Hydrocarbon degraders in the Lake Fryxell ice cover

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INTRODUCTION

The 17January 2003 crash of the Bell 212 helicopter 79U on the Lake Fryxell ice cover resulted in a mixed hydrocarbon spill comprised mostly of diesel fuel (Alexander and Stockton 2003). Preliminary assessment of the spill demonstrated our lack of knowledge of the fate of fuel in this pristine system, which serves as a site of long-term ecological research. There was a clear need to assess the impact of this hydrocarbon spill on the Lake Fryxell ice cover and its inhabitants. Towards this goal we have conducted a series of biological experiments to determine the fate of these hydrocarbons within the ice and its influence on biological activity and diversity.

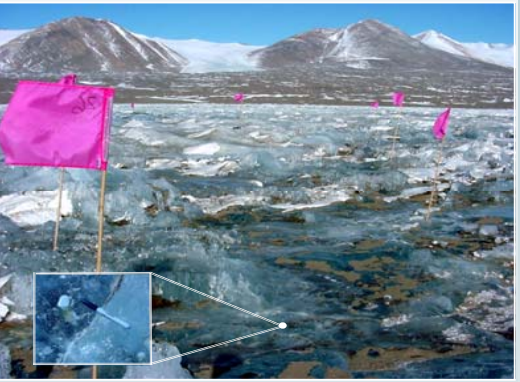

Figure 1. Lake Fryxell ice cover showing the location of flags marking the crash site, and a pull-out close-up of hydrocarbon pockets in the ice.

COLLECTION & PROCESSING OF SAMPLES

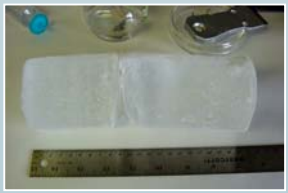

Figure 2 a, b. Ice cores were collected using a 10cm sipre corer from both contaminated and uncontaminated areas of the Lake Fryxell ice cover. Cores were extruded into flat wrap, stored in core tubes and returned to MSU for processing. Cores used in the experiments were scraped inside a -20˚C walk-in freezer using razor blades and clean techniques to remove any outside contaminants that may have been found on the surface of the cores. Several core sections were compiled to provide enough material for experiments (typically ~1500mls with a sediment weight of 2g/L).

METHODS & RESULTS

The goal of our experiments was to assess the ability of microorganisms from the Lake Fryxell ice cover to degrade hydrocarbons. Our hypothesis was that there are hydrocarbon degrading organisms present in the lake ice. In order to test this hypothesis we set-up a series of experiments to examine the degradation of JP8 jet fuel, as well as fractions of this fuel including naphthalene (aromatic) and nonane (C_9 alkane). Because reports in the literature (Aislabie et al. 1998; Kerry 1993) have shown enhanced degradation of hydrocarbons in Antarctic soils with the addition of nitrogen and phosphorous, our experimental treatments were run with and without additional N and P (see Table 1).

Table 1.

Experiments	Treatments	Parameters Monitored
^{14}C-naphthalene degradation	"Control" = ice core melt	TOC
		Bacterial cell counts
^{14}C-nonane degradation	"JP8" = 0.5% JP8	Hydrocarbons
		Chlorophyll-a
JP8 degradation (respirometry)	"JP8-NP" = 0.5% JP8 + 200μM NH_4NO_3 + 20μM H_2PO_4	Phylogenetic diversity (DGGE)

Testing for hydrocarbon degradation by the native ice microbial community was carried out using ^{14}C-naphthalene or ^{14}C-nonane in biometra flasks (Figure 3) over a period of x weeks. Melted ice core samples were spiked with a final concentration of 0.1μCi/mL of [Ul-^{14}C] naphthalene or nonane, sealed in the flasks with KOH as the CO_2 trap and incubated shaking at 6˚C under illumination.

Figure 3. Biometra flasks on a shaker table inside the illuminated incubator.

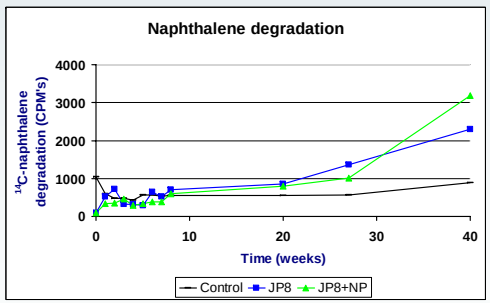

Figure 4. Naphthalene mineralization in samples from the Fryxell lake ice cover.

Samples were monitored weekly for a period of 8 weeks, and then over longer time intervals up to 40 weeks. During sampling 0.5mls of the CO_2 trapping agent (1M KOH) were removed, neutralized, and added to Cyotscint ES scintillation cocktail. Samples were allowed to sit for 24hrs before counting on a Beckman liquid scintillation counter in order to reduce any chemiluminescence. The amount of $^{14}CO_2$ trapped and counted was used as the measure of mineralization rates (Figure 4). By the end of 40 weeks it is clear that the native ice microbial community is capable of degrading naphthalene, and that the addition of N and P enhanced this mineralization. Studies are on-going using ^{14}C-nonane.

	Bacterial counts	TOC (mg/L)	Hydrocarbons present
t=0	1.6 x 10^5		?
Control t= week 1	4.82 x10^5	1.794	?
Control t= week 8	1.35 x 10^5	4.209	?
JP8 t= week 1	2.11 x 10^5	9.23	?
JP8 t= week 8	1.66 x 10^5	22.694	?

Table 2. Data from the ^{14}C-naphthalene degradation experiment

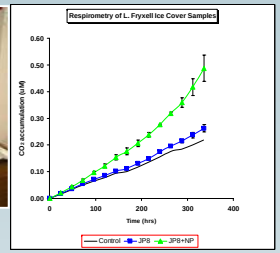

Figure 5 a, b. a.) Columbus Instruments Micro-Oxymax Respirometer set-up to run hydrocarbon containing samples at 4.5˚C. b.) Evolution of CO_2 from samples amended with JP8 and JP8+NP

Preliminary phylogenetic studies were conducted on ice cores collected from both hydrocarbon free and hydrocarbon contaminated sites in the Lake Fryxell ice cover. Figure 6 shows a TGGE gel. The first 3 lanes are from contaminated samples, while the next two are from clean cores. We are in the process of completing our phylogenetic studies on these lake ice samples.

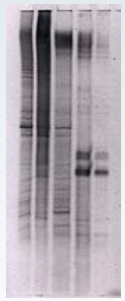

Figure 6. TGGE gel of clean and contaminated Fryxell lake ice samples. Primers 341F-GC and 534R were used to amplify the samples, which were subsequently run for 16hrs at 60V over a temperature gradient of 57.5-70.5˚C.

Ice core samples for microbial enrichments were collected prior to the fuel spill during October 2001 at a distance from all known LTER drilling operations. Melted ice core samples were enriched in R2A broth and then isolated on agar at 2-4ºC. Genomic DNA was extracted from isolates and partial sequence for the 16S rRNA gene was obtained. A neighbor-joining tree (Figure 7) was constructed using the Kimura 2-parameter model for estimating evolutionary distance between sequences. Phylogenetic analysis was conducted using MEGA 2.1 (Kumar, et al. 2001). Isolates from Lake Fryxell ice cover are shown highlighted in blue. Isolates from the *Beta-Proteobacteria* are closely related to known naphthalene degraders (Jeon et al. 2003), suggesting the ability to degrade complex hydrocarbons is present in the natural ice community assemblage.

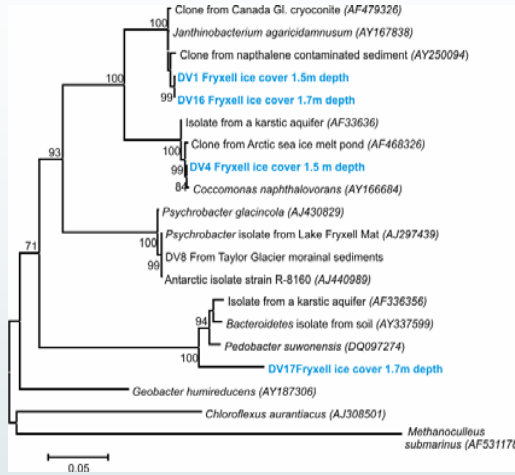

Figure 7. Neighbor-joining tree constructed using the Kimura 2-parameter model. Isolates from Lake Fryxell ice cover are shown highlighted in blue The scale bar represents 0.05 fixed substitutions per nucleotide position

CONCLUSIONS

The presence of hydrocarbon degraders in the ice cover of Lake Fryxell indicates the potential for biodegradation of these compounds *in situ*. This study demonstrates not only that the native ice community found in Lake Fryxell is capable of degrading JP8 jet fuel, and fractions therein, but also that these microbes were present before the helicopter crash in 2003.

Light fuels with a high vapor pressure, such as mogas and jet fuels, have been shown to volatilize from Antarctic soils (Aislabie et al., 2004 and refs therein), while heavier fractions such as engine oils are more viscous and less volatile. Based on our findings, bioremediation efforts should consider both volatilization and the role of added N and P on *in situ* mineralization.

LITERATURE CITED

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