More context, less content

Teaching mycology as a course-based undergraduate research experience



Geoff Zahn Utah Valley University

How can I maximize benefit when 90% of my students won't be mycologists, plant pathologists, etc.?



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The CURE: Course-based undergraduate research experience



Educational Equity and High-Impact Practice



1) Element of discovery with novel data

- 2) High level of collaboration
- 3) Students learn scientific process
- 4) Relevant topics with external interest



Benefits:

- Enhanced self-confidence in scientific thinking (Szteinberg and Weaver 2013)
- Development of scientific process skills (Brownell et al. 2015)
- Increased inclusion in science for unrepresented populations (Bangera and Brownell 2014)
- Improved retention in science and medicine (Hanauer et al. 2012)

Mycology is the perfect subject for a CURE!













A Mycological Investigation of Darwin's Naturalization Hypothesis

Peter Conley, Jacob Coulson, Jonathan Wasden, Dr. Geoffrey Zahn



(Figure 1 A phylogenetic tree showing the three isolates highlighted in yellow)

Who cares

This question has potential importance for agriculture and commerce. The growth and proliferation of a harmful or otherwise undesirable fungus may be inhibited by the presence and activity or metabolities of another less harmful species. Ideally, compounds could be isolated from the inhibitory fungus to control the malicious strains without the need to actually grow the inhibitor locally. Procedures could be implemented for many commercial benefits, but most obviously for increased crop yields in species that are regularly damaged by fungi and for predicting and controlling biological invasions.



Methods

Three fungal isolates were cultured from the environment, including one known outgroup.

Competitions were created by cultivating plates inoculated with each possible pairing of 2 isolates, as well as a set containing all 3 varieties. Five replicates were prepared reach test, measured every 3 days, and the rates of growth for each strain in a set were observed. These rates were expressed as a proportion to the baseline growth rate of isolates in pure culture.

DNA was extracted from each strain and the ITS1-28S region of the rDNA was sequenced to confirm species identification and determine phylogenetic relatedness.

Problem

Charles Darwin believed that colonization is less likely to be successful when the colonizing species are related to members of the invaded community, because evolutionary closeness intensifies competition among species that share similar resources. Much research on Darwin's Naturalization Hypothesis has been done based on plant or animal competition and his hypothesis has here done based on plant or animal competition and his hypothesis has remained unscattade by a majority of these studies. The question for this study, then, is whether or not this holds for fungal species as well. In particular we would focus on Asconrycete and Basidionyzete fungi.



Conclusion

Looking at the results, an interesting phenomenon occurred. Pops© (YPJ_6) inhibited the growth of the other two fungal isolates, and actually grew better in competition. Reduced growth rate for Silverback (YPJ_2) was subtle, while Oyster's (YPJ_4) growth inhibition was incredibly visible (see tig 2.4).

Sequencing data for Pops® revealed it to be the fungal parasite, Mycosymbioces mycenaphile, an Ascomycete. Both Oyster, Pleurotus ostreatus, and Silverback, Filobasidiella neoformans, are Basidiomycetes. We conclude that phylogenetic distance is not the primary factor in the competition of these isolates.

Further research would require additional species and the elimination of confounding factors, such as a parasitic outgroup. This would provide a more accurate analysis of the Naturalization Hypothesis among Fungal species.

Funding provided by UVU URSCA Award

Fungal endophyte communities during leaf senescence

Garrett Matthews, Parker Willett, Hailey Sommerfeld, Geoff Zahn



Endophytic fungi live inside plants' leaves. These fungi often confer many benefits to their host plant, even as acting as a form of "immune system" for the plant. But what do the fungi get out of this relationship?

One idea is that fungi are just waiting around for the leaf to die so they can be the first ones there to start decomposing fallen leaves.

This study investigated this "latent decomposer" hypothesis by looking at whether a community of endophytes present in healthy leaves were the same as those decomposing dead leaves.

METHODS

Leaves in varying stages of senescence were taken from a single mulberry tree on UVU's campus.

Fungi were isolated from these leaves and the ITS1-28S region of rDNA was sequenced to determine species identities.

Fungal endophyte communities were compared along a leaf age gradient

The second secon

RESULTS

There was no significant difference between endophyte communities between leaf ages. Diversity of endophyte communities did decline with leaf age, though.

The data suggest that endophytes may indeed be latent decomposers. However, this is only a small sample in a wider world, meaning more research is required to gain a better understanding of the situation

- Principle component analyses demonstrated significant overlap in endophyte communities. No single species drove observed differences between age groups.
- The fungi decomposing the leaves appear to be the same ones in healthy leaves

Funding for this work was provided by a UVU URSCA award



UTAH VALLEY UNIVERSI

Discovering New Fungal Species

Heather Moon, Alex Jones, Alex Raab, Clayton Rawson, and Kristina Nolff



Fungal Endophytes

Live within plant tissue

· Usually plant mutualists

Reduce herbivory

Nutrient exchange

diversity

Genus

Comparison of Fungal Endophytes in Conventional and Organic Brassicas Balderrama, Ashley; Clawson, Jordan; Christiansen, Colton; Cottle, Nathan; L'Ecuver, Katia and Dr. Geoffrey Zahn

Department of Biology, Utah Valley University



Introduction

- · We don't know much about fungi in extreme environments.
- The current databases we have on fungi don't have a lot of data.
- We wanted to find and describe fungal species in extreme environments.
- · Utah is an ideal area to do this research since it has many extreme environments within short distances.



Goals

- · We did a survey of fungal species in extreme environments in Utah.
- · We collected samples from 4 regions in Utah.
- · We hoped to discover a new species of fungi in these regions.



Materials and Methods

- · Collected varying samples of soil, water, and substrates from a wide variety of local Utah environments
- · Target environments of interest were soil and water from high saline waters, and soil samples from the local desserts from the east, west, and south sides of Utah. Other samples included leaf and bark samples from each region, and herbivore dung.
- · We created growth cultures from these samples on a general defined medium.
- · Cultured specimens then went through a series of isolation and reculturing until all specimens were separate and had abundant growth culture for further testing.
- · Samples found of interest then went under extensive DNA analysis with quantitative PCR and morphological categorization with extensive microscopic observation.
- · DNA was sent out for sequencing and its genus was determined to find if it can be considered a new species based on previous databases.

Conclusions

- We potentially found up to 15 new species of fungi!
- · In each region, we found several fungi species.
- genera in every environment. • This indicates these fungi can
- environments. Many also have morphological





Findings

- · We had 72 isolates and found 36 morphologically similar organisms.
- · Of the DNA that was sequenced, we matched 28 organisms.
- · Of those 28, we found 15 organisms that had matched only down to the genus, and the species was unknown.
- · This could indicate a new species within these genera.

Genera with unmatched species

- ♦ 6 in Alternaria ♦ 2 in Fusarium
- ◆1 in Cladosporium
- ♦ 3 in Phoma
- ♦1 in Dothideomycetes ◆1 in Mortierella
- 1 in Leptosphaeria



- · We found similar species and
- survive in a wide variety of
- similarities.
 - Part of the Mustard family Economically important



To compare and contrast the endophytic fungal species diversity within Brassica vegetables grown conventionally vs organically



Leaf fragments surface sterilized and plated on PDA

Methods



Endophytic fungi isolated via streak plate method



DNA extracted from fungal isolates using Extract-N-Amp protocol

PCR amplification using primers ITS1F and TW13



Sanger sequencing of amplicons

Fungal identification and

characterization using NCBI BLAST and FunGUILD databases

Results

Despite the contamination of e. coli that we experienced we were able to isolate and sequence 13 different kinds of endophytes. There wasn't a drastic difference in which vegetables grew fungi and which ones didn't. That once again was probably do to contamination. There seems to be more fungi in conventional vegetables but that is hard to say without further testing.

Conventional Vegetables	NCBI Sequence Blast	FunGUILD Results
Cauliflower	Penicillium Rubens	Saprotroph
Cauliflower	Galactomyces geotrichum	Pathotroph
Bok Choy	Penicillium Chrysogenum	Saprotroph
Bok Choy	Cladosporium sp.	Saprotroph
Kale	Galactomyces geotrichum	Pathotroph
Kale	Alternaria Infectoria	Saprotroph
Kale	Unknown ascomycetes	n/a
Cabbage	Penicillium Chrysogenum	Saprotroph
Organic Vegetables	NCBI Sequence Blast	FunGUILD Results
Organic Cauliflower	Cladosporium sp.	Saprotroph
Organic Bok Choy	Alternaria Infectoria	Saprotroph
Organic Kale	Dothideomycetes sp.	n/a
Organic Kale	Alternaria sp.	Saprotroph
Organic Kale	Cladosporium sp.	Saprotroph

Conclusion

- Fungi from the genus Alternaria and Cladosporium found in organic brassica vegetables were also found in conventional vegetables.
- · Penicillium and Cladosporium fungi were only found in conventional brassica vegetables.
- · All of the fungi isolated from organic brassica vegetables receive nutrients by breaking down dead host cells.
- · Some of isolated fungi in conventional brassica vegetables were pathotroph fungi. These fungi receive nutrients by harming the cells of the host.
- Due to the small sample size, our data is preliminary data and not an accurate representation of all conventional and organic brassica vegetables
- · Complications that could have affected our results was the limitation of analyzing fungi from organic cabbage, the low sample size of each type of vegetables, and the varied conditions that the vegetables were stored in

Acknowledgements

Research Grant: Utah Valley University, College of Science Scholarly Activities Committee (SAC) Laboratory Facility: Dr. Zhan for allowing us to use his lab







· Food, oils, condiments · Bok choy, kale, cabbage, cauliflower

Background

Increase resistance to pathogens

Produce medically useful compounds

Farming methods affect endophyte

Cruciferous Vegetables: Brassica



Study Objective



We Found Fungi That Kills MRSA

Branden Petersen, Jacob Warr, Baylor Steward, Sabrina Saley, Bryson Edwards, Kaz Horrocks, Geoffrey Zah



Create a living library of the MRSA antagonistic fungal samples that we identified
Continuation of research into the biochemical pathways used

Funded by UVU SAC grant

UTAH VALLEY

DU Department of Biore

Bioremediation of Plastics by Aspergillus

Natalia Backman, McKenzie Bell, Reagan Dodge, & G. Zahn

Introduction

Plastic typically takes thousands of years to biodegrade. In a process called bioremediation, *Aspergillus* species have been found to break down plastics. This project used varieties of this fungus to test feasibility and timeliness of bioremediation of plastic on a larger scale.



Results



Figure 3. Decomposition

between Aspergillus species

Species	Initial Mass (g)	Final Mass (g)	Difference
Native Aspergillus	0.0311	0.03	3.54%
Native Aspergillus	0.0291	0.029	0.34%
Native Aspergillus	0.034	0.0336	1.18%
Native Aspergillus	0.0295	0.03	-1.70%
Native Aspergillus	0.0351	0.0352	-0.28%
Native Aspergillus	0.0315	0.032	-1.59%
Native Aspergillus	0.0312	0.0305	2.24%
Native Aspergillus	0.0324	0.0333	-2.78%
Native Aspergillus	0.0329	0.0333	-1.22%
Native Aspergillus	0.0344	0.0345	-0.29%
Native Aspergillus	0.0302	0.0305	-0.99%
Native Aspergilfus	0.0292	0.0292	0%
Native Aspergillus	0.0328	0.033	-0.61%
Native Aspergillus	0.0347	0.0348	-0.29%
Native Aspergillus	0.0344	0.0345	-0.29%
Native Aspergillus	0.0363	0.0324	11.00%
Native Aspergillus	0.0306	0.0257	16.00%
Aspergillus Fumigatus	0.0341	0.0326	4.40%
Aspergillus Fumigatus	0.0337	0.0311	7.72%
Aspergillus Furnigatus	0.035	0.032	8.57%
Aspergillus Fumigatus	0.0335	0.03	10.45%
Aspergillus Fumigatus	0.0302	0.0292	3.31%
Aspergillus Fumigatus	0.0321	0.028	12.77%
Aspergillus Fumigatus	0.0305	0.03	1.64%
Aspergillus Fumigatus	0.0343	0.0299	12.83%
Aspergillus Fumigatus	0.0314	0.0297	5.41%
Aspergillus Fumigatus	0.0305	0.0298	2.30%
Aspergillus Fumigatus	0.0301	0.0291	3.32%
Aspergillus Fumigatus	0.0309	0.0299	3.24%
Aspergillus Fumigatus	0.0309	0.0298	3.56%
Aspergillus Fumigatus	0.0303	0.029	4.29%
Aspergillus Fumigatus	0.0311	0.0303	2.57%

Table 1. Data collected for initial and final mass of polyethylene for both Aspergillus species. Highlighted in green shows a positive decomposition result, while red shows a negative result.

Conclusion

Aspergillus fumigatus showed 5-10% more biodegradation of polyethylene plastic than the native Utah Aspergillus species.

Acknowledgements

- SAC Grant
- UVU Department of Biology
- Zahn Lab

Sometimes the research doesn't work.

Failure is good and healthy!

Student reflections at the end of the semester can synthesize learning from "failure"

Underwater Heroes: Finding a Chytrid Antagonist

Bryce Brunetti, Jordan Bayly, Geoffrey Zahn

Purpose:

Chytrid fungus is a large and extremely under-studied clade of fungi. Chytrids can be identified by their single-celled life cycle, and the characteristic flagella as part of their form. One species of Chytrid, *Batrachochytrium dendrobatidis*, poses a significant health risk to amphibian populations around the world. It alone is responsible for hundreds of species-level extinctions, and currently threatens the global amphibian population.

Because Chytrid fungi are so ubiquitous, it can be difficult to prevent the disease from spreading. The discovery of a nonthreatening fungus that controls Chytrid growth would be a vital step towards protecting global amphibian diversity.



Question: Are any local, culturable, aquatic fungi antagonistic to chytrid fungus?

Future Research:

- Begin with better established chytrid cultures
- Culture chytrids using liquid agar rather than Distilled Water

25.0 55.0

Analysis:

- Chytrid fungus is slower growing compared to competing aquatic organisms i.e. Oomycetes
- Pure chytrid culture used was not robust enough to compete against established microbe communities used for testing.
- Pine pollen is a affective bait for chytrid, but poor growth medium.
- Chytrids are notoriously difficult to culture.







The day before COVID closure







An example project from one team last semester...

"Can you get yeast to evolve greater alcohol tolerance?" "What fungi are found in urban mammal poo?" "Do commercial mycorrhizal products actually work?" "Does order of arrival affect wood decomposition?"

Three students selected this topic and teamed up.

Art Pre-dental Wildlife biology



Google Scholar Zotero Sci-hub Follow the citation trail

After two weeks of discussion, planning, and defending their rationale, the students turn in an annotated bibliography (20+ sources) that lays the groundwork for their project

They decide to complicate things a bit:

Comparing ADH knockouts to Wild-Type

Bibliography

B, Gallone, Steensels J, Prahl T, Soriaga L, Saels V, Herrera-Malaver B, Merlevede A, et al. "Domestication and Divergence of Saccharomyces Cerevisiae Beer Yeasts." *Cell* 166, no. 6 (September 8, 2016). <u>https://doi.org/10.1016/j.cell.2016.08.020</u>.

This article gives the history of the domestication of yeast giving insight to how we've gotten from the wild type to widely used industrial strains selected for desired traits. This helped us learn the background for yeast in general and why it's important to research evolutionary changes in yeast due to stress, especially alcohol stress as alcohol tolerance is one of the main characteristics consumers use in choosing a strain of yeast.

Becskei, A., Séraphin, B., & Serrano, L. (2001). Positive feedback in eukaryotic gene networks: Cell differentiation by graded to binary response conversion. *The EMBO Journal*, 20(10), 2528–2535. <u>https://doi.org/10.1093/emboj/20.10.2528</u>

This article defined and demonstrated a positive feedback gene network in a eukaryotic organism, which aided in understanding how the gene network for alcohol tolerance in yeast functioned.

Caspeta, L., Castillo, T., & Nielsen, J. (2015). Modifying Yeast Tolerance to Inhibitory Conditions of Ethanol Production Processes. *Frontiers in Bioengineering and Biotechnology*, **3**, 184. <u>https://doi.org/10.3389/fbioe.2015.00184</u>

This study gives us an example of how to grow a tolerance of alcohol in yeast. This will help us with the evolutionary recovery aspect of our project.

Denis, C. L. (1984). Identification of New Genes Involved in the Regulation of Yeast Alcohol Dehydrogenase II. Genetics, 108(4), 833–844.

This is important because it talks about some new genes that are found that regulate alcohol dehydrogenase 2. This dehydrogenase suppresses glucose negative control to make ethanol. This ADH2 is one of the genes we are going to knock out in our experiment.

They then prepare a grant proposal and submit it to the College of Science for internal funding.

Intellectual merit & broader impacts

Learning outcomes

Methods

Budget

Outcomes

Backup plan

Catalog number	Materials/Supplies	Cost
Y36236	ADH1 knockout yeast strain	\$50
Y30891	ADH2 knockout yeast strain	\$50
Y36217	ADH3 knockout yeast strain	\$50
Y35821	ADH7 knockout yeast strain	\$50
Y33866	SFA1/ADH5 knockout yeast strain	\$50
BY743	Wild Type strain	\$50
	Shipping for yeast strains	\$50
FB012931	100 pack of 96-well tissue culture plates	\$88.83
02-707-435	5-pack of 960-count pipette tips	\$254.90
21-377-171	1 case of large pipette tips	\$149.21
BP228184	4L of ethanol	\$96.74
	Sequencing for each yeast strain	\$51.00
	Total	\$990.68

Benefits

Impact on UVU: The proposal will train us to become more capable undergraduate researchers as we prepare for our professional careers. This proposal will conclude with a research article and research presentation, both of which relate to UVU's identity as an experience forward learning environment, and relates UVU's name with research in evolutionary studies and gene recovery

Impact on Students: Each student receives course credit for this semester of spring 2022. performance in this course is weighed heavily on the proposal and experimentation process. This project is part of a CURE for a mycology class. This learning based project will provide a high quality undergraduate research experience that will help us in our next research projects and in applying for individual post graduate programs.

Impact on Faculty: By presenting our research poster to the student body, we will increase the interest of other students in enrolling in the mycology course taught by Dr. Zahn, as well as overall interest in UVU's biology department.

Impact on Programs: This study will demonstrate the capabilities of the students enrolled in the science departments of UVU, and show that the program teaches practical skills that are valuable to answering questions pertinent to the field of biology. The experiment will also further our current understanding of the field, and this information will be open to any that request it. Impact on Community: The object of this study will reveal insights into the evolutionary processes of yeast, and how it adapts to environmental stress. This will also provide an analog to how similar yeast strains could be domesticated to tolerate higher ethanol levels. This topic is of particular interest to industries and groups that work with yeast and ethanol, such as the alcohol industry, chemical supply companies, and mycologists.

Methods

Once we have received the wild type and knockout strains of yeast, these will be plated and propagated in several petri dishes to preserve our base specimens. From this point, our experiment will consist of three sequential main phases.

Phase 1

The knock-out yeast strains and wild type will be transferred into well plates and organized in the configuration shown in the figure below, with the label indicating which gene has been disabled.





ADH1



Now, we're waiting for supplies, yeast strains, etc.

But there's plenty of stuff to do!

- Learn to pipette
- Discuss mycology papers
- Practice growing yeast and staying sterile
- Learn to run the plate reader and how to track growth
- Plan for the statistical analyses that we will do
- Learn some R skills and tidy data principles



Supplies arrive!

Students are ready to hit the ground running



The first real problems begin!

...And so does the real learning.

How to interpret data that don't make sense? What's going on?

Sound familiar?

This might be a new feeling for them...

WT growth curves under increasing EtOH %



The first real problems begin!

...And so does the real learning.

How to interpret data that don't make sense? What's going on?

Sound familiar?

This might be a new feeling for them...

"Did you take the lab notebook seriously?"



The poster.... the penultimate task







Evolutionary Recovery of Lost Alcohol Tolerance

Team lead Samuel Anaya, Peyton Bailey, and Lauren Semon

Data/Results

Introduction

Saccharomyces cerevisiae (brewers yeast) is fundamental as a widely studied model organism, most notably in the fields of DNA research. Between the short lifecycles and completely sequenced genome, S. cerevisiae makes an ideal organism for studying evolution and genetics. Many active fields of study involve engineering ethanol tolerance for commercial projects such as the production of ethanol for the alcoholic beverage industry, or as an alternative fuel to replace non-renewable fossil fuels.



Understanding evolution goes beyond production value, it is also insightful for understanding our world and how to improve our quality of life in the future. We looked to Jia(name) for much of our initial research. She discusses how evolution studies are centered around traditional mutagenesis, evolution engineering, random knockout, and genome shuffling. Our research follows this by engineering evolution by stressing our model yeast in an environment that favors alcohol tolerant mutants. Our experiment aimed to isolate the ADH gene pathway. This pathway leads to the coding of alcohol dehydrogenase, an enzyme that converts acetaldehyde into ethanol during fermentation, along with the reverse reaction. While there are many factors related to alcohol tolerance in S. cerevisiae, alcohol dehydrogenase is directly related to the detoxification of alcohols. Thus, alcohol dehydrogenase and the ADH genes play a vital role in cell survival.









References

Discussion

What was our preliminary hypothesis for this experiment?

We hypothesized that as the yeast underwent the evolutionary process and approached their maximum ethanol tolerance, their growth would begin to slow. However, due to evolutionary pressures, an alcohol tolerant mutation would be strongly selected for and cause an increase in fitness.

How did evolution present itself in our results?

During the evolutionary process we observed a quick decline in the fitness of the yeast at day 4 with 1% ethanol. This decline in fitness would continue for the remainder of the experiment unless a beneficial mutation occurred. When a mutation would occur we observed a significant increase in the number of yeast cells within the well plates.

Most of the evolved strains showed an increase in ethanol tolerance compared to the same pre-evolutionary strain. This brings into question whether the mutations are a re-evolution of the original knocked out ADH gene sequence, or if the yeast strain mutated a new sequence that alds in ethanol tolerance.

Future work

 Exploring the limits of evolved ethanol tolerance by continuing this experiment for a longer period of time and higher ethanol concentration.

 Knockout yeast strains that display alcohol tolerance could be sequenced to see if they evolved back to the original genetic sequence, mutated a new sequence, or adapted using another pathway.

 Recovery of knockout genes by inducing genetic mutations through radiation and chemical mutagens.

 Replicate this experiment with prokaryotic organisms or isolated tissue cells.

 Replicate this experiment using different stressors such as salinity or pH.

Acknowledgements

Utah Valley University SAC Grant Committee for providing funding for this project.

Dr. Geoffrey Zahn for guidance in experimental setup and aiding with statistical analysis.

+UVU science faculty for sharing their facilities.

Student Reflections (most important task)

"My career goal is to do scientific illustration, so the only research based experiences I will have will be through my undergraduate degree. I realized how important it is to collaborate with people with different specialties."

"As a student preparing to attend graduate school, I had very little information regarding how academic research was actually conducted prior to this project. This opportunity helped me learn about the unspoken details of conducting research, such as how to plan out the budget for a research project, and to identify key supplies that will be needed throughout the process."

"This class got me excited for my future as I hope to be a part of research projects in dental school. This project has required me to apply the knowledge I have gained in other classes, in order to design the experiment, and make correct calculations"

Student Reflections (most important task)

"[we should do] possible rotations into other groups to learn concepts outside our specific research"

"I wish we could have read more papers and talked to more mycologists about their work"

"I wish we could have spent some more time up front learning about fungi before jumping into research papers and our projects"

Logistics for a full-semester CURE

- Start with a list of broad topics that you're ready to facilitate - Describe the goals, potential methods, and what skills they will learn!

- Pick groups and projects on day 1 (time is short!)
- Spend quality time with each group (get to know them as people!)
- Have groups share with each other (ideas and materials)
- Keep a bunch of short lessons ready
- Have a contract, but give students a lot of leeway
- Money laundering is useful and good :)
- Find room for fun activities when you can (Guests, paper discussions, classic experiments)
- Assessments based on effort and student reflections

Summary

Introduce full research process to students who would never otherwise be exposed to it

Get a wide range of students excited about mycology

Discovery-based research is "real" science, as opposed to lab exercises that have set outcomes

Failure of some sort is almost guaranteed!

Little time to cover much domain knowledge; Nobody will know what a Spitzenkörpor is

Takes getting used to for both teacher and students

Assessment of learning is more difficult and subjective

Acknowledgements:

UVU Scholarly Activities Committee UVU Office of Teaching and Learning UVU Mycology Students







gzahn.github.io/mycology/

What ideas do you have for incorporating research into mycology?

What's a good balance between domain knowledge and scientific training?

How could this work with large course sections?