ABSTRACT

In the biology Junior Year Writing class of Fall 2019, we are proposing a class research experiment into investigations of slime mold behavior and characteristics. The purpose of the research is to successfully investigate the behavior *Physarum Polycephalum* and its response to varying temperatures. This can be achieved by observing growth in different controlled environments with set temperatures. The experiment performed will be placing slime molds with food sources on different heat sources from ice baths and heat plates for the entire class time. The growth will then be observed over the time trial as well as photographed prior to and after treatment. To analyze the data, the size of the slime mold will be noted before and after exposure to different temperatures and the growth will be estimated in relation to the other temperature trials. A determination can then be made about which temperature is best suited for *Physarum Polycephalum.* This is significant research because it enables the students to complete a research project from conception to completion and presentation.

SPECIFIC AIMS

Slime molds have been a topic of interest in our class for a scientific project. Current in-class research has focused on the plasmodium form of different slime molds. These plasmodia function as a single celled organism, with multiple nuclei and a lack of membranes separating these. Many slime molds prefer the same temperatures that are ideal for human comfort to grow and develop. What is not known, however, is the effect of increased or decreased temperatures on the structure of the plasmodium. Performing such an experiment will provide further insight into the function of slime molds, and allow for further branching and experimentation on other aspects of slime mold life and development.

Our goal in this experiment is to determine the effects of temperature on slime mold plasmodium form. Specifically, we will be using the slime mold *Physarum Polycephalum* as a test subject. This will carried out through the utilization of an ice bath to reach a temperature of 0ºC, and a hot plate to reach temperatures of 40ºC, 50ºC, 60ºC, and 70ºC. A control experiment at room temperature will also be performed. *Physarum Polycephalum* plasmodium form and spacing will be assessed after 2.5 hours.

Our aims for this experiment include the following: 1) A better understanding of the *Physarum Polycephalum* species of slime mold, 2) Performing the entire breadth of the experimental process, including proposal, setup and performance of the experiment, and subsequent data analysis, 3) Successfully investigate a process of the model organism. Through this methodology, we believe this pathway of experimentation to be an easy, inexpensive method of modeling the full process of scientific experimentation.

BACKGROUND

Slime molds are unicellular protist organisms that were previously classified as fungi until they were discovered to be unrelated. Slime molds are lacking the general characteristics of fungi such as having chitin in their walls and not being able to move in any form of their life cycle. The specific slime mold species that will be experimented, *Physarum Polycephalum,* is able to live in the haploid and diploid form. In starvation, the diploid will sporulate and the haploid spores then sexually reproduce to increase genetic variation in the species as if favored by natural selection. The diploid form is able to grow plasmodium which is the characteristic appearance of slime molds, forming large branching structures that expand across a surface.

Research has been performed on this species and scientists have made conclusions about its intelligence, organization and memory. It has been shown to exhibit a collective behavior where several single celled organisms cluster and form temporary tissues that move together in times of resource deprivation. Also slime molds have been found to grow in any direction to find food, then upon discovery of a food source, reduces the non beneficial branches that did not land on a food source and thickens the successful branch.

RESEARCH DESIGN

The species *Physarum Polycephalum* will be investigated *in vitro* over the duration of three classes. Firstly, it is required that the model organism is ordered from a scientific supply company. The company identified that is able to provide for the organism is the Carolina Biological Supply Company located at 2700 York Road, Burlington, NC. Two day shipping is provided for live stock and it can be mailed on plates for $14.70 or in a box with several samples to be plated separately for $14.70, before shipping. The optimal media for the organism is 2% Agar with Old Fashioned Quaker® Oats as a food source. Making media in class will require deionized water, agar, a scale, and petri dishes. Medium size 100mm petri dishes can be purchased sterile on Amazon for $20 for 20 petri dishes. Agar would be used at 2% weight with water to make the media, and reagent grade agar powder can be obtained for $23.95 for 100g on Amazon as well.

Beyond maintaining the life of the organism, the experiment will require methods by which to control the environment at specific temperatures. Available ways to achieve this include ice baths that will be made by obtaining ice buckets borrowed from 153 biology labs on campus and using the research grade ice from the bucket down the hall from the Biology Computer Resource Center. Also heat plates will be used to grow plates on top of a heat source that will be controlled for temperature using the digital display for the temperature of the plate. Plates can be obtained by purchase on amazon for approximately $230, or by borrowing from the biology department or chemistry department that has many in use and some not in use. A simple heat plate would be required as each class time would be a different temperature trial marking the growth of different plates over the time of the class period.

Culturing cells and performing the various experiments

IMPACT/SIGNIFICANCE

This research proposal would reinforce students' time management skills, laboratory experience, knowledge of non-mammalian life cycles and maintaining cultured species. It also allows students to go through the process of modern science, including the phases of proposal, experimentation, and data analysis and conclusion. For those in a career path involving biological research, or research in general, this project will enable students to practice their scientific writing skills and deepen their understanding of performing proper science. We believe that through our methodology of experimentation, students will be able to experience this through an inexpensive, straight-forward pathway and present their findings through a poster presentation to the class.

OUR RESOURCES PLEASE IGNORE

Methods Reference

Reid CR, Latty T, Dussutour A, Beekman M. **Slime mold uses an externalized spatial "memory" to navigate in complex environments**. Proceedings of the National Academy of Sciences [Internet]. 2012 ;109(43):17490 - 17494. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1215037109https://syndication.highwire.org/content/doi/10.1073/pnas.1215037109

Huynh TTM, Phung TV, Stephenson SL, Tran HTM. Biological activities and chemical compositions of slime tracks and crude exopolysaccharides isolated from plasmodia of Physarum polycephalum and Physarella oblonga. *BMC Biotechnol*. 2017;17(1):76. Published 2017 Nov 9. doi:10.1186/s12896-017-0398-6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5679387/

Review Reference

Reid CR, Latty T. **Collective behaviour and swarm intelligence in slime moulds** Gibbs K. FEMS Microbiology Reviews [Internet]. 2016 ;40(6):798 - 806. Available from: https://academic.oup.com/femsre/article-lookup/doi/10.1093/femsre/fuw033http://academic.oup.com/femsre/article-pdf/40/6/798/10741808/fuw033.pdf

https://herbarium.usu.edu/fun-with-fungi/slime-molds

- Plasmodia in good conditionals

Proposal Projects

https://www.carolina.com/teacher-resources/Interactive/think-single-celled-organisms-are-simple-think-again-the-slime-mold-physarum-polycephalum-a-single-celled-organism-for-student-investigations/tr41405.tr
https://www.carolina.com/slime-molds/physarum-polycephalum-plasmodium-living-plate/156193.pr#

Research Questions

1. How do slime molds respond to different food sources
2. How do slime molds respond to light
3. Are slime molds able to solve a maze
4. Can slime molds grow vertically to find food
5. Can slime molds heal a cut wound?
6. How do two slime mold individuals interact?
7. Slime mold response to temperature

Requirements:

Petri dishes:
https://www.amazon.com/Sterile-Plastic-Petri-Dishes-100mm/dp/B001S4D36A $20
Hot Plate: $230