Hydrocarbon Contamination and Biodegradation Within the Permanent Ice Cover of Lake Fryxell, Antarctica

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Research Site:

During the summer of 2003, a helicopter crashed onto the permanent ice cover of Lake Fryxell contaminating the surface with several hundred liters of jet fuel (JP8). Lake Fryxell is one of the MCM LTER sampling sites providing us with a research framework to examine the influence of this spill on the microbial community that resides within the ice cover.

Fig.1 a. Helo flying over Lake Fryxell b. Crash site c. Fuel spill on ice surface

Sample Collection:

Ice cores were collected using a 10cm SIPRE corer from both contaminated and uncontaminated areas of the Lake Fryxell ice cover in December of 2003.

Fig.2 Drilling ice cores with 10cm SIPRE corer

Decontamination:

Ice cores were shipped back to MSU were they were decontaminated by scraping in a -20°C walk-in freezer using clean techniques to remove any outside contaminants.

Fig.3 Ice core obtained from Lake Fryxell ice cover

Materials and Methods:

Respiration Experiments

Goal: To test the hypothesis that there are hydrocarbon degrading organisms present in the natural lake ice community. These experiments measured the degradation of JP8 jet fuel, as well as fractions of this fuel including naphthalene (aromatic) and nonane (C9 alkane). Studies have shown enhanced degradation of hydrocarbons in Antarctic soils with the addition of N and P, therefore our experimental treatments were run with and without additional N and P.

Fig.4 Biometric flasks containing 14C naphthalene and 14C nonane degradation experiments. Control, JP8, JP8+NP

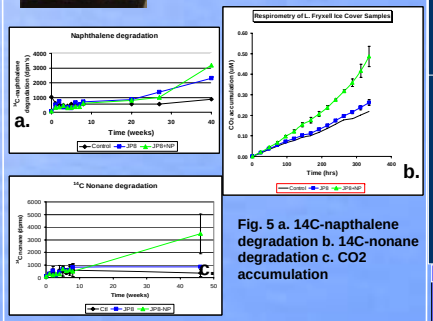


Fig. 5 a. 14C-napthalene degradation b. 14C-nonane degradation c. CO2 accumulation

Temperature Gradient Gel Electrophoresis (TGGE):

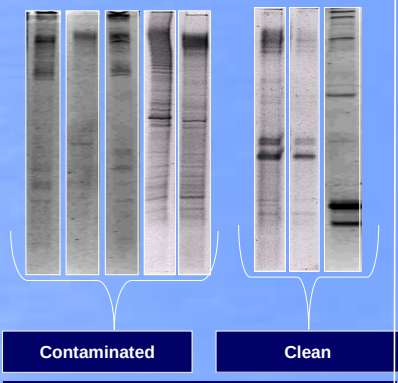


Fig.6 TGGE analysis of 16S rDNA (*E.coli* # 341F- 534R) from clean and contaminated Lake Fryxell lake ice sediment.

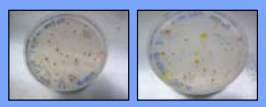
Isolates Capable of Bioremediation at 15°C

Table 1. Isolates were cultured from uncontaminated ice cores on 1/10 solid R2A agar, isolates shown were capable of degrading JP8 fuel as the only carbon source. Genomic DNA was extracted, PCR was preformed using several different primer sets (Table 2). PCR product was sent to Tgen for sequencing. Sequences were aligned with Bioedit and NCBI's blastn tool was used to find the closest relative.

Strain ID	Cell Type	Degrade Jp8 Jet Fuel @ 15ºC	Color	Isolated From	Alignment With Closest Relative	% Similarity	Closest Relative	Genbank Accession Number	Info on Closest Relative
T=0-8	Bacteria	✓	Yellow	Lake Fryxell Lake Ice	724/725	99%	Nocardioides sp.	AY571807	Fuel Sites in Scott Base, Antarctica
T=0-9	Bacteria	✓	Red	Lake Fryxell Lake Ice	696/702	99%	Uncultured bacterium clone ARKCRY2 Uncultured *Flexibactera-cea bacterium*	AY198110 DQ418532	Artic Sea Floes Puruoga-ngri ice core
T=8-2	Eukarya green algae	✓	Green	Lake Fryxell Lake ice enriched with JP8 Jet Fuel	531/555	95%	Scenedesmus obliquus	N/A	No Info
T=8-10	Bacteria	✓	Off White	Lake Fryxell Lake ice enriched with JP8 Jet Fuel	564/573 558/573	98% 97%	Mycobacterium sp. Mycobacterium vanbaalenii	AY439252 AY636002	Deep Greenland glacier ice core Polycyclic aromatic hydrocarb. degrader

Sequencing	Primer	Direction	Sequence 5'- 3'
	27F	Forward	AGAGTTTGATCCTGGCTCAG
	515F	Forward	GTGCCAGCMGCCGCGGTAA
	926R	Reverse	ACCGCTTGTGCGGGCCC
	1391R	Reverse	GACGGGCGGTGTGTRCA

Table 2. List of primers used for sequencing



Before fuel addition | After fuel addition

Fig. 7 (Above) Uncontaminated and contaminated ice core was melted and plated onto solid 1/10 R2A agar. While many of the same colony types existed on both plates, several different colony types were observed on the contaminated plate.

Fig 8. Isolates cultured from the uncontaminated and contaminated ice were tested for Jp8 degradation capabilities by inoculation in a liquid media containing JP8 as the sole carbon source.
Left: Blank containing media with no inoculum
Right: Isolate T=0-9 ***Flexibacteraceae* sp.** capable of growth on Jp8 fuel

Conclusions:

Our experiments show that the native ice community found in Lake Fryxell is capable of degrading JP8 jet fuel and fractions of the fuel. Respirometry experiments showed that addition of N and P increased the rate of degradation, this may be due to the fact that this environment is limited in these nutrients. A change in community and diversity was observed in both the TTGE analysis and culturing methods suggesting that the hydrocarbon spill changed the community structure of the lake ice.

Acknowledgements:

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