AP Research

Comparison of *Physarum Polycephalum* Growth Rate on Various Nutrients

Word count: 4,003

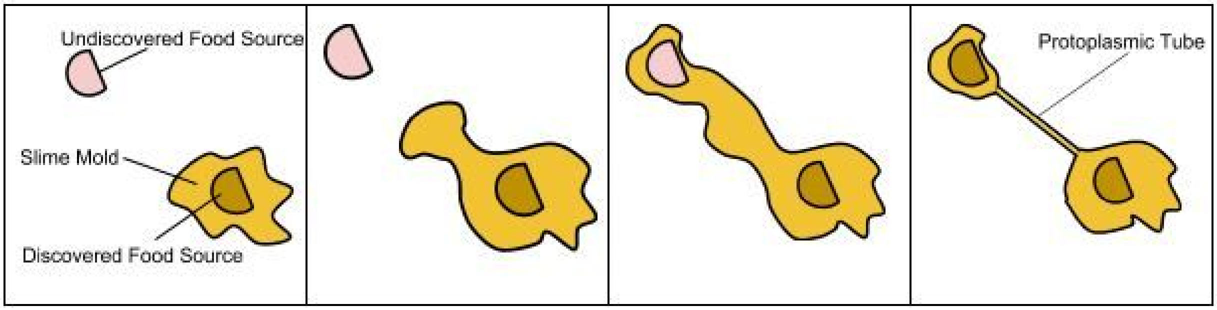
Abstract

The growth rate of *Physarum Polycephalum* (slime mold) was measured to determine what type of food results in the largest increase in surface area across a petri dish. Old fashioned oat flakes, the most commonly used nutrient for slime mold, was compared against 5 other food sources to determine if there is a food other than oat flakes that could yield a higher growth rate. The growth rates were measured with time lapse cameras over the course of three days. Over the duration of the recorded growth, it was concluded that the old fashioned oat flakes yielded the highest growth rate.

Introduction

Slime mold is a single celled protist that is visible to the naked eye. Slime mold is naturally found in the dark, moist climates that exist on decaying leaves and logs in forest areas. The protist consumes fungal spores, bacteria, and other microbes by means of engulfing (Dagamac 2015). To find its prey, slime mold is able to explore its environment “by spontaneous and self-organised oscillatory contractions” (Jones 2015). This means that the slime mold is able to contract its body in an organized way, allowing the slime mold to slowly push itself in all directions. When a slime mold encounters a new food source, it is able to connect the new food source to it’s pre existing ones. To conserve mass, the slime mold gradually funnels the link between the food sources to a single ‘protoplasmic’ tube. This protoplasmic tube has the ability to exchange not only nutrients, but also information throughout the entire network the slime mold creates. This allows the slime mold to conduct a logical and efficient search of it’s surroundings to decide what the most efficient method of utilizing its resources is.

Figure 1. -Slime Mold Protoplasmic Tube Formation



In early stages of slime mold development, the Slime mold exists in a feeding phase, in which the slime mold remains on an already discovered food source, and increases in mass. The slime mold will then gradually transition into a more explorative phase, where the slime mold discovers new sources of food and prepares to create fruiting bodies, which eventually create more slime mold (Dagamac 2015). Slime mold is a puzzling organism because it “possesses no neural tissue yet, despite this, are known to exhibit complex biological and computational behavior”(Jones 2015). This aspect of slime mold allows it to be used in many scientific experiments that further the understanding of thought and decision making skills for single celled organisms. “The plasmodium can solve computational problems with natural parallelism, such as finding the shortest paths and hierarchies of planar proximity graphs, plane tessellations, and planar shapes, execution of logical computing schemes, and natural implementation of spatial logic and process algebra, unconventional hybrid wetware and hardware, and prototypes of microfluidic logic gates” (Adamatzky 2015). In one example testing *Physarum Polycephalum* learning capabilities, slime mold was incubated in a container with a temperature that fluctuated at a constant rate. The slime mold’s growth would slow when it became colder, and returned to normal when the temperature rose again. Eventually, the fluctuations ended, yet slime mold continued to slow its growth when the temperature would have dropped if the temperature changes had continued. This shows that the slime mold was able to anticipate a potential change in the temperature based off of previous experience (Saigusa 2008). Another aspect of *Physarum Polycephalum* is it's form of responding to stimuli. One method used to interpret responses from slime mold is through measuring the electric potential of the organism. To do this, the slime mold must be grown with two colonies, each on one end of a conductivity probe, with a single protoplasmic tube connecting the two colonies. When the slime mold is exposed to different stimuli, unique responses can be measured by the oscillations of the the electrical conductivity through the slime mold's protoplasmic tubes (Adamatzky 2015). This research is useful because it shows a way of immediately recording the response that slime mold has to stimuli in a quantitative form of data. Prior to this method, growth rate was the only form of retrieving quantitative data for a slime mold's response to stimuli, a relatively slow method that and requires days and weeks as opposed to seconds.

My initial research plan was to correlate slime mold's ability to understand and predict changes in temperature (Saigusa 2008) with it's ability to interpret different stimuli (Adamatzky 2015). The slime mold's response to being stimulated with two different stimuli would be measured by its electrical potential.The goal of this research would test if the slime mold was capable of correlating the two forms of stimuli, meaning the slime mold is capable of some form of associative learning. I would began this research by stimulating an experimental group of slime mold with two different stimuli at the same time. Two control groups would also be created. Each control group would be stimulated with one of the two forms of the stimuli. The experimental group would be stimulated with both stimuli simultaneously for many sessions.

The expected response, measured by the oscillations in electrical conductivity, was expected to be unique in all three groups. At the end of the sessions, the experimental group would be stimulated with only one of the two stimuli. If the slime mold is capable of associative learning, the response from the experimental group will be the same when it is stimulated with only one of the stimuli, yet different to both of the control groups. After collecting all of the supplies required to perform this project, I began my experiment. Unfortunately, I was unable to complete this research because the conductivity probes I used did not yield reliable measurements of the slime mold's electrical conductivity. After several weeks of working with the probes, I was forced to change my focus to a different research project.

Experimentation with slime mold requires a large enough amount of the protist to be readily available in the case of a failed culture. Due to the fact that slime mold grows in the units of hours and days, having a food source that yields the highest growth rate is useful for ensuring that an extra culture can always be made. The most commonly used food source to grow slime mold is old fashioned oat flakes. This is likely due to their high amounts of carbohydrates and protein.

Purpose

The key purpose of this project was to determine if there is a possible food source that stimulates a higher slime mold growth rate than Old Fashioned Oat Flakes. If the highest growth rate from a food source is not the oat flakes, it is possible that this food source could be implemented as a more efficient food source for growing *Physarum Polycephalum*. This could lead to an easier accessibility to slime mold for further research to be undertaken on understanding thought and decision making skills for single celled organisms, and further correlated to neural networks in more complex organisms, such as humans.

Hypothesis

Old fashioned oat flakes will yield the highest growth rate for *physarum polycephalum* relative to the other food sources.

Null Hypothesis

The *physarum polycephalum* will yield the same growth rate on all food sources.

Materials

- *Physarum Polycephalum* (Slime Mold)
- Petri Dishes (6)
- 2 % Bacteriological Agar (120 mL)
- *Avena sativa* (oat flakes)
- Cracklin Oat Bran (Oat cereal)
- goPro Hero+ (2)
- *Persea american*a (Avocado)
- *Theobroma cacao* (Chocolate Chips)
- *Cyanococcus* (Blueberries)
- *Anacardium occidentale* (Cashews)
- Lamp (120V, 420mA)

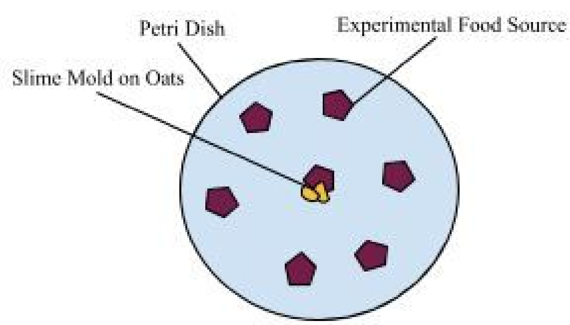
- Tweezers
- 125 mL flask
- DI water
- Parafilm
- Microwave
- Ohaus GA110 analytical balance
- Rack (To hold cameras)

Methods

Old fashioned oat flakes were used as the control for this experiment. This is because they are the most commonly used food source for slime mold, and are high in carbohydrates and protein. An oat cereal called Cracklin Oat Bran was used as one of the experimental food sources due to its high levels of carbohydrates and similarity to to the commonly used oat. The Cracklin Oat Bran could potentially be an effective food source because it is non perishable and the significantly higher amounts of sugar could potentially increase the slime mold's growth. Chocolate chips were used as one of the experimental food sources due to their high levels of saturated fat and sugars. The chocolate chips were used to differentiate between whether the high levels of sugars would allow the slime mold to grow better with carbohydrates similar to the oat flakes, or with the saturated fats present in chocolate chips. Cashews were used due to their high levels of calories with soluble fiber. The Blueberries were used due to their high levels of natural sugars. Avocados were used for their high levels of dietary fiber and saturated fat.

The first step in executing this project was to age the slime mold. In order for the slime mold to move into the more explorative phase of it's life, the slime mold was left to grow for several weeks. The slime mold took 3 to 4 days to fill the entire petri dish, so the colony was constantly being transitioned into new petri dishes. In order to ensure the slime mold survived and grew in natural conditions, new oat flakes were placed in the petri dishes daily, and the lids of the petri dishes were squirted with DI water to keep the environment moist. The petri dishes were filled with 2% bacteriological water agar, as this type of agar is non nutritional, allowing the oat flakes to be the only source of food during this growth period. The 2% bacteriological water agar is also useful because it is far less prone to becoming contaminated with foreign bacteria. The constant opening and closing of the petri dish in order to add more oat flakes and moisture to the slime mold makes non nutritional agar an important factor for preventing the slime mold from getting contaminated by other bacteria. To prepare the petri dishes, 0.5 grams of bacteriological powder was measured with an Ohaus GA110 analytical balance. The bacterial powder was then placed in a 125 mL flask, and DI water was added to the flask to fill up to the 25 mL mark. This created a solution with 2% bacteriological agar. To fully dilute the solution, parafilm was stretched over the flask, and the solution was microwaved on high in 10 sessions. In each session, the microwave was turned on for 15 seconds, and the flask was thoroughly stirred. These sessions were used to prevent the solution from boiling, and spilling out of the beaker. Once the solution was entirely diluted, all 25 mL of the solution were poured to fill a single petri dish. The agar was then left out for ten to fifteen minutes to solidify. This process was repeated for all six petri dishes. To fill the petri dishes with nutrients, the experimental food sources were measured to fill approximately the same surface area of the petri dish. This turned out to be approximately 2 grams for all of the food sources, with the exception of the oat flakes, as their density was too low to mass 2 grams and take up a similar portion of a petri dish. The 6 different food sources were then placed in individual petri dishes, with the food positioned with one piece in the center, and the rest of the pieces forming a circle around the center.

Figure 2. -Arrangement of Food on Petri Dish



The petri dishes were kept under low lighting to allow for the slime mold to remain healthy. 1 cm by 1 cm Graph paper was put beneath the petri dishes to allow for a frame of reference for how far the slime mold grew. All of the slime mold colonies originated from the same colony, growing on oat flakes. In order to start the new colonies, two oat flakes that were being digested by the slime mold were removed from the parent colony using tweezers, and placed in the center of the new petri dish with the experimental food source. To record slime mold growth, two goPro Hero+ cameras were used with a time lapse interval of 60 seconds. To ensure the cameras were not disturbed while recording, a frame was created around the petri dishes. The frame was enclosed with paper towels to prevent direct light from hitting the photosensitive slime mold, but still allow the petri dishes to be well lit for recording with the cameras. A 120V, 420mA lamp was used to ensure constant lighting while the recording was taking place. The lamp was placed above the frame, pointed towards a cabinet, that reflected the light back into where the slime mold was. The light was positioned in this manner to allow the cameras to record the growth of the slime mold while not oversaturating the slime mold with direct light. The goPros were attached to the top of the setup by a large test tube rack. The holes in the rack were large enough for the lens to record the images of the slime mold's growth. Footage was recorded on the cameras until the micro sd cards were full. This took approximately 3 days. To retrieve quantitative data from the images collected, the number of grid spaces occupied by slime mold was counted. To measure the growth over time, I measured the surface area of the slime mold in increments doubling every time, starting with 1 hour and ending at 32 hours.

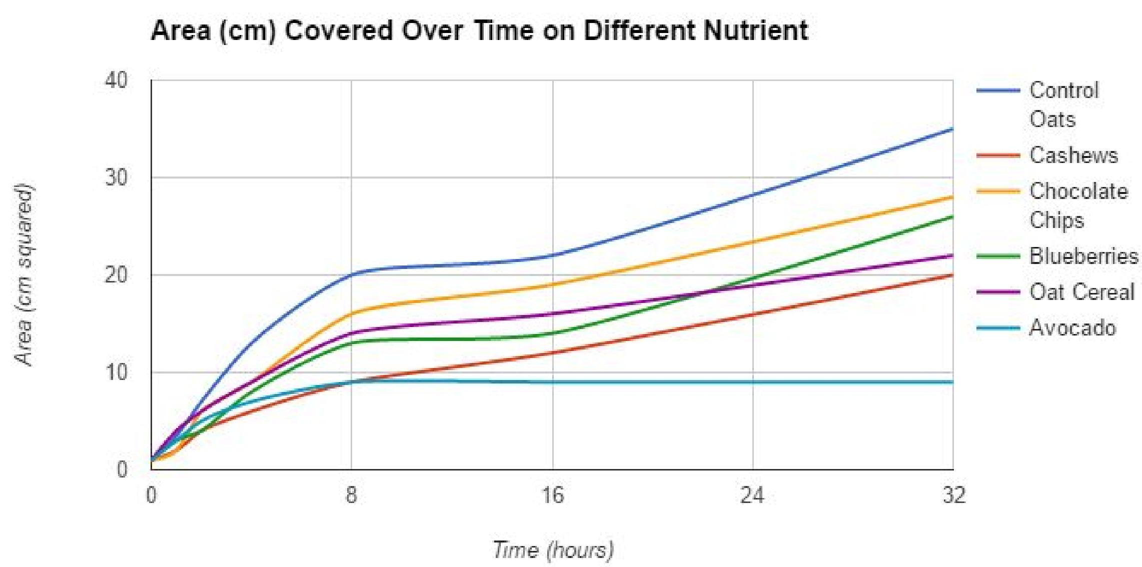
Results

Table 1. -Mass of Nutrients Placed in Petri Dish	
Food	Mass
Control oat flakes	0.5
Cashews	2.1
Chocolate Chips	2.1
Blueberries	2.3
Oat Cereal	2.0
Avocado	2.2

This data was collected to exaggerate the growth rates of slime mold by putting the growth rate over the mass of the food. If there was not a distinct difference between data sets, these values could help reach a conclusion as to what food source is most efficient.

Table 2. -Surface Area (cm²) Over Time							
Food	**0 Hours**	**1 Hours**	**2 Hours**	**4 Hours**	**8 Hours**	**16 Hours**	**32 Hours**
Control oat flakes	1	3.5	7	13	20	22	35
Cashews	1	2	4	6	9	12	20
Chocolate Chips	1	2	6	9	16	19	28
Blueberries	1	3	4	8	13	14	26
Oat Cereal	1	4	6	9	14	16	22
Avocado	1	3	5	7	9	9	9

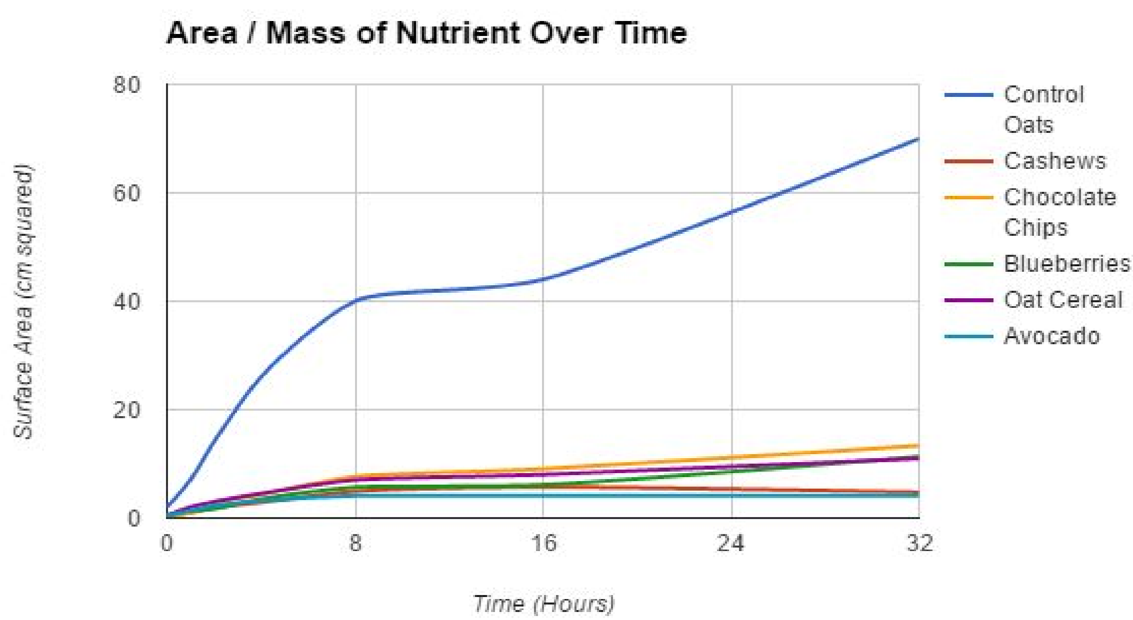
Figure 3. -Surface Area (cm²) Over Time



The table shows raw data measured based off of the time lapse images taken. The graph provides a visual aid that clearly shows that the Control oat flakes yielded the highest slime mold growth rate.

Table 3. -Surface Area (cm^2)/Mass of Nutrition Over Time							
Food	**0 hrs**	**1 hr**	**2 hrs**	**4 hrs**	**8 hrs**	**16 hrs**	**32 hrs**
Control oat flakes	2	7	14	26	40	44	70
Cashews	0.48	0.95	1.9	2.86	4.9	5.71	4.76
Chocolate Chips	0.48	0.95	2.86	4.29	7.62	9.05	13.33
Blueberries	0.43	1.3	1.7	3.48	5.65	6.09	11.3
Oat Cereal	0.5	2	3	4.5	7	8	11
Avocado	0.46	1.37	2.27	3.18	4.09	4.09	4.09

Figure 4- Surface Area (cm^2)/Mass of Nutrition Over Time



In this data set, each row was divided by the mass of the nutrient. This shows an exaggerated form of the data by adding an extra variable to the calculation.

Table 4 -T-test analysis					
Food	1 Hour	2 Hours	4 Hours	8 Hours	T-test
Control oat flakes	3.5	3.5	3.25	3.5	1
Cashews	2	2	1.5	1.13	0.000195
Chocolate Chips	2	3	2.25	2	0.003705
Blueberries	3	2	2	1.63	0.005335
Oat Cereal	4	3	2.5	1.75	0.237142
Avocado	3	2.5	1.75	1.13	0.018036

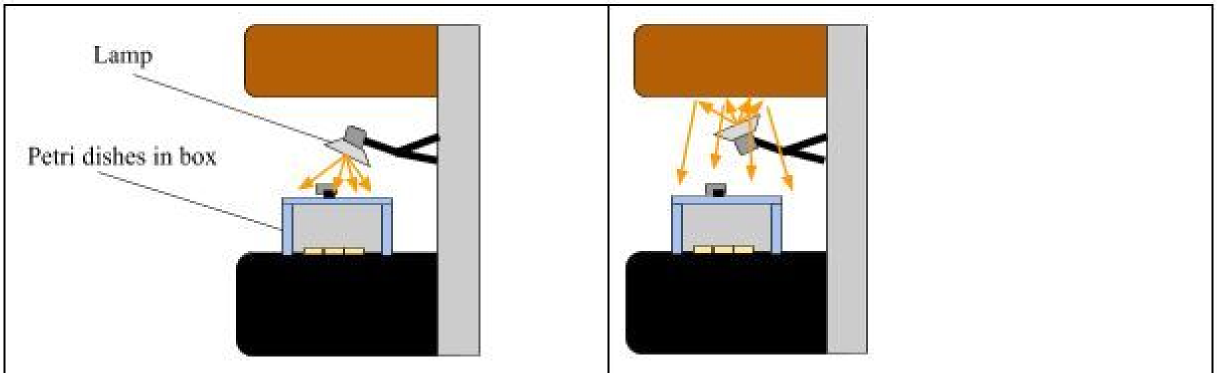
The T-test analysis is used to show if there is a significant difference in the data. In order to create valid values that would create units that could be computed in a t-test, the surface area of the slime mold was divided by the time the slime mold had been growing. Only the first 8 hours of data were used for the t-test because after that, all of the petri dishes yielded a slower growth rate that would invalidate the t-test. The experimental food sources were measured against the control oat flakes for a significant difference. The higher the t-test value, the less significant the difference. The only food source that did not have a significant difference was the oat cereal. This is likely because the cereal had a very high initial growth rate, before slowing down later on.

Discussion

Not only did the oat flakes yield the highest growth rate, but they also yielded the highest growth rate to mass ratio. This means that the oat flakes were able to provide the slime mold with more energy even with the least amount of mass. In addition to this, the oat flakes are also the least perishable food source, further showing that oat flakes are the best food source for growing slime mold. While the oat flakes yielded the highest growth rate, chocolate chips yielded the second highest growth rate. This indicates that there is the possibility that the slime mold can yield a higher growth rate with higher levels of saturated fats and sugars. The avocado, which also has high levels of saturated fats came in last for the food sources tested. This could be because the colony simply failed for reasons other than the food source, but because the blueberries, with high levels of sugars had a growth rate slightly slower to the chocolate, it appears that there is some nutritional benefit of sugars in slime mold growth. This brings up the question of why the oat cereal underperformed. Because the oat cereal has little nutritional value beyond high levels of sugar, it is possible that the cereal simply did not have enough variance in nutrients to allow the slime mold to have a high growth rate. The cashews yielded a relatively low growth rate in comparison with the other food sources, indicating that high levels of calories with saturated fats may not be as nutritional for slime mold growth.

There were several setbacks during the process of completing this research. While creating the petri dishes, a miscalculation by changing a decimal place caused me to create petri dishes with a concentration of 0.2% bacteriological water agar, resulting in petri dishes that did not solidify. To fix the problem, I added the remaining 1.8% required for my normal lab procedure, and was able to still create successful petri dishes for experimentation. Once the entire setup had been constructed, and I had recorded the slime mold's growth over the previous days, I discovered that none of the slime mold colonies, including the oat flakes, had grown. I concluded that due to the slime mold's photosensitivity, the light from the lamp was too bright, preventing the slime mold from growing. To fix the problem, the light was turned in the opposite direction of the container, reflecting off of a cabinet before illuminating the container. This decreased the amount of direct light hitting the slime mold, but still kept it bright enough for the cameras to take pictures for me to record my data.

Figure 5- Light Arrangement



Failed Growth	Successful Growth

Sources of Error

Some possible sources for error include the size of the data, the lighting, and condition of the food source. While measuring data in any scientific experiment, it is important to have multiple trials to base a conclusion off of. Due to the fact that a large portion of the time permitted was spent on a project that ended up unsuccessful, I did not have enough time to run multiple tests to make further verification that my data was accurate. My t-test values do help counter this problem, as the difference in the data sets was considered to be significant. The lighting is another potential source for error. The first test yielded no growth because of too much light. The second test yielded growth, and so this is the data I used for analysis. It is highly likely that the slime mold would yield an even higher growth rate if the slime mold was left to grow in a darker environment. Another potential source of error is the condition of the food. Due to the fact that all of each experimental group came from a single source, there is a possibility that the food yielded an inaccurate representation as to how the slime mold would normally grow on the food source. Because all of the slime mold experimented on originated from a colony that was growing on oat flakes, it is also possible that the slime mold had adapted to grow the most efficient on oat flakes, meaning it would yield the highest growth rate on the food it had already grown accustomed to.

Conclusion

In conclusion, the old fashioned oat flakes yielded the highest growth rate, and are the most effective for further experiments. In addition to this, the oat flakes have far less mass, and are less perishable than the other foods experimented, giving them an additional benefit over the other food sources tested. It is likely that the slime mold had the highest growth rate on the oat flakes due to the high levels of carbohydrates and protein that is present in them. While the other food sources did not yield a higher growth rate, this does not mean that the oat flakes are the absolute best food source for slime mold. The other food sources were still capable of yielding a growth rate that was relatively close to the growth rate of the oat flakes. This means that it is entirely possible that another food source could yield an even higher growth rate.

Further Work

To further expand off of this research, multiple trials could be done to verify my findings. Because I only ran one trial, there could be some inconsistency in the growth rates that I measured. If I were to repeat the experiment over, to improve the accuracy of the data, I would keep the slime mold entirely in the darkness, and only expose the slime mold to light briefly while taking the periodic photos to measure the slime mold's growth. I would also have had the experimental slime mold never come in contact with oat flakes, as to prevent them from potentially growing accustomed to a food source that is not being experimented. Another way that the growth rate could be accelerated is by maintaining a more moist environment. Other further work could include measuring other sources of food for a higher growth rate. Fungal spores, bacteria, and other microbes could be measured for a higher growth rate because this is what they naturally eat. Instead of using 2% bacteriological water agar, another more nutrient filled agar could be used to have an increased growth rate. My initial research proposal is also still a valid research project for further work, as I was unable to prove or disprove my hypothesis.

Acknowledgements

I would like to give A special thank you to Dr. Malhotra for her supervision and guidance through my project. Without the use of her lab, I would not have been able to even attempt my initial project. Another thanks to Sonya Lehto, who works at Amgen, who allowed me to use her own personal equipment, as well as gave me excellent advice and motivation to push on even when I thought there was no hope for a successful project. My classmates and peers deserve a thank you as well for their consistent help by giving me recommendations on how to improve my research.

(4,003)

Sources

Adamatzky, A. (2015). A Would-Be Nervous System Made from a Slime Mold. *Artificial Life*, *21*(1), 73-91. doi:10.1162/ARTL_a_00153

Experiments done to test the properties of slime mold. This article served as my inspiration to connect slime molds ability to learn with its ability to detect chemical and optical stimulation.

Adamatzky, A. (2013). Slimeware: Engineering Devices with Slime Mold. *Artificial Life*, *19*(3/4), 317-330. doi:10.1162/ARTL_a_00110

Explores the possibility of using slime mold as a computational device by stimulating the slime mold in various forms.

Atangana, A., & Vermeulen, P. D. (2014). Modelling the Aggregation Process of Cellular Slime Mold by the Chemical Attraction. *Biomed Research International*, 1-9. doi:10.1155/2014/815690

Berzina, T., Dimonte, A., Cifarelli, A., & Erokhin, V. (2015). Hybrid slime mould-based system for unconventional computing. *International Journal Of General Systems*, *44*(3), 341-353. doi:10.1080/03081079.2014.997523\

Berzina, T., Dimonte, A., Cifarelli, A., & Erokhin, V. (2015). Hybrid Slime Mold - Containing Systems for Unconventional Computing. *AIP Conference Proceedings*, *1648*(1), 1-3. doi:10.1063/1.4912532

Dagamac, N. A., Rea-Maminta, M. D., & dela Cruz, T. E. (2015). Plasmodial Slime Molds of a Tropical Karst Forest, Quezon National Park, the Philippines. *Pacific Science*, *69*(3), 411-422. doi:10.2984/69.3.9

Jones, J. D. (2015). Exploiting Environmental Computation in a Multi-Agent Model of Slime mold. *AIP Conference Proceedings*, *1648*(1), 1-4. doi:10.1063/1.4912814

Explains how slime mold is able to explore its environment and why slime mold can learn from its surroundings. This article can provide me with background information on slime mold.

Reid, C. R., Latty, T., Dussutour, A., & Beekman, M. (2012). Slime mold uses an externalized spatial "memory" to navigate in complex environments. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, *109*(43), 17490-17494. doi:10.1073/pnas.1215037109

S. (2008, November 1). Amoebae Anticipate Periodic Events. *Physical Review Letters, 100(1): 01810*. Retrieved from (c) 2008 American Physical Society.

The basis of my hypothesis. this paper explains how slime mold can learn and anticipate patterns from the environment.

Umedachi, T., Idei, R., Ito, K., & Ishiguro, A. (2013). A Fluid-Filled Soft Robot That Exhibits Spontaneous Switching Among Versatile Spatiotemporal Oscillatory Patterns Inspired by the True Slime Mold. *Artificial Life*, *19*(1), 67-78. doi:10.1162/artl_a_00081