ISOLATION AND CHARACTERIZATION OF RECALCITRANT MATERIALS FROM *ASPERGILLUS* USING FTIR

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Abstract

With the imminent change in global climate patterns, and the fact that a large reservoir of carbon is contained within soils, it is important to understand carbon cycling in soils. This study is aimed to determine whether or not fungi themselves produce recalcitrant organic material that adds to the bank of sequestered carbon in soils.

Fourier transform infrared (FTIR) and elemental analysis (EA) were used to characterize the recalcitrant material present in the wild-type soil fungi *Aspergillus* after stepwise solvent extractions and acid hydrolysis. The material left over did not resemble polysaccharides, proteins, or lipids. It also had a higher ratio of carbon to nitrogen compared to that of the parent material. The recalcitrant material accounted for approximately 3% of the total dry mass of Aspergillus.

Introduction

In recent years, climate change has become a topic of concern and widely studied in science. The increase of carbon dioxide in the atmosphere has lead to an increase in the greenhouse effect, thereby creating a change in climate patterns that are potentially harmful to Earth and its inhabitants. Because climate change is dependent on carbon cycling, we are interested in further understanding the mechanism in which carbon from the atmosphere is entrapped in soil, also known as carbon sequestration.

Since fungi are one of the largest sources of biomass in soils, they might contribute to the process of carbon sequestration[1]. Fungi are symbiotic organisms that aid plants in collecting water and nutrients[2]. In return plants provide fungi with nutrients.

In this study we wanted to see if any biopolymers in the fungi, *Aspergillus,* were capable of surviving solvent extractions and acid hydrolysis. If so, this non-extractable and non-hydrolysable material may also persist in its natural environment, thereby serving as a form of sequestered carbon.

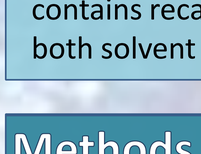
Hypothesis

It is hypothesized that soil fungi (*Aspergillus*) contains recalcitrant material that will survive both solvent extractions and acid hydrolysis.

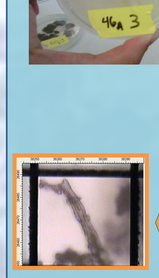
Approach

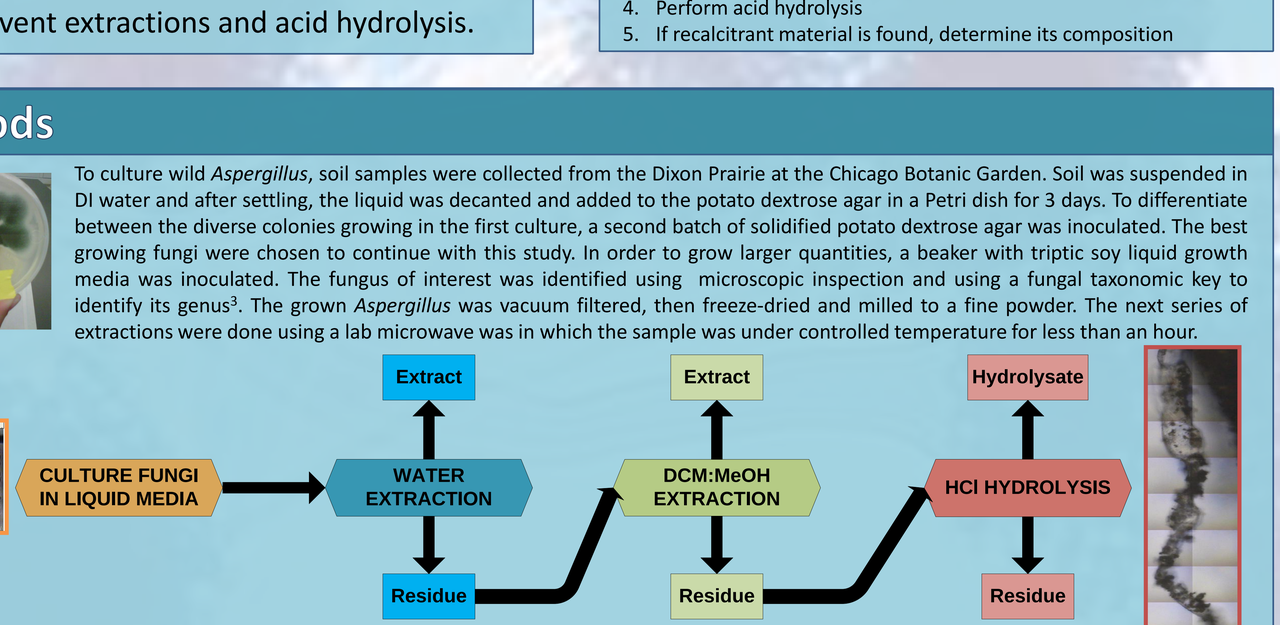
1. Isolate fungi from soil
2. Identify genus of fungi
3. Perform water and solvent extractions
4. Perform acid hydrolysis
5. If recalcitrant material is found, determine its composition

Methods



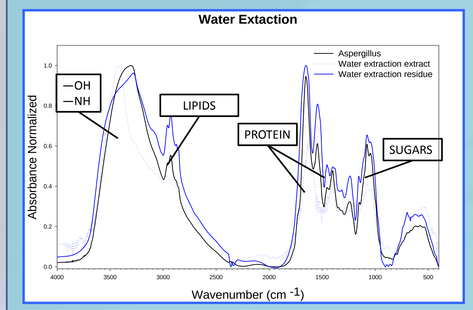
To culture wild *Aspergillus*, soil samples were collected from the Dixon Prairie at the Chicago Botanic Garden. Soil was suspended in DI water and after settling, the liquid was decanted and added to the potato dextrose agar in a Petri dish for 3 days. To differentiate between the diverse colonies growing in the first culture, a second batch of solidified potato dextrose agar was inoculated. The best growing fungi were chosen to continue with this study. In order to grow larger quantities, a beaker with triptic soy liquid growth media was inoculated. The fungus of interest was identified using microscopic inspection and using a fungal taxonomic key to identify its genus[3]. The grown *Aspergillus* was vacuum filtered, then freeze-dried and milled to a fine powder. The next series of extractions were done using a lab microwave was in which the sample was under controlled temperature for less than an hour.



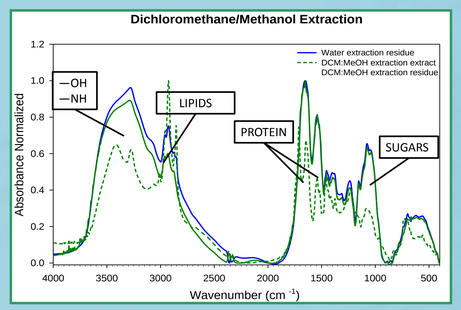


FTIR Spectra

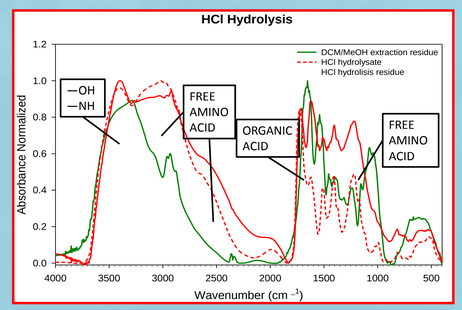
In Fourier transform infrared spectroscopy (FTIR), infrared light passes though the sample and is absorbed by molecular functional groups. Each compound will have a unique set of absorption patterns corresponding to the vibrations of the bonds it contains. Peak patterns were comparable to other fungal FTIR spectra found in the literature[2,4,5,6,7,8].



- Some –OH was extracted
- No lipids and only some sugars were extracted



- Mostly lipids extracted
- No proteins extracted
- Minimal amount of sugars removed
- ~12% of the residue from the water extraction were removed by DCM:MeOH extraction



- No protein or carbohydrates in residue.
- Free amino acids present in both residue and extract, further washing of residue is needed.
- ~80% of the DCM:MeOH residue was removed by the acid hydrolysis
- The yield of the recalcitrant material over the entire extraction process was ~3% of the freeze-dried *Aspergillus* starting material

Elemental Analysis

Material	%C	%N	Atoms C/N
Freeze-dried Fungi	42 ± 1	10 ± 2	5 ± 1
Water Residue	47.32 ± 0.02	8.50 ± 0.02	6.50 ± 0.02
Solvent Residue	44.2 ± 0.4	9.5 ± 0.2	5.44 ± 0.4
Acid Hydrolysis	45 ± 6	4.1 ± 0.1	12 ± 1

The acid hydrolyzed residue contains 2.5 times more carbon per nitrogen atom than starting material. Acidic hydrolysis causes peptide bonds from the protein to break and most nitrogen is removed from the sample as soluble amino acids, leaving a nitrogen-poor residue.

Conclusions and Future Work

- Recalcitrant material was found after chemical extractions and hydrolysis.
- The recalcitrant biopolymer did not resemble proteins, polysaccharides or lipids
- About ~3% of the recalcitrant material was extracted in the overall process..
- Further washing of the recalcitrant material is needed to remove possible residual hydrolysate.
- EA data corroborates that the recalcitrant material contains an increased carbon to nitrogen ratio
- Further analysis (pyr-GC-MS, NMR) needs to be done to better understand the composition and structure of the recalcitrant material.
- Are chemical extractions comparable to enzymatic degradation?

Acknowledgments

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