Microbial Communities in Methane Hydrate-Bearing Sediments from the Alaskan North Slope

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Abstract

High latitude soils and sediments often contain large quantities of methane as well as microbial communities capable of producing and consuming the methane. We studied the microbial communities collected from hydrate-bearing sediments on the Alaskan North Slope to determine how abiotic variables (e.g., grain size, hydrate presence, original depositional environment) may control the type and distribution of microbes in the sediments. The cores were acquired from sub-permafrost, Eocene (35-36 million years ago [MYA]) sediments laid down as a marine transgressive series within which hydrates are believed to have formed 1.5 MYA. Forty samples, eight of which originally contained hydrates, were acquired from depths of ca. 606-666 meters below land surface. Five samples from drilling fluids acquired from the same depth range were included in the analysis as a control for contamination during the drilling and handling of cores. DNA was extracted from the samples (typically <1 ng DNA/g sediment was recovered) and then amplified using polymerase chain reaction with primers specific for bacterial and archaeal 16S rDNA. Only bacterial DNA amplicons were detected. Terminal-restriction fragment length polymorphism (t-RFLP) was used to measure bacterial diversity in the respective samples. Non-metric multidimensional scaling (NMDS) was then used to determine the abiotic variables that may have influenced bacterial diversity. NMDS analysis revealed that sediment samples were distinct from those obtained from drilling fluids suggesting that the samples were not contaminated by the drilling fluids. All samples had evidence of microbial communities and sample depth, temperature, and hydrate presence appeared to have some influence on community diversity. Samples sharing these environmental parameters often shared common t-RFLP profiles. Further examination of selected samples using clone libraries should help to identify the key taxa present in these unique sediments and yield a better understanding of the biogeochemistry of these gas-bearing systems.

Objective: Determine how abiotic variables (e.g., grain size, hydrate presence, original depositional environment) may control the type and distribution of microbes in subpermafrost sediments.

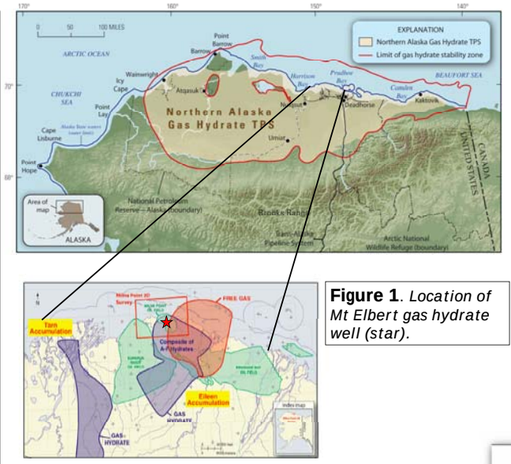
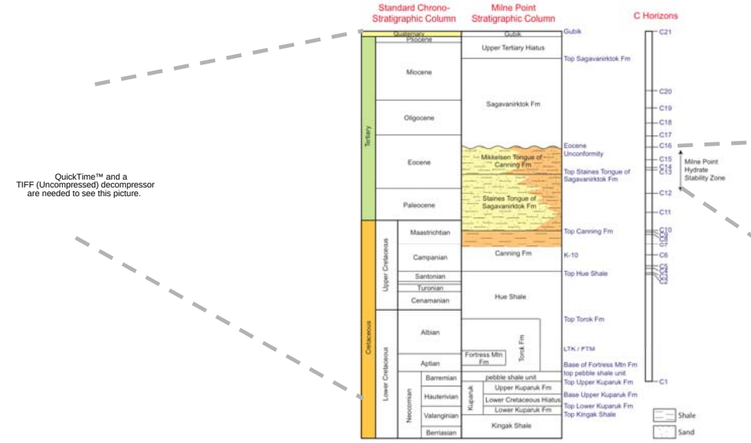


Figure 1. *Location of Mt Elbert gas hydrate well (star).*



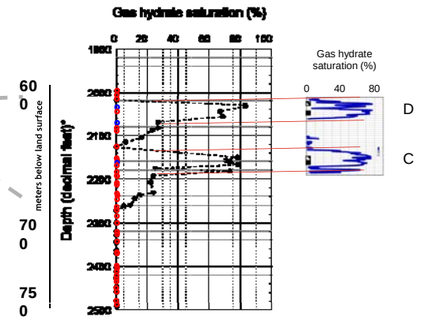


Figure 2. *Depth profile for samples acquired from the Mt Elbert gas hydrate well showing geologic formations and microbiology sample locations (red circles, far right). Figure is partly from Masterson et al. (2001).*

Methods

Sample site and sample collection
- Mt Elbert drill site on the Alaskan North Slope (Figure 1)
- From 154 m of vertical core, 40 samples preserved and shipped in liquid N_2 (Figure 2)
- Eight samples contained hydrate, all of which were from between 607 and 666 mbls
- Cores were subcored to obtain pristine material

Molecular methods
- DNA was extracted (MoBio Power Soil kits) from six grams of each sediment sample
- DNA yield was < 1 ng DNA/g sediment
- PCR amplification (bacterial primers 8F and 926R; 35 cycles) and dilution of the DNA (Figure 3) indicated that PCR inhibitors were minimal
- t-RFLP amplification (Figure 3) was followed by restriction with Hin6I, MspI, and BsuRI
- t-RFLP patterns from BsuRI restrictions were analyzed using non-metric multidimensional scaling (NMDS) to determine natural groupings of the communities and how such groupings may be controlled by abiotic parameters

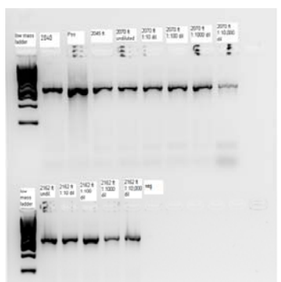


Figure 3. *Results of PCR amplification following dilution of two selected samples.*

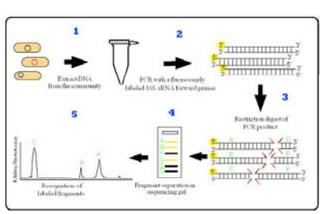


Figure 4. *Generalized strategy for terminal restriction length polymorphism (t-RFLP) analysis of DNA from complex communities (from Gruntzig et al. 2002. Mich. State Univ.)*

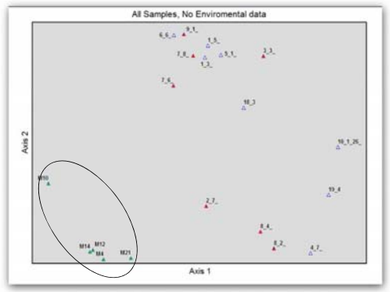


Figure 5. *NMDS plot of t-RFLP data. Samples from hydrate zones (red), non-hydrate zones (open), and drilling fluids (green; circled) are shown.*

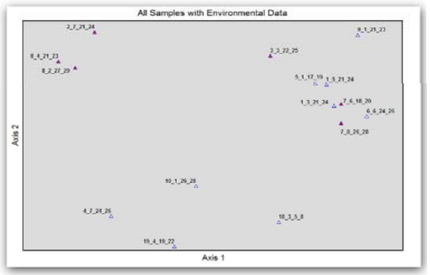


Figure 6. *NMDS plot of t-RFLP data (drilling mud samples excluded). Environmental factors included in the analysis include depth, hydrate presence, grain size, density-derived porosity, temperature, mud gas methane, drilling rate, and gamma log. Environmental factors that align consistently with axis 2 are depth (R=0.63), hydrate presence (R=0.66), and temperature (R=0.62).*

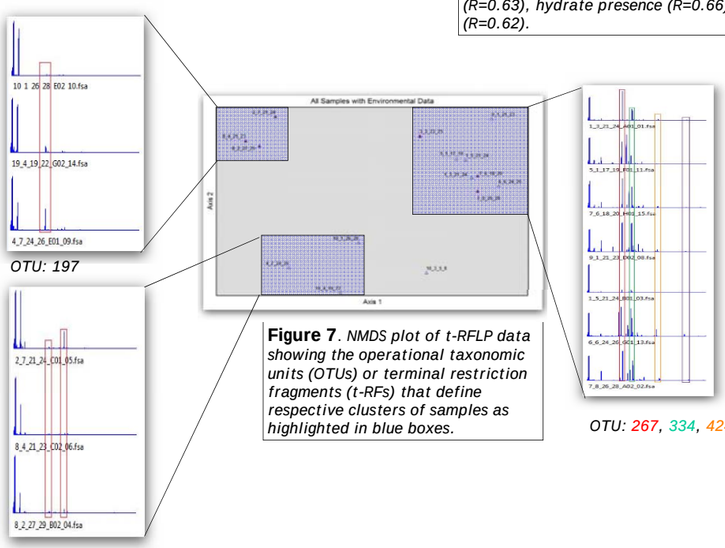


Figure 7. *NMDS plot of t-RFLP data showing the operational taxonomic units (OTUs) or terminal restriction fragments (t-RFs) that define respective clusters of samples as highlighted in blue boxes.*

Summary
- Microbial communities present in drilling fluids are distinct from those in the cores
- Depth, hydrate presence and temperature exert some control over the microbial community members present in the sediments, as determined by t-RFLP profiles from extracted, amplified DNA.
- Some samples are clustered together in NMDS plots by virtue of microbes that are common to these samples. The presence of these microbes is evident by the characteristic length of their terminal restriction fragments which represent unique operational taxonomic units (OTUs) in the sediments.

Next Steps and Future
- Completion of 16S rDNA clone libraries developed from DNA extracted from selected or representative cores.
- Consideration of sample mineralogy to determine importance of this factor in structuring microbial communities.
- Contiguous coring and sampling from the surface through the permafrost and into the hydrate zone beneath would provide a complete picture of microbial communities and their functional potential in this sensitive environment.

References

Masterson WD, Dzou LIP, Holba AG, Fincannon AL, Ellis L (2001) Evidence for biodegradation and evaporative fractionation in West Sak, Kuparuk and Prudhoe Bay field areas, North Slope, Alaska. Organic Geochemistry 32:411-441

Acknowledgements

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