Physiological characteristics of fungi associated with Antarctic ice

Priyanka S. Kudalkar[1], Gary Strobel[2], Cathy Cripps[2] and John Priscu[1]

[1]Land Resources and Environmental Sciences and [2] Department of Plant Sciences Montana State University, Bozeman, MT 59717, USA

Abstract

The permanently ice covered lakes of Antarctica's Dry Valleys region harbor a consortia of phototrophic and heterotrophic microorganisms that metabolize during the short summer months when solar radiation produces melt inclusions within the ice. Our study focused on the physiology and function of fungi in lake ice ecosystems of the McMurdo Dry Valleys. Laboratory cultures obtained from ice cores taken from three lakes were tested for growth characteristics under various temperature and nutrient regimes. Partial ITS- DNA sequencing was used to identify novel fungal types that are unique to this region. Our results show that axenic cultures were successfully obtained from the permanent ice cover of the lake ice. Temperature response experiments revealed that the isolated fungi were psychrotolerant and growth rates were greatest at 25°C. In addition, the isolated organisms possess wide-spectrum antifungal activity against several plant pathogens suggesting potential antimicrobial activity. The metabolic potential and preferred substrate utilization was examined by exposing fungal isolates to a variety of substrates in a 96 well "Biolog" plate.

Objectives and Hypotheses

Overarching Objective:

Fungi that live in the permanent ice covers of McMurdo Dry Valley lakes possess novel physiological characteristics allowing them to grow in this environment.

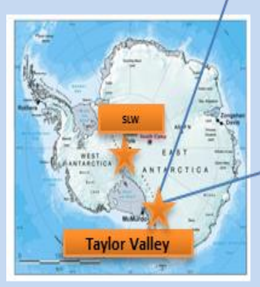
Hypotheses:

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
4. Fungi have a preference for labile carbon substrates

Methods

Field Sampling:

Ice core samples containing microbial mats were collected during the 2012- 2013 summer season from the permanent ice covers of Lake Chad and East Lake Bonney in Taylor Valley (Figure 1). The sample from Subglacial Lake Whillans (SLW), which lies 800 m beneath the surface of the Whillans Ice Stream in West Antarctica, was collected in January 2013. The sediment sample from SLW was collected with sterile tools and frozen immediately.



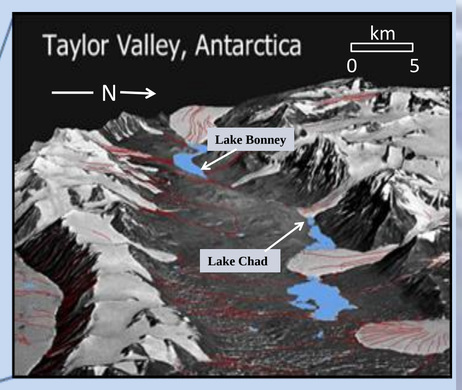


Figure 1: Location of SLW and the study lakes in the Taylor Valley

Decontamination Protocol:

Ice cores were decontaminated using the protocol of Christner et al., 2005 in a Class 1000 Cold Clean lab and microbial mats were retrieved.

Isolation and Culturing:

Fungi were isolated from mat samples by cultivating on Potato Dextrose agar (PDA) medium containing antibiotic (Cyclodextrin) to restrict bacterial growth.

Taxonomic identification:

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

Growth Rates:

Growth rates were determined for cultures grown on PDA plates at 0, 4, 7, 10, 15, 20 and 25°C

Antimicrobial Activity of Fungi:

Dual culture assay

An agar block of a 10-day old fungal culture was placed in the center of a PDA plate and the pathogens- *Pythium ultimum, Phytophthora cinnamoni, Sclerotinia sclerotiorum and Botrytis cinera* were inoculated at 2 cm juxtaposed to the fungus and tested for bioactivity (Figure 2). An unidentified heterotrophic bacterial isolate was streaked onto the test plate, and growth was scored as positive if colony development eventually occurred.

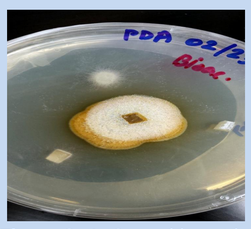


Figure 2: A 9cm wide petri dish plated with the fungus and pathogens placed in periphery

Measuring substrate utilization using Biolog Assay:

1) The Biolog technique is a redox system where microorganisms oxidize substrates present in the wells and simultaneously, reduce colorless tetrazolium dye to a violet formazan that can be measured spectrophotometrically at 490 nm (A.Stefanowicz, 2006).
2) This technique was used to estimate metabolic potential of microbial isolates.
3) The microtiter plates used in this experiment contained 95 different carbon substrates in the wells, and a control well.

Results

1. Taxonomic identification

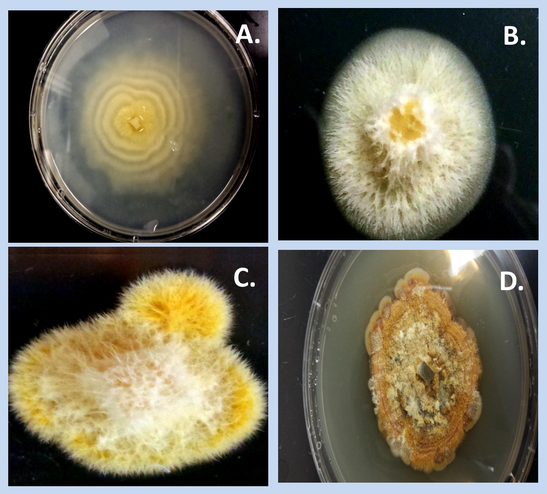


Figure 3: Petri plates supporting the growth of mycelial colonies of 10 day old axenic cultures. A)*Tetracladium ellipsoideum* from Lake Chad , B) *Mucorales sp.* from East Lake Bonney, C) Unidentified isolate from East Lake Bonney , and D) *Lecythophora hoffmannii* from the sediments of Subglacial Lake Whillans. Based on the morphology and DNA analysis it was confirmed that these isolates were axenic.

2. Growth Rates

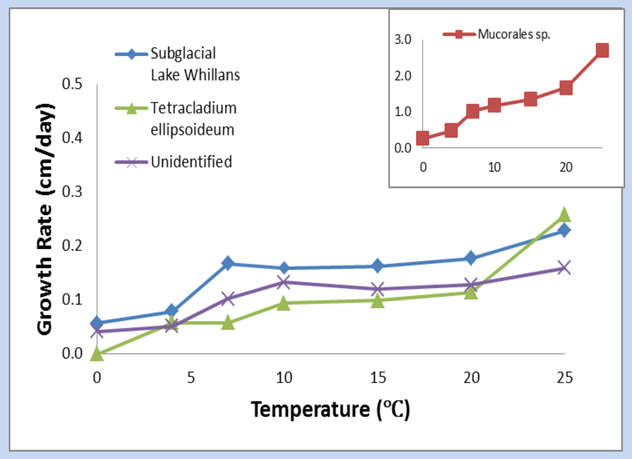


Figure 4: Effect of temperature on the radial growth of fungal isolates

3. Antimicrobial Activity of Fungi

Table 1. Effects of the bioactive compounds produced by the isolates on fungal pathogens and bacteria from Subglacial Lake Whillans (SLW**).** The inhibition values were calculated as percentage growth inhibition as compared to an untreated control. The tests were repeated three times with comparable results

Isolates	Percent Inhibition after 48 h exposure with test organism				
	Pythium ultimum	*Phytophthora cinnamoni*	*Sclerotinia sclerotiorum*	*Botrytis cinera*	SLW bacterial strain
Tetracladium sp.	17.46	91.89	96	70	0
Lecythophora hoffmanii	92.78	20.21	30.62	0	0
Mucorales sp.	0	0	0	0	0
Unidentified	0	0	0	0	0

4. Substrate Utilization

Table 2. The substrate utilization profile of *Mucorales sp.* at 4°C and 24°C comparing the common substrates. Additional substrates were utilized at 24°C but are not shown.

Substrates	At 4°C incubation time (days)				At 24°C incubation time (days)			
	1	2	3	4	1	2	3	4
α-Cyclodextrin	0.34	0.36	0.34	0.35	0.24	0.20	0.21	0.21
Adenosine	0.29	0.26	0.25	0.27	0.13	0.08	0.14	0.13
Sebacic Acid	0.20	0.19	0.20	0.26	0.30	0.23	0.33	0.46
Glycogen	0.19	0.27	0.24	0.24	0.15	0.15	0.18	0.21
L-Phenylalanine	0.18	0.10	0.14	0.14	0.11	0.13	0.16	0.16
D-Ribose	0.17	0.16	0.14	0.15	0.19	0.19	0.23	0.24
N-Acetyl-D-Glucosamine	0.13	0.15	0.16	0.24	0.23	0.56	0.89	1.02
Dextrin	0.16	0.18	0.17	0.19	0.21	0.22	0.26	0.28
N-Acetyl-D-Galactosamine	0.16	0.15	0.16	0.14	0.16	0.17	0.19	0.20

More respiration to less respiration

More absorbance to less absorbance

Conclusions

1. Axenic cultures were successfully obtained from the permanent lake-ice cover
   - Tetracladium *ellipsoideum* from Lake Chad
   - *Lecythophora hoffmaanii* from Subglacial Lake Whillans
   - *Mucorales sp.* from East Lake Bonney
   - Unidentified isolate from East Lake Bonney
2. *Tetracladium ellipsoideum* and *Lecythophora hoffmannii* posses antifungal activity against known plant pathogens that has not been previously reported from fungi isolated from Antarctic lake ice. 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.
3. Temperature response experiment revealed that these organisms were psychrotolerant and grew most rapidly at 25°C.
4. The Biolog experiment showed that -
   - At 4°C, the isolate *Mucorales sp*. has maximum utilization of the sugar molecule a-cyclodextrin; at 24°C the same organism shows preference for the monosaccharide N-Acetylglucosamine.
   - The fastest growth occurred on several monosaccharides, amino acids, 2-keto-D-gluconic acid and glycogen.
   - At the higher temperature, the results indicated that 24 substrates were being utilized which is considerably higher than the 8 substrates utilized at the lower temperature.
   - This indicates dependence of growth rate on temperature.

References

- Christner BC, Mickucki JC, Formeman CM, Priscu JC, 2005. Glacial cores: a model system for developing extraterrestrial decontamination protocols. *Icarus* 174.2
- Stefanowicz, A ., 2006. The Biolog Plates Technique as a Tool in Ecological Studies of Microbial Communities. *Polish Journal of Environmental Studies* , 15.5 : 669-76
- White TJ, Bruns T, Taylor JW, 1990. Amplification of direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, 315–324

Acknowledgements

This work was supported by NSF grants 0839075, 1346249, 1115245 to JCP, a research grant from the Montana State Institute of Ecosystems, and a travel grant from Ohio State University. We thank ASC for logistical support, PHI for air support in the Dry Valleys, and the Priscu Research Group for field and laboratory support.