Spring 4-27-2018

Newall Glacier Nucleic Acid Analysis

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Honors Project

Submitted to the Honors College
at Bowling Green State University in partial fulfillment of the
requirements for graduation with University Honors

May 5, 2018

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Abstract

The Newall Glacier is located in Antarctica between Mount Newall and Mount Weyant, at approximately 77°30′S, 162°50′E. Having existed for millions of years, and being rarely touched by human populations, glaciers are a major source of information on climate and life in the past. During the past 5 decades, a multi-country team of scientists have collaborated to drill into many of Antarctica's glaciers and ice fields, removing ice cores for scientific investigation. The ice core section chosen for this project was drilled from the Newall Glacier in 1988 and its depth was from 100.670 to 101.000 m. The purpose of this study was to identify any viable organisms in the ice approximately 100 m below the surface of the Newall Glacier. The results were limited to the identification of a single isolate of a species similar to *Conocybe brachypodii*.

Introduction

The Newall Glacier is located in Antarctica between Mount Newall and Mount Weyant, at approximately 77°30′S, 162°50′E (National Geospatial-Intelligence Agency, 2016). Having existed for thousands of years and being rarely touched by human populations, glaciers are a major key to information on climate and life of the past. In the 1980s, a team of scientists collaborated to drill deep into several of Antarctica's glaciers and removed cores of ice in order to investigate information that glacial ice can provide (Britannica Illustrated Science Library, 2013). The ice core section chosen for this project was drilled from the Newall Glacier in 1988 and its depth was approximately 100.670 m to the top of the core section and 101.000 m to the bottom of the core section.

Often the information glaciers can provide are unseen. Microscopic organisms can be left behind or deposited or transplanted by wind (Dam, 2013) and then buried deep beneath the surface. Such unseen organisms and molecules have been found deep in other Antarctic ice cores, thus the purpose of this study was to isolate and identify any viable organisms or molecules from approximately 100 m into the Newall Glacier. This is deeper than firn ice, and therefore any viable organisms at this depth would have been isolated from the atmosphere for hundreds or possibly thousands of years.

Materials and Methods

The ice core section examined for nucleic acids was melted in layers in 2011, according to the method described in Rogers et al. 2004. Each layer was given a "shell" number, frozen again, and stored at -20˚C. Shell number 12 was used for this project. This originated from deep inside the ice core section.

Shell 12, contained in a 2059 falcon tube, was removed from the freezer and placed in wet ice in order to allow the ice core shell to melt while remaining at an appropriate temperature to preserve possible organisms and biological molecules within. Within a sterile biosafety hood (Labconco Purifier Class II Biosafety Cabinet, model: 36208/36209, Kansas City, MO), 200 ul of melted ice core water was pipetted onto 20 Luria-Bertani (LB) petri plates (25 mg/ml LB medium, 15 mg/ml agar) and 23 malt extract agar (MEA) petri plates (20 mg/ml malt extract, 20 mg/ml dextrose, 6 mg/ml peptone, 15 mg/ml agar) and spread with sterilized glass rods. While plating the melt water, two LB plates and two MEA plates were left open in the hood, one in each corner, for controls. All sample plates remained closed with the exception of time spent

spreading the sample. All plates were sealed with Parafilm after plating, labeled, inverted, and incubated at 15˚C. The plates were checked weekly for growth. After approximately 10 days, a single fungal colony was discovered on MEA plate #10.

After allowing the colony to grow for approximately two weeks undisturbed, a portion of the colony was transferred in a sterile hood onto six new MEA plates using a sterilized wire loop. All six new malt plates were sealed with Parafilm, labeled (#24-29), inverted, incubated at 15˚C, and allowed several more weeks for growth.

Two samples were taken from the colony on MEA plate #26 (tubes #26-1 and 26-2) and two samples were taken from MEA plate #28 (tubes #28-1 and 28-2) for DNA isolation using a CTAB (cetyltrimethylammonium bromide) extraction method (Rogers and Bendich, 1985; Rogers et al. 1989). In this method, cell membranes of fungi tissue were disrupted using CTAB and proteins removed with chloroform/isoamyl alcohol. DNA was then precipitated with isopropanol and there proteins were discarded. CTAB buffer was used to re-precipitate the DNA then a high salt buffer (10 mM Tris (pH 8.0), 1mM EDTA (pH 8.0), 1M NaCl) was used to replace CTAB cations with sodium ions so DNA could be precipitated out of solution in ethanol. All four resulting pellets were individually rehydrated in 0.1xTE (1 mM Tris (pH 8.0), 0.1 mM EDTA) and stored at -20˚C.

The resulting four DNA samples, along with two fungal tissue samples, were amplified with primers ITS3, ITS4, and ITS5 (White et al. 1990). Each tube contained 20 ul master mix (100mM dNTP, 175 ul 10x PCR buffer, 5 U/ul Taq DNA polymerase, 280 ul RNase free water), 2.5 ul primer #1, 2.5 ul primer #2 (10 uM), and 22 ul RNase free water. Tubes #7 and 9 contained small fungi tissue samples from transfer MEA plate #28, tubes #8 and 10 contained 2 ul of DNA solution from extraction tube #26-1, and tubes #11 and 12 contained 4 ul of DNA solution from extraction tube #28-2. Mineral oil (35 ul) was added to the top of each sample and placed in a Thermocycler (Peltier Thermal Cycler-100, Hercules, CA) using the set PCR cycling program (94˚C two minutes, 55˚C two minutes, 0.1˚C/s to 72 ˚C, hold 72˚C for two minutes; 35 cycles repeated with one minute at 94˚C, after 35th cycle hold samples at 72˚C for an additional 10 minutes and indefinitely at 4˚C). The reactions were then run for visualization on a 2% agarose gel (2 g agarose, 100 ml 1x TBE, 10 mg/ml ethidium bromide) then viewed with UV and photographed.

The PCR products were transferred into new tubes without mineral oil and the approximate volume was measured using a pipette (with the exception of tube #11 which was discarded due to low DNA yield). A 5M NaCl solution was added to each tube, $1/10^{th}$ of the total volume (0.45M NaCl final concentration), and 2.5 volume of 100% ethanol was added to each tube, to precipitate the DNA samples for sequencing. The samples were stored overnight at -20˚C to aid in precipitation. The next day, the tubes were centrifuged at 11,000 xg for 5 min and the supernatants pipetted off. One volume of -20˚C 80% ethanol (equal to the amount of 100% ethanol added the day before) was added to each pellet then centrifuged for 4 min and the supernatants discarded again. All five pellets were dried in a SpeedVac concentrator (Eppendorf Vacufuge plus, catalog #022820001, Hamburg, Germany) for 15 min then stored at -20˚C.

To prepare each pellet for sequencing (via University of Chicago Comprehensive Cancer Center), the dried pellets were rehydrated with 10 ul nuclease free water. An aliquot of 5 ul of sample was pipetted into two tubes and 2 ul of primer was pipetted into the respective separate tubes and packaged for shipping alongside the samples. All tubes were placed in sealed petri dishes for protection, labeled, placed on ice, and shipped to Chicago.

The resulting sequences were used in a Basic Local Alignment Search Tool (BLAST) search of the National Center for Biotechnology Information (NCBI) database to determine any potential taxonomic affiliations of the samples.

Results

All sequences determined from the University of Chicago Comprehensive Cancer Center were subjected to BLAST similarity searches utilizing the NCBI GenBank database. All sequences showed a close relationship to the genus *Conocybe*, a basidiomycete, with a 97% similarity to the internal transcribed spacer (ITS) regions of *Conocybe brachypodii*. However, the exact species is unknown. Sequences were aligned and phylogenetic analysis was performed using MEGA on the returned sequences, alongside 34 other fungal sequences from NCBI's GenBank, in order to create a tree displaying the potential relationships among the species.

Discussion

Conocybe species are a type of basidiomycete fungi with hundreds of species worldwide, most growing on dead plant matter. *Conocybe brachypodii* is said to be a particularly variable species with wide ecological amplitude and are most often found in grassy areas (Amandeep, 2015). *C. brachypodii* has been studied worldwide from Russia and Ukraine, across Europe, and into India (Prydiuk, 2007; Amandeep, 2015; Malysheva, 2011). These studies report that the average spore size of *C. brachypodii* is 7.2-9.5(-10.5) x 4.1-5(-5.5) μm (Prydiuk, 2007), unable to be seen by the human eye.

While it is unknown for certain how a fungi similar to *C. brachypodii* could be isolated from 100 m beneath the surface of a glacier residing in Antarctica, it is likely that a single fungal spore or several fungal spores entered the global airstream and traveled far from their place of origin (Dam, 2013) until deposited on the Newall Glacier hundreds or thousands of years ago before becoming buried with new layers of ice and snow until drilling in 1988. In fact, Dam asserts that while several spores are deposited near their place of origin, the majority travel upward to enter the global airstream and are deposited elsewhere by precipitation (2013). As mentioned above, *C. brachypodii* release spores smaller than 20 μm, making them able to easily move between continents throughout the course of a year (Wilkinson, 2012), and it is possible that the species isolated in this project possessed similar spore characteristics.

When spores travel they can reach high altitudes, some being detected as high as 18-27 km, where they are exposed to low temperatures, extreme dryness, and even solar radiation (Henis, 1987). Such details suggest that fungal spores can remain dormant under extreme conditions and remain viable for later growth when conditions improve. While opposite conditions of extreme heat and humidity appear to decrease viability (Schein, 1965), the Henis study and fungal growth resulting from this study suggest that cool and dry conditions preserve viability of spores.

It is unknown whether the fungal growth found in this study is solely one species of *Conocybe* or multiple/dependent species together. Upon finishing phylogenetic analysis using MEGA it appears that more diversity is possibly present. Three of the returned sequences appear to be within *Conocybe* (see figure 1, samples #1, 5, 9) while the others were very divergent from the rest of the tree, thus not likely within the genus *Conocybe*.

Moving forward, this research could be expanded by identifying and compiling more sequences of *Conocybe* species in order to better identify the organism isolated in this study. Additionally, isolating single spores from the resulting fungal growth in order to extract DNA and sequence the results would better determine if the growth is composed of a mixture of species.

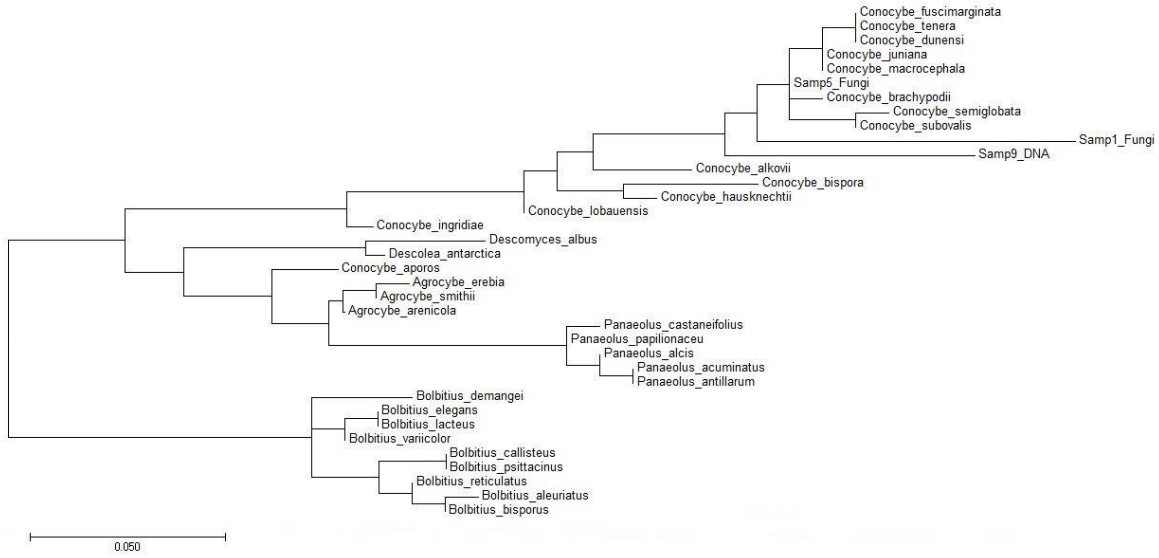

Figure 1. Phylogenetic tree of fungal species and resulting sequences from DNA extraction.

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