Environmental Factors Affecting the Distribution of Ammonia-Oxidizing Archaea in the Mississippi River Plume

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Abstract

Marine Crenarchaeota have recently been found to be capable of oxidizing ammonia via an Archaea-specific ammonia monooxygenase (*amoA*) gene. Archaeal *amoA* genes have been observed in marine environments at 10-1000 times greater abundance than the *amoA* homologue from ammonia-oxidizing Bacteria (AOB), suggesting that the ammonia-oxidizing Archaea (AOA) play a major role in the marine nitrogen cycle. Pelagic AOA are most abundant below 100 m depth in the water column and at higher latitudes, especially near the poles. Reasons for this distribution remain largely unknown, but previous studies have pointed to environmental factors, such as salinity, light, temperature, oxygen, and sulfide. Competition with bacteria or phytoplankton has also been thought to influence abundance of AOA in the environment.

This study compares the distribution of AOA and AOB in the Mississippi River Plume using quantitative PCR (qPCR) analysis of 16S rDNA and *amoA* genes from each group combined with different environmental factors measured at the same stations. Crenarchaeal acetyl-CoA carboxylase gene (*accA*) abundance was also measured to look at distribution of autotrophic metabolism. The enzyme translated from the *accA* gene is involved in the 3-hydroxypropionate pathway, which enables MG1C to assimilate carbon autotrophically. Surface and deep (200m) samples were also amended with 3H-leucine to measure protein synthesis rates in the Mississippi River Plume; samples from these emendations will also be used for microautoradiography fluorescent *in situ* hybridization (MAR-FISH) analysis to look at incorporation by single cells, in order to investigate potential heterotrophic metabolism by AOA.

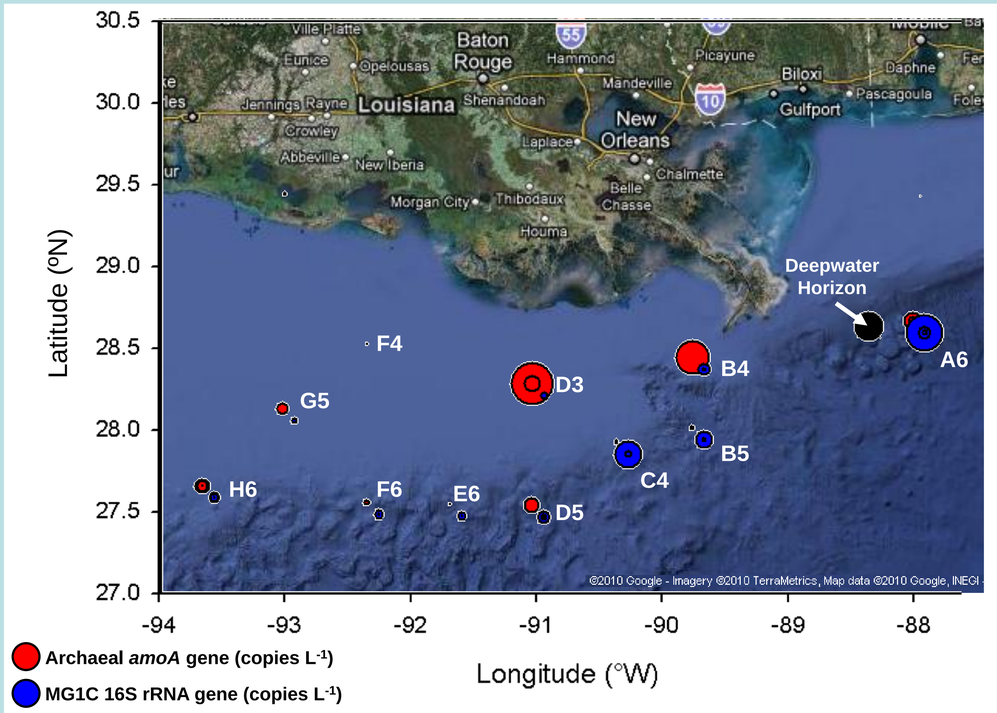
Methods

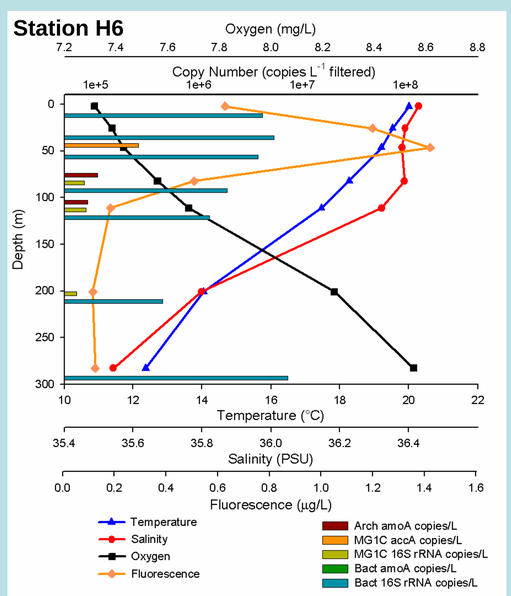
Samples were collected from CTD casts in the Gulf of Mexico (30º 07' N 088º 02' W to 27º 39' N 093º 39'W; Figure 1) in March 2010, prior to the Deepwater Horizon oil spill. Environmental data (temperature, salinity, etc.) was obtained from CTD bottle casts. Approximately 1 L of water from each depth was filtered onto 0.22 µm Durapore filters (Millipore) and frozen. DNA was collected from filters by using a phenol-chloroform extraction method (Bano and Hollibaugh, 2000), and eluted in Tris-EDTA buffer (pH 8) for storage. Relative abundances of Archaeal *amoA*, *accA*, and 16S rRNA genes as well as Bacterial *amoA* and 16S rRNA genes were determined with qPCR using either SYBR® Green I dye (BioRad) or TaqMan® (Applied Biosystems) chemistries for detection. Primers and probes used in this study are listed below (Table 1). qPCR reactions were set up in triplicate and run against a range of standards (10^1-10^7 copies µL^{-1} DNA) as described in Kalanetra *et al.* (2009), with the exception of Archaeal *accA* gene amplification, which followed protocols described in Yakimov *et al*. (2009).

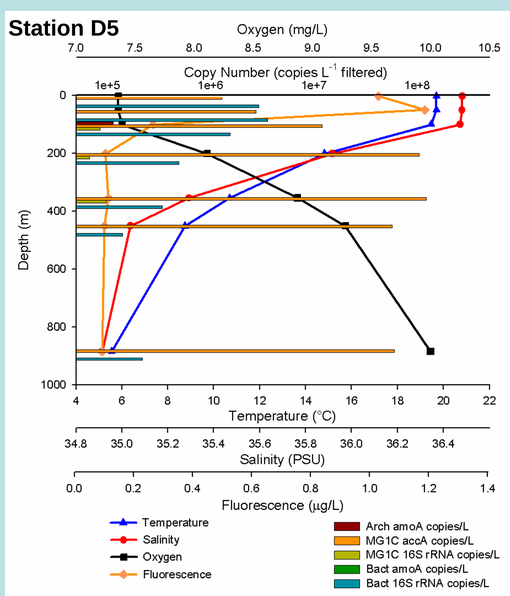
Prokaryotic activity rates were measured from three stations (A6, D5, H6) from both surface (2m) and deep (200m) samples. Each of these samples was amended with 10nM 3H-leucine and incubated at *in situ* temperature for 6-7 hours. Samples were then filtered onto 0.22 µm nitrocellulose filters (Millipore) for scintillation counting or 0.22 µm polycarbonate filters (Whatman) for MAR-FISH analysis. Nitrocellulose filters were washed three times with cold 5% trichloroacetic acid to remove unincorporated leucine, dissolved in ethyl acetate, and counted for 5 minutes each.

R/V *Cape Hatteras*, Duke University Marine Lab

Figure 1: GulfCarbon 5 Cruise Track
Cruise track taken by the R/V Cape Hatteras in March 2010. Stations where samples were collected are labeled in white. Distribution of MG1C and AOA are shown by qPCR counts of 16S rRNA (blue circles) and *amoA* (red circles) genes. Size is proportional to copy number (copies L^{-1}).







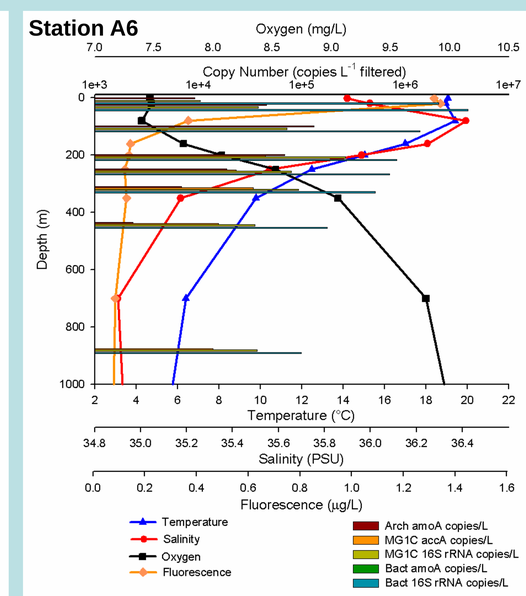


Table 1: Primers and Probes Used in this Study

Target Gene	Primer/ Probe	Sequence (5'→3')	Reaction	Detection Limit	Reference
Archaeal 16S rDNA	21F	TTC CGG TTG ATC CYG CCG GA	PCR and Cloning	N/A	DeLong *et al.*,1992
	958R	YCC GGC GTT GAM TCC AAT T			
MG1C 16S rDNA	G1_751F	GTC TAC CAG AAC AYG TTC	qPCR	1.63 x 10^1 copies/µL	Mincer *et al.*,2007
	G1_956R	HGG CGT TGA CTC CAA TTG			
	TM519AR	TTA CCG CGG CGG CTG GCA C			Suzuki *et al.*, 2000
pSL12 16S rDNA	pSL12_750F	GGT CCR CCA GAA CGC GC	qPCR	4.27 x 10^1 copies/µL	Mincer *et al.*, 2007
	pSL12_876R	GTA CTC CCC AGG CGG CAA			
Bacterial 16S rDNA	BACT1369F	CGG TGA ATA CGT TCY CGG	qPCR	4.56 x 10^1 copies/µL	Suzuki *et al.*, 2000
	PROK1492R	GGW TAC CTT GTT ACG ACT T			
	TM1389F	CTT GTA CAC ACC GCC CGT C			
Archaeal *amoA*	ArchamoAF	STA ATG GTC TGG CTT AGA CG	qPCR	5.74 x 10^1 copies/µL	Wuchter *et al.*, 2006
	ArchamoAR	GCG GCC ATC CAT CTG TAT GT			
Bacterial *amoA**	amoA-1F	GGG GTT TCT ACT GGT GGT	qPCR	6.50 x 10^1 copies/µL	Rotthauwe *et al.*, 1997
	amoA-r New	CCC CTC BGS AAA VCC TTC TTC			Hornek *et al.*, 2006
Archaeal *accA*	Crena_529F	GCW ATG ACW GAY TTT GTY RTA ATG	qPCR	5.01 x 10^1 copies/µL	Yakimov *et al.*, 2009
	Crena_981R	TGG WTK RYT TGC AAY TAT WCC			

*Bacterial *amoA* primers amplify *amoA* genes from β-Proteobacteria only, and not γ-Proteobacteria

Figure 2: Depth Profiles of Stations H6, D5, and A6
Environmental and qPCR data is plotted against depth for each station. Temperature (ºC), salinity (PSU), oxygen (mg/L), and fluorescence (µg/L) data was collected from CTD bottle cast data. Gene copies per liter of sample filtered were determined from qPCR amplifications of archaeal and bacterial 16S rRNA and *amoA* genes, as well as the Crenarchaeal *accA* gene. Missing bars for gene copies indicate qPCR reactions with results below the limit of detection (LD) for a particular gene (see Table 1 for LD for each reaction).

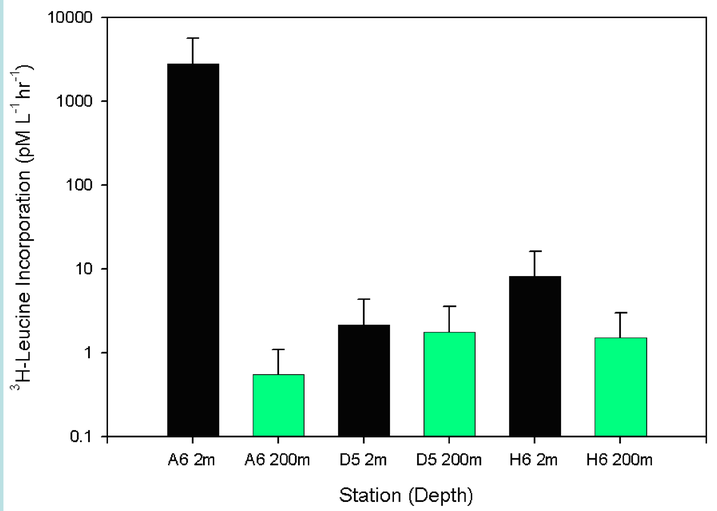


Figure 3: Prokaryotic Activity
The amount of ^{3}H-leucine incorporated (pM L^{-1} hr^{-1}) by prokaryotes in both surface and deep is shown for Stations A6, D5, and H6. Each bar represents the average of four replicate samples; error bars represent standard deviation.

Conclusions

This data indicates a wide distribution of MG1C and AOA in the northern Gulf of Mexico. Both archaeal 16S rRNA and *amoA* genes were found at abundances up to 10^5 copies L^{-1}. However, very few copies of AOB *amoA* were found in these waters (most 10^1 or LD). This may indicate a lack of primer specificity, an abundance of ammonia-oxidizing γ-Proteobacteria not picked up by our primer set, or simply low numbers AOB in the sample area. Ratios of archaeal *amoA*:16S rDNA, used to indicate the amount of Archaea that are AOA, were considerably high at some stations (up to 6.5 *amoA* for each 16S rDNA). This could indicate the presence of a population of AOA with multiple *amoA* copies per genome or another group of archaea capable of oxidizing ammonia (such as the pSL12-like clade). Finally, this dataset represents a broad sampling of MG1C in the Gulf of Mexico, and also gives a snapshot of the microbial community in this region prior to the oil spill, which could prove useful for future studies on the impact this has on microorganisms and their recovery.

References

Bano, N. and J.T. Hollibaugh (2000). Diversity and Distribution of DNA Sequences with Affinity to Ammonia-Oxidizing Bacteria of the β Subdivision of the Class *Proteobacteria* in the Arctic Ocean. *Appl. Env. Microbiol.* **66**: 1960-1969.

Kalanetra, K.M., N. Bano, and J.T. Hollibaugh (2009). Ammonia-oxidizing Archaea in the Arctic Ocean and Antarctic coastal waters. *Env. Microbiol.* **11**: 2434-2445.

Yakimov, M.M., V. La Cono, and R. Denaro (2009). A first insight into the occurrence and expression of functional *amoA* and *accA* genes of autotrophic and ammonia-oxidizing bathypelagic *Crenarchaeota* of Tyrrhenian Sea. *Deep Sea Res. II.* **56**: 748-754.

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