STRUCTURE AND FUNCTION OF FUNGI IN ANTARCTIC LAKE ICE

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ABSTRACT

The permanently ice covered lakes of Antarctica's Dry Valleys region harbor a diverse group of microbes that live in unique liquid water habitats. In particular, the lake ice hosts microbial communities that form functional ecosystems. My research focuses on the functional role of fungi in the permanent ice covers of lakes in the McMurdo Dry Valleys. Laboratory cultures obtained from ice cores taken from two lakes were tested for growth characteristics under various temperature and nutrient regimes. Partial ITS- DNA sequencing was used to screen for functional genes and to identify novel fungal types unique to this region of Antarctica. Our results show that axenic cultures were successfully obtained from the permanent ice cover of the lake ice. Temperature experiments revealed that these organisms were psychrotolerant, but grew most rapidly at 20°C. In addition, the isolated organisms possess antifungal activity that has not been previously reported from fungi isolated from Antarctic lake ice. Results from the study will be the first to address the structure and function of fungi in these sub-zero habitats and the potential for eukaryotic life to exist in icy worlds beyond Earth.

OBJECTIVES AND HYPOTHESES

Overarching Objective:

To study the structure, function and distribution of fungi in the lake ice ecosystem of the McMurdo (MCM) Dry Valley lakes.

Hypotheses:

1. Fungi are present in the lake ice and are viable as spores and mycelium
2. Fungi in the lake ice are Psychrophilic
3. Fungi in the lake ice produce bioactive volatile compounds

STUDY SITE

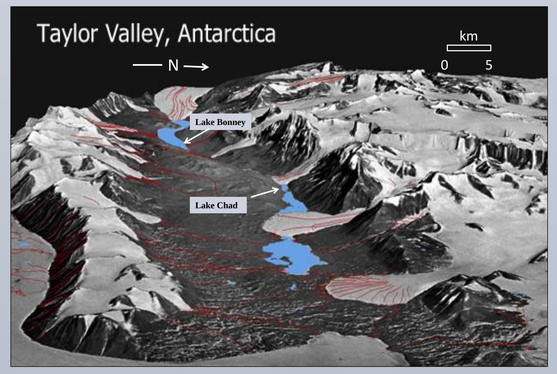

Figure 1: Location of the MCM Dry Valleys (77°S, 163 °E) and the study lakes in the Taylor Valley

METHODS

Field Sampling:

Samples were collected during the 2012- 2013 field season from several lakes in Taylor Valley (Figure 1).

Decontamination Protocol:

Ice cores were decontaminated using the protocol of Christner et al., 2005 in a Class 1000 Cold Clean lab (Figure 2)

Figure 2: Processing Ice Samples in the Subzero facilities (Picture by: Alex Michaud)

Isolation and Culturing :

Fungal mats were isolated and cultivated on Potato Dextrose Agar (PDA) medium.

Phylogenetic identification:

1. DNA extraction using DNeasy Plant Mini Kit (Qiagen)
2. Phylogenetic analysis was carried out by the acquisition of the ITS- 5.8 S ribosomal gene sequence
3. The ITS regions of the fungus were amplified with the universal ITS primers, ITS1F and ITS4 using PCR (White et al. 1990)
4. Sequencing was performed by Functional Biosciences (Madison, WI)
5. Sequences were organized and queried against the NCBI database using an in house program (SeqTrace)

Growth Rates:

The isolated cultures grown on PDA plates were tested for growth characteristics at several different temperatures

Antimicrobial Activity of Fungi:

Dual Culture assay

An agar block of a 15-day old culture of fungus was placed in the center of PDA plate and the pathogen was inoculated at 2 cm juxtaposed to the pathogen and tested for bioactivity (Figure 3)

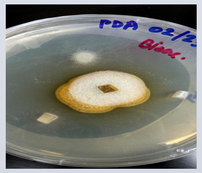

Figure 3: A 9cm wide petri dish plated with the fungus and pathogens placed in periphery

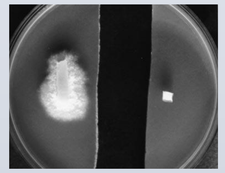

Figure 4: A 9cm wide petri dish plated with the fungus and the pathogen to check its bioactivity (Picture by Dr. Gary Strobel)

Gas test

An agar strip 2.5 wide was completely removed from the mid-portion of a Petri plate of PDA. Then, the test organism was inoculated and grown on one side while The pathogen was placed on to the agar half-moon strip on the opposite side of the plate (Figure 4)

RESULTS

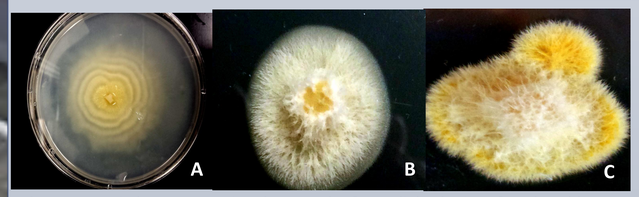

Figure 5: Petri plates supporting the growth of mycelial colonies of 10 day old axenic cultures

→ Figure 6 demonstrates the dependence of growth rate on temperature

→ At 0°C, the growth is the slowest and most rapid at 20°C

→ Figure 5 demonstrates isolation and characterization of fungi from the lake ice was achieved

→ Based on the morphology and DNA analysis it was confirmed that these isolates were axenic

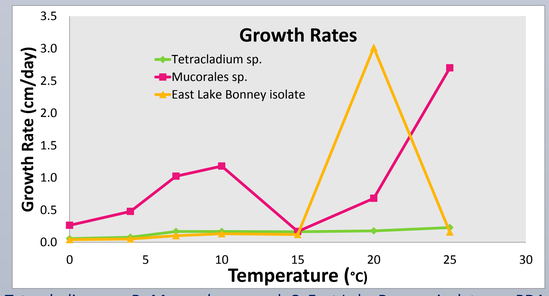

Figure 6: Effect of temperature on the radial growth of fungal isolates identified as A .*Tetracladium sp.,* B. *Mucorales sp.* and C. East Lake Bonney isolate on PDA medium.

Isolates	Inhibition (%) after 48 h exposure with test organisms			
	Pythium ultimum	*Phytophthora cinnamoni*	*Sclerotinia sclerotiorum*	*Botrytis cinera*
Tetracladium sp.	17.46	91.89	96	70
Mucorales sp.	0	0	0	0
Unknown	0	0	0	0

Figure 7: Effects of the bioactive compounds produced by the isolates on fungal pathogens

$$\%\text{Inhibition} = \frac{\text{Growth on Control} - \text{Growth on Treatment}}{\text{Growth on Control}} \times 100\%$$

→ The isolates exhibited antagonistic properties against the most detrimental pathogens

CONCLUSIONS

1. Axenic cultures were successfully obtained and identified from the permanent lake-ice cover:
   - *Tetracladium sp.* from Lake Chad
   - *Mucorales sp.* from East Lake Bonney
   - *Unknown* from East Lake Bonney
2. Temperature experiments revealed that the isolated organisms were psychrotolerant. Growth rate was greatest at 20°C
3. The isolated organism *Tetracladium sp.* possess antifungal activity against known plant pathogens that has not been previously reported from fungi isolated from Antarctic lake ice

SIGNIFICANCE

Isolating and characterizing fungi in the icy ecosystem of the dry valleys provides a better **understanding of the component members of the ecosystem that have been not studied previously.** These fungi from the polar region are capable of inhibiting the growth of detrimental pathogens. This could have several **promising applications in the field of medicine and agriculture.**

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