Archaeal populations and their associations with geochemical conditions on the Iberian Margin

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Introduction:

IODP Expedition 339 drilled sediments from the Iberian Margin and surrounding slope environments near the Mediterranean Outflow. Site U1385 on the margin shelf has a well defined paleo-history (Figure 1). Also known as the Shackleton site, after Nick Shackleton's conclusions on millenial scale glaciation events by correlating oxygen isotopes in benthic foraminifera to those in the Greenland ice cores (Shackleton et al 2004), site U1385 offers a good comparison site to other well studied continental margin boreholes, particularly site 1229 on the Peru Margin. However, while site 1229 has been well characterized geologically and biologically, U1385 has had no previous microbial ecology work focused there. In this study, we present results from the first down-core molecular survey of this borehole. Archaeal populations were investigated using targeted 16S rDNA primers at 5 depth horizons. Operational taxonomic units (OTUs) abundance were compared with geochemical data and significant associations were searched for using the BIOENV procedure in the ecological statistics software PRIMER-E (Clarke and Gorley 2006). A non-significant, yet compelling correlation between archaeal population distribution and iron and organic carbon content was observed. Abundance of OTUs identified as the Deep Sea Archaeal Group (DSAG) is highest where iron and organic carbon levels are highest. This supports recent work showing DSAG archaea associating with high iron and organic carbon horizons in sediment cores from the Arctic mid-ocean spreading ridge (Jorgensen et al 2013). Jorgensen and colleagues suggest a possible metabolism of iron reduction tied to organic carbon oxidation. Data presented here supports this and further work is on-going that may definitively identify the metabolism of this important member of marine deep subsurface microbial communities.

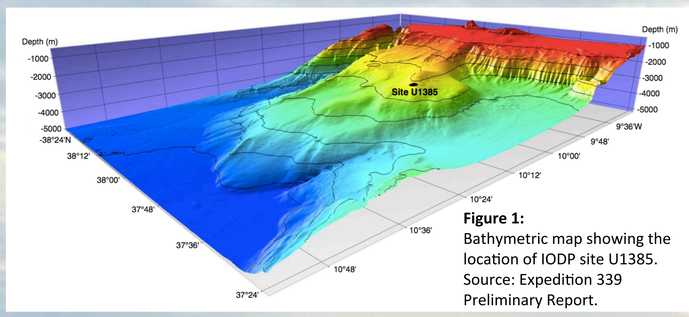

Figure 1: Bathymetric map showing the location of IODP site U1385. Source: Expedition 339 Preliminary Report.

Methods:

DNA was extracted from 5 grams of sediment from 5 separate depths (2H, 6H, 8H, 10H, 14H) ranging from 10 meters below seafloor (mbsf) to 123 mbsf using the FAST-DNA Spin Kit for Soils (MP Biomedicals, Santa Ana, CA, USA). Archaeal 16S rDNA was amplified using archaea specific primers U519F and 806R tagged with 10 basepair barcodes specific to each depth horizon. PCR products were pooled in equal concentrations and sent for 454 pyrosequencing at Selah Genomics (Columbia, SC, USA) using Titanium chemistry. Sequences were denoised and checked for chimeras using the Qiime version 1.7 pipeline. Further taxonomic classifications were also done through the Qiime v1.7 workflow using default parameters except for the alignment database, which was changed to the SILVA database (release 115). An OTU table was generated at a 97% similarity cut-off level. This OTU table along with shipboard geochemistry data was imported into PRIMER-E v. 6.0 (Plymouth, UK). Biological data was square-root transformed and a Bray-Curtis distance matrix was calculated. MDS plots were generated from this matrix. Geochemical data was normalised and a Euclidean distance matrix calculated. Both distance matrices were imported into the BIO-ENV procedure to calculate correlation statistics.

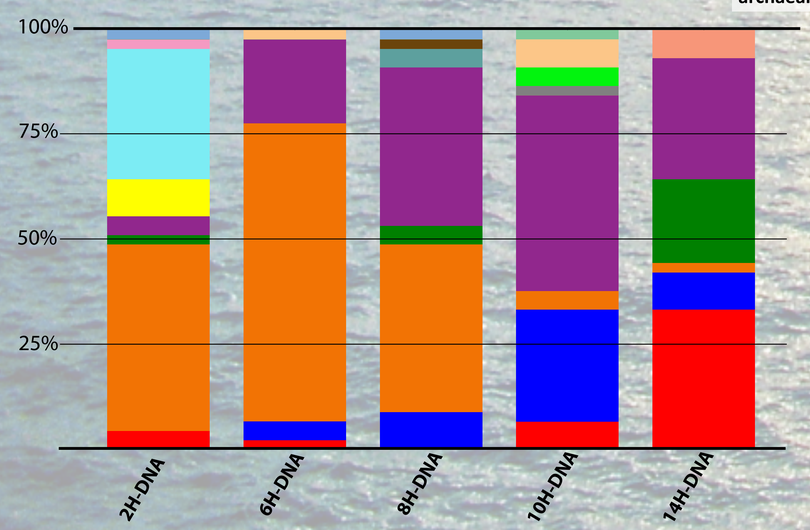

Figure 2: Taxonomic profiles of down-core archaeal populations at IODP site U1385.

Table 1: U1385 Geochemistry

	2H	6H	8H	10H	14H
Meters below seafloor	10	48	68	87	123
Methane (ppmv)	4	107	11600	68269	68375
Sulfate (mM)	17.4	0	0	0	0
Strontium (uM)	122.5	114.8	140.4	145.9	155.5
Manganese (uM)	16.7	12.2	12.5	12.3	13.6
Iron (uM)	90.9	23.7	22.4	16.4	17
Organic Carbon (wt %)	1.04	0.82	0.54	0.56	0.26
Nitrogen (wt%)	0.08	0.06	0.06	0.07	0.26
C/N	12.1	13.27	9.1	8.6	3.8
CaCO3 (wt%)	30.2	38.6	25.3	29.7	31.9
Ammonium (uM)	1272	2789	3516	4004	4482

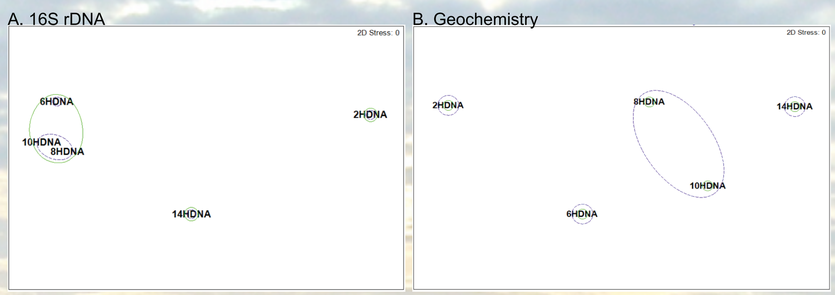

Figure 3: MDS plots of OTU abundances (panel A) and geochemical conditions (panel B) amongst the 5 sampled depths. Note the similarities in distribution between biological and geochemical data. Blue dashed lines indicate 60% similarity level. Green lines indicate 40% similarity level.

Geochemical Variables	Correlation statistic (rho)
Iron + organic carbon	0.842
iron + nitrogen + ammonium	0.818
manganese (uM)	0.648
iron (uM)	0.612
ammonium (uM)	0.6
sulfate (mM)	0.569
organic carbon (wt%)	0.564
methane (ppmv)	0.552
C/N	0.224
nitrogen (wt%)	0.214

Table 2: Geochemical variables and the strength of their correlation statistic (rho) to the total archaeal population distribution down-core at site U1385.

Results:

- **DSAG archaea are the most abundant single taxa at 10 and 48 mbsf**
- **At 68 mbsf, DSAG and MCG comprise the majority of the population**
- **MCG become more abundant at depth**
- **Using the BIOENV procedure in Primer-E (6th Edition), a non-significant, yet compelling correlation between iron and organic carbon content and archaeal population distribution was observed (Table 2). The correlation statistic (rho-value) was 0.842. The significance (p-value) was 0.1.**
- **Iron and organic carbon levels are highest in the shallower depths, corresponding to higher abundance of DSAG archaea. This supports previous work by Jorgensen et al. 2013, which showed significant associations of DSAG archaea with iron and organic carbon at the Arctic mid-ocean spreading ridge via qPCR assays.**
- **Further work is ongoing to more robustly prove this association at site U1385 via qPCR and enrichment/cultivation studies.**

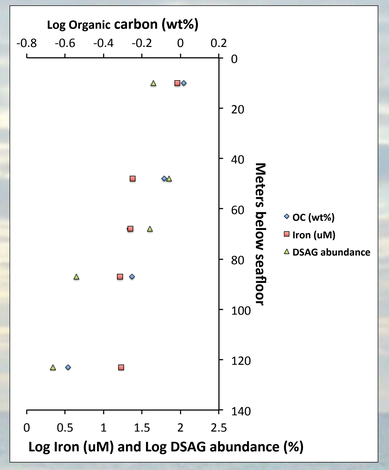

Figure 4: Graph of down-core iron and organic carbon content with DSAG abundance (all Log(10) transformed).

Discussion:

- There is a compelling correlation between total archaeal population distribution and iron and organic carbon content. DSAG abundance is highest where iron and organic carbon content are highest (Figure 4), supporting recent observations at the Arctic mid-ocean spreading center (Jorgensen et al. 2013)
- The DSAG abundance spike at 48 mbsf may be due to a "sweet spot" level of iron and organic carbon content where they can out-compete other archaea.
- To investigate DSAG response to iron and organic matter, a range of iron and organic carbon content will be used in enrichment experiments paired with DSAG qPCR assays. The ultimate goal of these enrichments is to obtain the first isolate of a member of the Deep Sea Archaeal Group and definitively determine the metabolic strategies of this cosmopolitan member of the marine deep subsurface.

References:

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