MUSHROOM BIODIVERSITY AND THE ABILITY OF FUNGI TO SYNTHESIZE GLUCOSE FROM CELLULOSE

Devin D'Agostino, Kaila Gagne

Abstract:
We believe the ability of mushrooms to synthesize cellulose from glucose (to aid in cellulosic ethanol production) can be isolated and traced through the use of a phylogenetic tree. Whether the trait has developed multiple times, is present in a common ancestor, or leads to the conclusion that our current system of phylogeny for mushrooms is outdated and must be revised according to genetic data, can be determined through future research. However, at this point, we are interested in examining the diversity of mushrooms based on enzyme activity. We are curious to see if the ability to convert cellulose to glucose can be present in multiple species of mushroom in the same area.

Introduction:

Studies suggest that the world supply of "fossil fuels" (crude oil, coal, and gas) will be completely diminished in less than a century (Shafiee). Fossil fuel emissions have also been linked to climate change (Marland); which if allowed to continue would cause the ice caps to shrink, sea levels to rise, global temperatures to increase, insects and disease to spread and agricultural yields to reduce ("The Current and Future").

Ethanol is a clean fuel source synthesized from glucose ("Biofuel Enzyme Kit"). Iit is a renewable source of energy made from plant matter, burning it does not give off as many harsh pollutants as other fuel sources, and it can be produced domestically ("Ethanol").

Ethanol production consists of multiple steps that convert the cellulose of plant matter into a usable energy source, with the use of plant matter. Enzymes are then added to this powder to synthesize cellulose,a long chain of linked sugar molecules, or polysaccharides, which form the cell wall, and give a plant its strength into simple sugars. These sugars are then fermented into alcohol through Microbial Fermentation ("How Ethanol is Made").

Ethanol does not produce nearly as much energy as in gasoline, and it is primarily used as an additive to gasoline to deplete the amount of pollution given off through its use ("Ethanol"). Also the primary source of ethanol is corn and grain. To create a significant amount of ethanol would require the cultivation of large amount of these plants, taking up space and resources that could be used for foodstuff (Layton). There is an alternative to this traditional cultivation of ethanol known as cellulosic ethanol.

The enzyme used to convert cellulose into sugar is known as cellulose ("Cellulase"). Organisms on the macroscopic scale are usually unable to produce their own cellulase, and use microbial fermentation to break down cellulose. Certain species of fungi produce their own cellulase and are able to convert cellulose into glucose, a procedure often used in the decomposition process (Sun). By using these species of fungi, ethanol producers can collect plant sugars from what the fungi gives off, which would allow them to skip the cultivation of crops and manual enzymatic addition and go right to fermentation. This would save money and resources, and decrease the amount of pollutants given off during the production of ethanol (Layton).

Not all fungi are able to breakdown cellulose. It is an energy intensive process, and the ability to do so becomes a tradeoff because the resources it uses takes away from the resources used in reproduction (Allison). This experiment sets out to determine genetic similarities between mushroom species that are efficient in the synthesis of glucose from cellulose. Through genetic analysis we hope to not only observe the prominence of said traits, how many mushrooms in an ecosystem exhibit and utilize this process, but also determine where phylogenetically the trait developed.

Methodology:

Part 1: Collection

Long Island *Splish Splas*h: Permission was recently gained to collect mushrooms in *Splish Splash* water park. The area contains a variety of species of mushrooms; at this point it is important to collect as large of a sample size as possible. 20 specimens are to be collected from this location.

Upon isolation of a mushroom for collection, a 1 meter quadrat square is placed around the specimen with a numbered identification card next to the organism. Pictures of the specimen and the surrounding area are then taken along with latitudinal and longitudinal coordinates. The quadrat is used for both identification based on where the specimen is found and for comparison to see if mushrooms with different levels of enzyme activity and genetic similarity are located in similar or different areas.

Using a shovel, mushroom specimens will be removed from underneath the root. Specimens are to be transported in a cooler in a ziploc bag. In the laboratory, pictures of the mushrooms gills will be taken with numerical identification cards placed next to them. Small portions of each mushroom will be taken and frozen for DNA analysis. The rest of each specimen is to be placed in brown paper bags and refrigerated until they are ready for enzyme activity analysis.

Part 2: Identification of Enzyme Activity

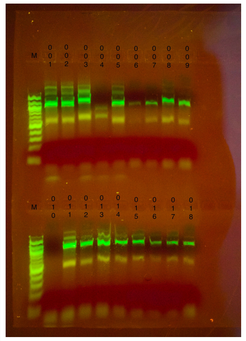
The goal of this portion of the experiment is to establish which species of mushrooms are most efficient at enzyme activity to be later used in comparison.

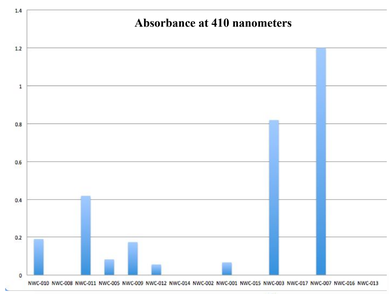
- Determine the reaction rate in presence and absence of the enzyme
- Test the ability of the mushroom sample to increase the reaction rate through light spectrometry

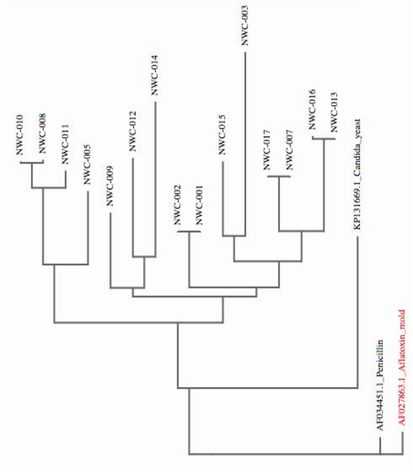
Part 3: Genetic Sequencing

Part 4: Comparing Genomes

Results:







BLAST Search Results

NWC-001: Agaricus subrutilecens
NWC-002: Agaricus sp.
NWC-003: Armillaria mellea
NWC-005: Russula sp.
NWC-007: Amanita multisquamosa
NWC-008: Uncultured fungus
NWC-009: Corinarius vibratilis
NWC-010: Uncultured fungus
NWC-011: Uncultured Fungus
NWC-012: Suillus sibiricus
NWC-013: Amanita sp.
NWC-014: Scleroderma polyrhizam
NWC-015: Cyathus striatus
NWC-016: Amanita sp.
NWC-017: Amanita multisquamosa

Discussion:

At this point in the experiment, there is no clear evolutionary link between mushroom with high enzyme ability. However, this question cannot be definitively answered until more trials are performed on enzyme activity. The samples were frozen and thawed out between collection and enzyme analysis. During this period the integrity of species may have been damaged. Future analysis of enzyme activity should be taken to verify results.

However, if enzyme analysis is assumed accurate, there are a few things that can be surmised from the data. Since the ability to breakdown cellulose is not isolated to one of our mushroom population, it is possible the trait is an ancestral one, that multiple genus of mushrooms contain but only some exhibit. This would make sense considering the wide variety of mushrooms present in a single ecosystem and the limited capacity of said biome to hold multiple species who fill a specific niche. It would also explain why relatively closely related types of mushroom do not both exhibit the trait. As previously stated, cellulose decomposition is an energy intensive process, which may not be beneficial to all species of mushroom in a single ecosystem. Again the trait is not isolated to a single branch of mushroom, therefore it may be surmised that cellulosic ethanol capabilities developed early on in mushroom evolution.

The data can also be used in analysis of mushroom biodiversity. The environment collected provides a unique location to collect from. It is a water park built on a forested area, leading to both natural and artificial features in play. There appears to be some amount of genetic diversity in the area. At least seven unique genus of mushroom were found, and many species and subspecies branching off. The high diversity may be due to a variety of species being introduced during the construction of the park with supplies and materials originating from all over North America. Also the unique openings a water park (from high traffic areas to different chemicals from pools splashing onto the surrounding area) may provide for species to fill is interesting to consider, and may explain the variety of specimens collected. Further research may investigate just how many different species of mushrooms are present in this kind of unique area, and the variations in species between the park and surrounding forest spheres.

The most exciting part of this experiment is the possibilities that come from the isolation of a branch with the ability to produce glucose from cellulose. With selective breeding and the growing field of genetic engineering, scientist can use this gene to create different species of mushrooms, or even bacteria, with the ability to change cellulose into glucose. Farms of these new mushrooms can be bred and used by the ethanol industry to create a usable and plentiful source of clean energy. We can stop our dependence on fossil fuels and use a cleaner and more available source of energy. The practicable applications of this experiment are plentiful for both the ethanol industry and the clean energy.

Acknowledgements

Thank you to Splish Splash Water Parks for allowing us access to collect samples and explore the park off season. Thanks to Barcode Long Island and Cold Springs Harbor for supplies and the grant to allow us to perform our experiment and pursue our interests in science and familiarize ourselves with the scientific process and methods.

Special thank you to Jok Kommer, Steven Lacker, and of course Frank Diehl, for their guidance, support, and genuine fascination with our research. The lessons you taught us will continue to guide us through college and our future careers in the sciences. Thanks.

References

Allison, Stephen D., et al. "Evolutionary-Economic Principles as Regulators of Soil Enzyme Production and Ecosystem Function." *Soil Biology: Soil Enzymology* 22 (2011): 229-43. *The Allison Lab*. Web. 2 Jan. 2015. <http://allison.bio.uci.edu/publications/allison2010c.pdf>.

Biofuel Enzyme Kit. Hercules: Bio-Rad, 2009. Print.

Bowen, R. A. "Microbial Fermentation." *Hypertexts for Biomedical Science*. Colorado State University, 20 Feb. 2010. Web. 2 Jan. 2015. <http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/largegut/ferment.html>.

"Cellulase." *GMO Database*. GMO Compass, 17 July 2010. Web. 15 Jan. 2015. <http://www.gmo-compass.org/eng/database/enzymes/82.cellulase.html>.

Chen, Yinliang, et al. "Anaerobic yeast fermentation for the production of ethanol in a versatile lab fermentor." *Nature Methods* 5.12 (2008): 445-53. *Nature Methods*. Web. 2 Jan. 2015. <http://www.nature.com/nmeth/journal/v5/n12/full/nmeth.f.228.html>.

"The Current and Future Consequences of Global Change." *Climate Change: Vital Signs of the PLANET*. Ed. Holly Shaftel. NASA, 2014. Web. 2 Jan. 2015. <http://climate.nasa.gov/effects/>.

DNA Subway. N.p.: Cold Springs Harbor Laboratory, n.d. *DNA Subway*. Web. 23 Jan. 2015. <http://dnasubway.iplantcollaborative.org/files/pdf/DNA_Subway_Guide.pdf>.

"Ethanol." *Fuel Economy*. US Department of Energy, 2014. Web. 2 Jan. 2015. <http://www.fueleconomy.gov/feg/ethanol.shtml>.

"Experiment Protocol." *Cold Spring Harbor Laboratory's DNA Barcoding 101*. Cold Spring Harbor Labs, 2014. Web. 9 Jan. 2015. <http://www.dnabarcoding101.org/protocol_collecting_specimens.html>.

"Gut Fermentation in Herbivorous Animals." *Map of Life*. University of Cambridge, 5 Oct. 2007. Web. 2 Jan. 2015. <http://www.mapoflife.org/topics/topic_206_Gut-fermentation-in-herbivorous-animals/>.

"How Ethanol is Made." *Renewable Fuels Association*. Renewable Fuels Association, 2006. Web. 2 Jan. 2015.

Kindler, Ken, ed. *Hiking Long Island*. Hiking Long Island, 2004. Web. 14 Jan. 2015. <http://www.hike-li.org/index.html>.

Layton, Julia. "Is Ethanol Really More Eco-Friendly than Gas?" *How Stuff Works*. N.p., 24 Jan. 2010. Web. 2 Jan. 2015. <http://auto.howstuffworks.com/fuel-efficiency/alternative-fuels/ethanol-facts.htm>.

Marland, G., T.A. Boden, and R.J. Andres. 2008. Global, Regional, and National Fossil Fuel CO2 Emissions. In Trends: A Compendium of Data on Global Change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenn., U.S.A.

Senese, Fred. "What is Cellulose?" *General Chemistry Online*. Ed. Fred Senese. Frostburg State University, 15 Feb. 2010. Web. 2 Jan. 2015. <http://antoine.frostburg.edu/chem/senese/101/consumer/faq/what-is-cellulose.shtml>.

Shafiee, Shahriar, and Erkan Topal. "When Will Fossil Fuels Reserves be Diminished?" *Energy Policy* 37.1 (2009): 181-89. Abstract. *Science Direct*. Web. 2 Jan. 2015. <http://www.sciencedirect.com/science/article/pii/S0301421508004126>.

Sun, Ru-Cang, et al. "Structural evaluation and bioethanol production by simultaneous saccharification and fermentation with biodegraded triploid poplar." *Biotechnology for Biofuels* 6 (2013): n. pag. *Biotechnology for Biofuels*. Web. 2 Jan. 2015. <http://www.biotechnologyforbiofuels.com/content/6/1/42>.