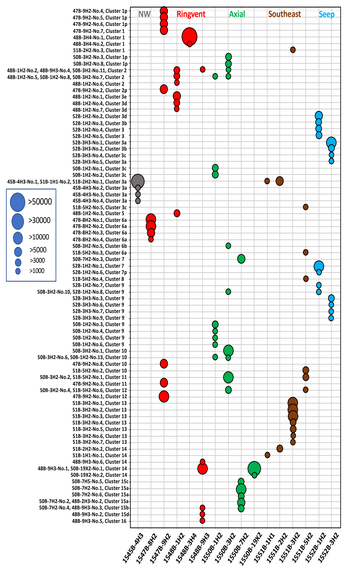

Figure 5: Bubble plot representing the sequence frequency of ANME-1 lineages

Future Direction

Matching archaeal 16S rRNA datasets are being investigated and will complement the *mcrA* gene phylogenies for a comprehensive PCR-based survey of methane-cycling microbial communities in the Guaymas Basin subsurface. Additionally, there is the possibility of linking our *mcrA*-based phylogenies to genome-based phylogenies of ANME-1(Laso-Pérez et al., 2023).

Citations

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