Oil & Natural Gas Technology

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Quarterly Progress Report

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Characterization of Methane Degradation and Methane-Degrading Microbes in Alaska Coastal Water

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EXECUTIVE SUMMARY

This National Energy Technology Laboratory (NETL) project consists of an expedition to the Beaufort Sea in September 2009 and analyses of samples collected from that expedition. The main activity of the last quarter was to continue the molecular analysis of microbes and genes coding for methane degradation enzymes in sediment cores collected during the cruise. We were notified that more metagenomic sequence data will arrive in late October 2010. In addition to providing insights into methane degradation not otherwise possible, the new data could lead to modifications of the assays originally planned as part of the NETL project. Consequently, the assays originally proposed for the project have been delayed until the sequence data have been fully analyzed. A six month extension of the project has been approved. Our preliminary analysis of the metagenomic data revealed a complete pathway for sulfate reduction at depths where other data indicated active sulfate reduction. Only about 40% of the expected methane degradation genes in the metagenomic data have been detected so far, perhaps due to deficiencies in standard metagenomic sequence analyses. Ultimately, the metagenomic and 16S rRNA gene data will be combined with other microbial and biogeochemical data to obtain the most complete picture possible of methane degradation and other biogeochemical processes in these methane-rich sediments.

Progress Report

Task 1: Project Management Plan

This task was completed. During the last quarter, the milestones for the project were revised and resubmitted to reflect the delay in receiving the second year of funds.

Task 2: Cruise Logistics and Planning

This task was completed.

Task 3: Oceanic Cruise

This task was completed.

Task 4 - Methane Degradation Analysis

Methane degradation rates have been calculated, but detailed analyses and synthesis of the data remain to be done. The incubations for methane degradation were done on the ship as soon as possible after the sediment cores were available. The incubations were then killed and subsampled on the ship. As planned, these subsamples have been analyzed in Germany by our collaborator, Dr. Tina Treude. We are in the process of comparing data sets.

Task 5 - DNA Sequence Analysis

This task continues to be the main focus of this phase of the project. During the end of the first year of this project, we had analyzed microbial and biogeochemical data from several sediment cores in order to select a couple for more detailed analyses by gene sequencing. The microbial data include genes for aerobic (*pmoA*) and anaerobic (*mcrA*) methane degradation. From these data, two cores were selected for detailed analysis of the 16S rRNA gene, which is used to identify microbes. About 40 samples were analyzed, a number set by DOE's Joint Genome Institute (JGI) who did the sequencing. We are still analyzing the 16S rRNA data to obtain a complete picture of which microbes were in these cores.

As discussed in the previous quarterly report, data on the methane-rich cores were examined to select five samples for metagenomic sequencing, i.e. a complete list of genes found in the microbial communities. The number of samples (five) was set by JGI. The data used to select these samples included methane and sulfate concentrations and PCR-based assays for methane degradation genes (Fig. 1). Surprisingly, genes for aerobic methane degradation (*pmoA*) were found through the sediment core, even in the top layer were sulfate reduction is high (data not shown) and in the bottom layers were oxygen is absent. In contrast, genes for anaerobic methane degradation (*mcrA*) were detectable only from about 75-225 cm, even though this process was measurable by 14C-degradation experiments. It is not clear if these genes are truly absent or if there is a problem with the PCR assay; one potential problem is that the PCR primers, which were taken from previous studies, do not match the actual genes in these samples. The metagenomic data would help answer this question.

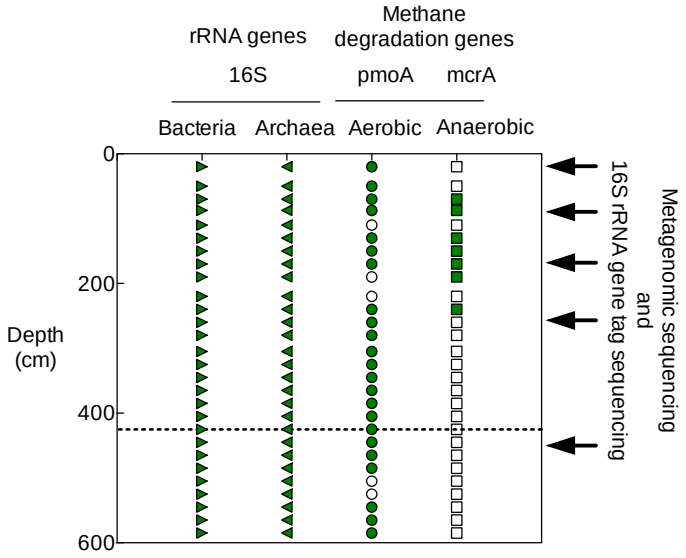

Figure 1. PCR-based assays for rRNA genes (for identifying microbes) and methane degradation genes. The filled-in symbols indicate a positive reaction.

Overview of metagenomic data We received the first installment of the metagenomic data from JGI in June 2010 and have been promised the final installment of about the same size in late October 2010. Dr. Matt Cottrell visited JGI (October 18-22, 2010) to attend a data analysis workshop and to talk with JGI colleagues about the data. Although detailed analysis of these data has been delayed until all of the data arrive, we can make a few observations based on our preliminary analyses using the standard JGI automated data analysis tools.

The main purpose of the metagenomic sequencing effort is to gain insights into methane degradation and related processes, but the metagenomic data can also be used to address basic questions about the types of microbes present in these samples. We have three ways to address that question: two from the metagenomic data and a third from the "tag 16S rRNA sequences" generated from PCR-based assays. In contrast, metagenomic data are not generated by PCR.

All three approaches indicated that organisms in the Bacteria domain dominated the 20 cm sample from the depth core (Table 1), but the three approaches differed for the other assignments. Taxonomic identity deduced from protein sequences suggested that Archaea made up over 3% of the total, substantially higher than the estimates based on the metagenomic or the tag 16S rRNA sequences. Also, a high fraction (16%) of the tag 16S rRNA sequences were said to be from eukaryotes, which is not seen in the metagenomic data. Finally, high percentages (13 and 28%, respectively) of the protein and 16S rRNA genes could not be classified at all ("other" in Table 1). These data indicate potential problems with the standard JGI analyses for metagenomic data. The standard analyses may not be appropriate for anaerobic organisms.

Table 1. Classification of sequences at the domain level. The 16S rRNA gene is the "gold standard" for classifying organisms, but taxonomic identity can be deduced from protein sequences.

	Metagenome		Tag
	Protein	16S rRNA	16S rRNA
Bacteria	83	72	82
Archaea	3.5	0	0.04
Eukaryota	1.3	0	16
Other*	13	28	3.9

*"Other" means that the sequence could not be assigned to one of the three domains of life. There are no domains other than Bacteria, Archaea and Eukaryota.

Another indication of possible problems with the standard JGI analyses comes from examining sulfate reduction and methane degradation genes (Fig. 2). ^{35}S assays

indicated that sulfate reduction was occurring at 20 cm, one of the depths selected for metagenomic analysis. Tag sequence analysis revealed the presence of *Desulfatibacillum alkenivorans* AK-01 and many other members of the Deltaproteobacteria known for sulfate reduction. Consistent with this analysis, the complete dissimilatory sulfate reduction pathway was found. Anaerobic methane oxidation is dependent on sulfate reduction, but we have not so far found all of the methane oxidation genes at this depth, even though methane degradation was occurring, according to ^{14}C assays. We suspect this apparent absence is not real but rather is due to problems with the standard JGI analyses, which are not designed for these organisms

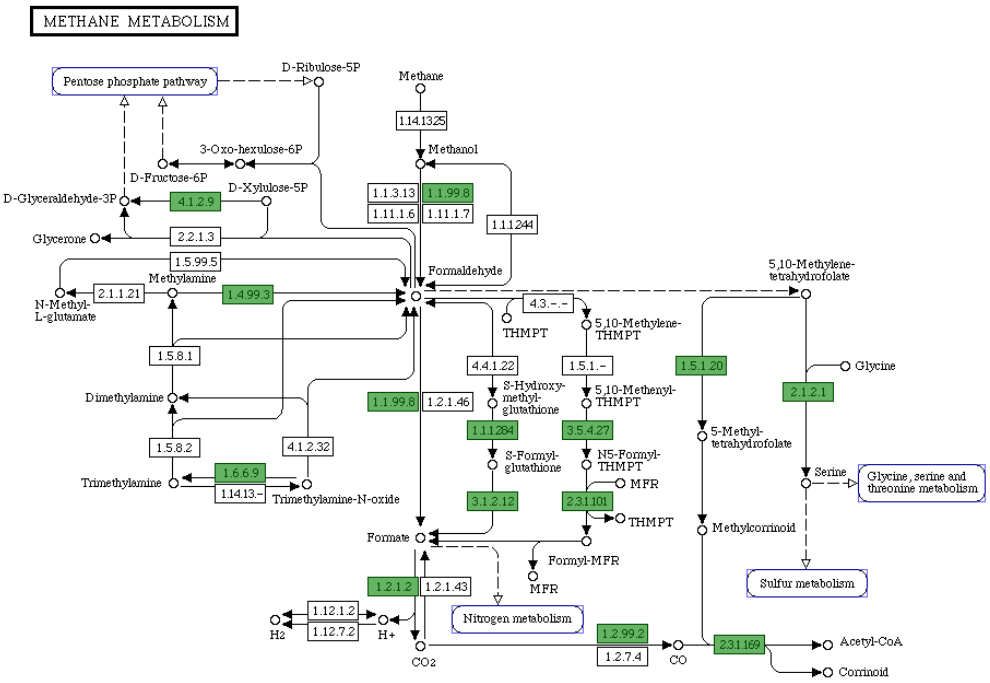

Figure 2. Aerobic methane degradation pathway at 20 cm. The filled-in green boxes indicate genes that were detected while the open boxes are expected genes that have not been detected. A complete dissimilatory sulfate reduction pathway was found at 20 cm.

Other Tasks

The remaining parts of Task 4 and Tasks 5-7 are scheduled to be completed during the next year of the project.

Conclusions

Preliminary analyses of the metagenomic and tag data indicate the potential of this approach for revealing new insights into methane degradation and related processes, like sulfate reduction, but it also pointed to problems with the standard automated sequence analyses. We are working at correcting these problems as we wait for the final installment of the metagenomic data. Although this delays the planned PCR assays for methane degradation genes, the wait is worth it. This information will help us design better methods (better PCR primers) to analyze these genes and to gain more insights into methane degradation in the Beaufort Sea.

Cost Status

The table below gives the project expenses for the third quarter of Year 2 as originally budgeted ("Original") and actual expenditures ("Actual"), as of Oct 14, 2010.

Third Quarter of Second Year Budget

	Original	Actual
Personnel	$5,426	$23,858
Benefits	$1,489	$7,230
Permanent Equipment	$0	$0
Expendable Supplies	$921	$290
Travel	$0	$250
Subtotal	$7,836	$31,628
Indirect costs (53%)	$4,153	$16,763

The large difference between actual expenditures and the original budget is due to personnel costs. The difference between the original and actual this quarter (-$18,432) is about the same as the surplus last quarter ($13,866). As explained in last quarter's report, in the original proposal, all expenditures were projected to be high initially and then to decrease during the year. We now expect a much more even rate of expenditures over the second year of this project. This more even rate reflects how the University distributes personnel money (especially for Kirchman), but it also is closer to how the work actually proceeds.

Products

• Revised Web site

A Web site outlining work in the Arctic by Kirchman lab, including the NETL project (http://www.ocean.udel.edu/cms/dkirchman/Arctic/), has been revised.

• Poster presentation, “Methane-degrading microbes in Arctic sediments”, Oct 18-Oct 22, 2010, JGI Metagenomic Workshop, Walnut Creek, CA.

• Contacts and lectures with the general public

Kirchman’s seminars on climate change in polar environments are available on his Arctic web site.

• Inclusion of information and pictures from the Beaufort cruise in a general course for undergraduates and graduate students on marine biology and biological oceanography taught by Kirchman.

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