### Factors that Affect the Exploration Activity of Slime Mold, *Physarum*

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### Abstract

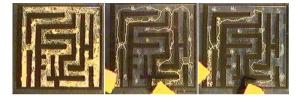
Slime Mold *Physarum* is known to take the shortest path in 2D mazes according to a previous research (2006). However, one study (2018) made it clear that *Physarum* in 3D mazes takes several paths including the shortest path. Our research aims to find out the reason why Physarum cannot solve 3D mazes in the shortest path. To find the reason for this, we searched for factors that may affect the exploration activity of *Physarum* and focused on the gravity effect. Two kinds of experiments using hemispheres and inclinations were done. As a result, the tendency that *Physarum* prefers to go against gravity was seen and it became obvious that gravity affects the exploration activity of *Physarum* cannot solve 3D mazes in the shortest path because *Physarum*. Therefore, we concluded that *Physarum* cannot solve 3D mazes in the shortest path because *Physarum* chooses going upwards more than taking the shortest path, saving the energy to move.

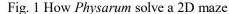
### 1. 研究の概要

粘菌(モジホコリ)は、二次元の迷路では餌と餌を最短経路で結ぶが、三次元迷路では最短経路を含む 複数の経路を残すことが先行研究(2018)で明らかとなっている。本研究では、粘菌が三次元迷路を解けな い理由を探ることを目的としている。研究に際して、粘菌の探索行動に影響する要因を探究し、重力の影響に着目した。粘菌の探索行動に及ぼす重力の影響を解明するために、傾斜や半球を用いた2つの実験を 行った。実験の結果、粘菌は傾斜のある位置の餌に向かう傾向が見られた。つまり、粘菌は三次元迷路で は、水平方向の移動より負の重力方向に探索行動をするため、複数の経路が残ることが示唆される。

#### 2. Introduction

*Physarum* is known to take the shortest path in 2D mazes (Fig. 1) and this special feature of *Physarum* is used as a bio-computer and actually helps to theorize urban structure.





On the other hand, *Physarum* in 3D mazes takes several paths including the shortest one. If *Physarum* can solve 3D mazes in the shortest path, this feature can be applied to avoid crowded places in three-dimensional buildings

like shopping malls. Therefore, the purpose of our experiment is to find out the reason why *Physarum* cannot solve 3D mazes in the shortest path and apply *Physarum* to our daily life.

Our hypothesis was set up for these reasons:

- *Physarum* has negative photo-taxis. Also in the natural environment, there is more light at higher places than lower places.
- It seems obvious that going upwards takes more energy than going horizontally.

Therefore, we expect that *Physarum* cannot solve 3D mazes in the shortest path because it prefers to move horizontally.

From the hypothesis, we focused on the effect of gravity on the exploration activity of *Physarum* and two kinds of experiments were done.

### 3. Method and Results

2% agar medium and oatmeal were used in all experiments and when *Physarum* was cultured.

### <Experiment 1>

This experiment was about *Physarum*'s activity on hemispheric agar. The purpose was to verify whether gravity affects *Physarum's* activity or not.

*Physarum* and oatmeal spots were put on hemispheric agar as the photo shows (Fig.2). All of the lengths between *Physarum* and oatmeal were the same, no

matter which path they took, because it was a hemispheric agar. Therefore, in this experiment, only gravity affected their movement.



Fig.2 Photo of Experiment 1

Four kinds of hemispheric agars, each of which had a different length of radius, were used in the experiment. The radii were 0.9cm, 1.2cm, 1.6cm, and 2.1cm. We expected that *Physarum* would go horizontally which means they would not go up the hemispheric agar regardless of the radius.

We took pictures to record their movement with a camera which enabled us to do a fixed-point observation.

### <Result from Experiment 1>

The number of experiments using each agar was as follows. 0.9cm was used 3 times, 1.2cm was 33 times, 1.6cm was 6 times, and 2.1cm was 3 times, as shown in Fig. 3. Out of four different hemispheres, the experiments using 1.2cm radius agar were done the most. Therefore, the result of the experiments with agar which has 1.2 radius should be more reliable than others.

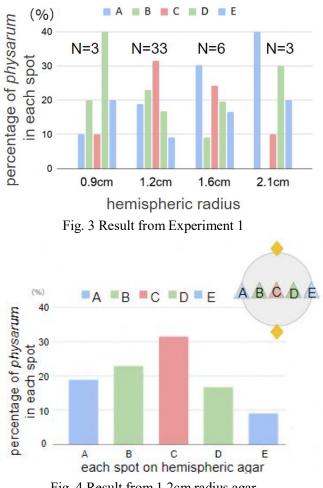


Fig. 4 Result from 1.2cm radius agar

As Fig. 4 shows, more than 70% of *Physarum* went towards the oatmeal of B, C, and D which were located in higher places. On the other hand, only about 30% of *Physarum* went towards the oatmeal of A and E which are located on the same height as the height *Physarum* was first put. This result is perfectly opposite from our hypothesis in which we expected that *Physarum* prefers to go horizontally.

In this experiment, the tendency of going upwards the agar was seen, but we weren't sure which inclination *Physarum* would prefer. Therefore, we had the Experiment 2 about various angle inclinations.

#### <Experiment 2>

This experiment is about *Physarum*'s activity on inclinations. The purpose of this experiment was to find which inclination *Physarum* would prefer.

All sides of *Physarum* were surrounded with  $0^{\circ}$ , 22.5°, 45°, and 90° inclination agars and the length of all the agar was 3cm as the figure below shows (Fig.5). The lengths between *Physarum* and oatmeal spots on each agar, which was the nearest from *Physurum*, was the same.

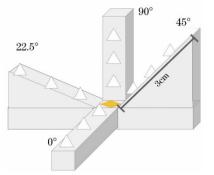
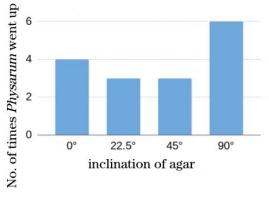
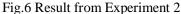


Fig. 5 Figure of Experimental Device

### <Results from Experiment 2>

The experiment was performed 6 times.





The numbers of *Physarum* which went to  $0^{\circ}$  agar, 22.5° agar, and 45° agar were almost the same and all *Physarum* went up to 90° agar (Fig. 6). Although this experiment was performed only 6 times and there may be an error, it can be said that this result shows that *Physarum* has a tendency of going to higher degree inclinations. Therefore, this tendency is thought to affect the exploration activity of *Physarum*.

### 4. Discussion

In our hypothesis, we expected *Physarum* could not solve 3D mazes in the shortest path because *Physarum* preferred to move horizontally. However, against our hypothesis, the tendency of moving upwards of *Physarum* was seen in the experiments. There are two possible reasons why *Physarum* went upwards in the experiments.

One possible reason is because we could not reconstruct the natural environment in the experiments (Fig. 7). Although it is obvious that *Physarum* has tendency of going towards moisture, the 2% agar medium on which *Physarum* was cultured might have been too moist for *Physarum* compared to the natural environment.

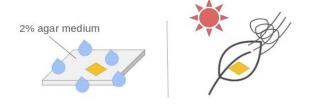


Fig. 7 Difference of moisture between experiments and the natural environment

The other possible reason is that *Physarum* had a tendency of going against gravity originally. There are two possible benefits for *Physarum* to go upwards in the natural environment.

First, it is to leave their descendants in a wider range of locations. Moreover, *Physarum* form sporangium when they are exposed to sunlight or dryness, which they need to do in order to leave descendants.

The other reason is for habitat segregation (Fig. 8). In the natural environment, *Physarum* eats mushrooms, fungi, dead leaves, and so on. But there are many creatures that eat those things so their food and habitat are limited. Therefore, to separate their habitat from other creatures, *Physarum* seems to go against gravity.

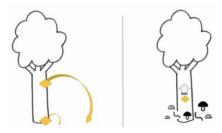


Fig. 8 benefits of going upwards

### 5. Conclusion

From these two experiments, we concluded that *Physarum* cannot solve 3D mazes in the shortest path because *Physarum* has the tendency of going upwards rather than moving horizontally. Moreover, when *Physarum* was cultured, *Physarum* sometimes formed sporangium which is one part of *Physarum*'s life cycle. It was usually when *Physarum* was exposed to light and dryness. Therefore, mainly three factors: light, moisture, and gravity, affect *Physarum*'s exploration activity. But, of course, these factors are closely related to each other in the natural environment. In such conditions, we expect that *Physarum* changes their needs depending on their biological life cycle (Fig. 9).

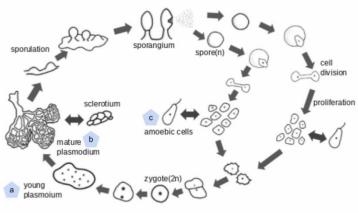


Fig. 9 Physarum biological life cycle

For example, young plasmodium (2n) needs moisture for moving around and predation (Fig. 9-a). On the other hand, when they are in the stage of mature plasmodium (Fig. 9-b), they give priority to searching light or going against gravity over moisture in order to form sporophytes (2n). Once spores germinate, and become amoebic cells, they inhabit where there is much moisture to conjugate with gamete (Fig. 9-c). This is how we thought *Physarum's* needs change depending on their biological life cycle.

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# The Factors that Affect the Exploration Activity of Slime Mold Physarum

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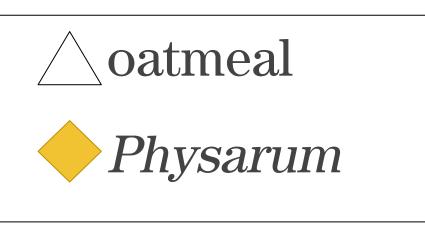
# I. Introduction

From previous research(2018), *Physarum* is known to take the shortest path in 2D mazes. On the other hand, *Physarum* in 3D mazes takes several paths including the shortest path. We wondered why Physarum couldn't solve 3D mazes in the shortest path. To find out the reason, we searched for factors that affect *Physarum*'s activity.

[Expt.2] *Physarum*'s activity on inclinations.

PURPOSE: To find which inclination *Physarum* prefer.

 $45^{\circ}$  $22.5^{\circ}$ 





### II. Purpose

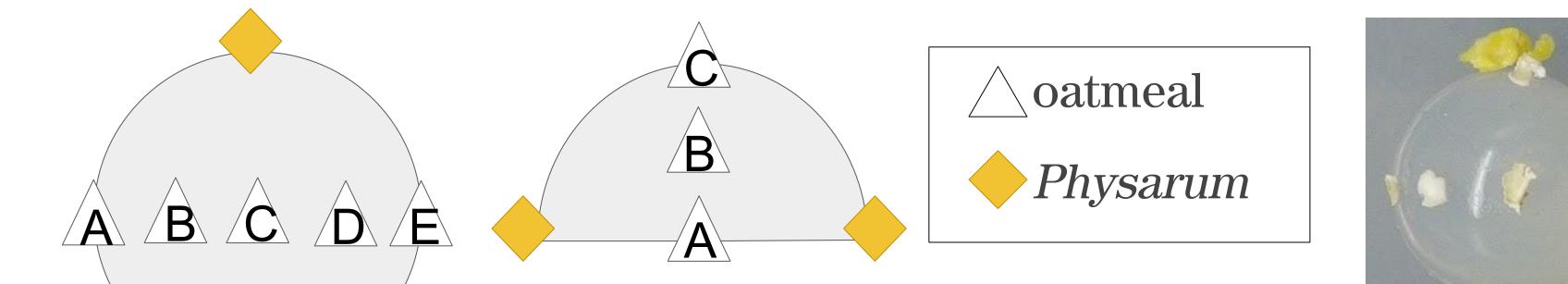
The purpose is to find the reason why *Physarum* couldn't solve 3D mazes in the shortest path by searching for factors that affect *Physarum's activity*.

# III. Method & Result

2 kinds of experiments were done.

[Expt.1] Physarum's activity on hemispheric agar

PURPOSE: To determine if gravity affect *Physarum*'s activity.



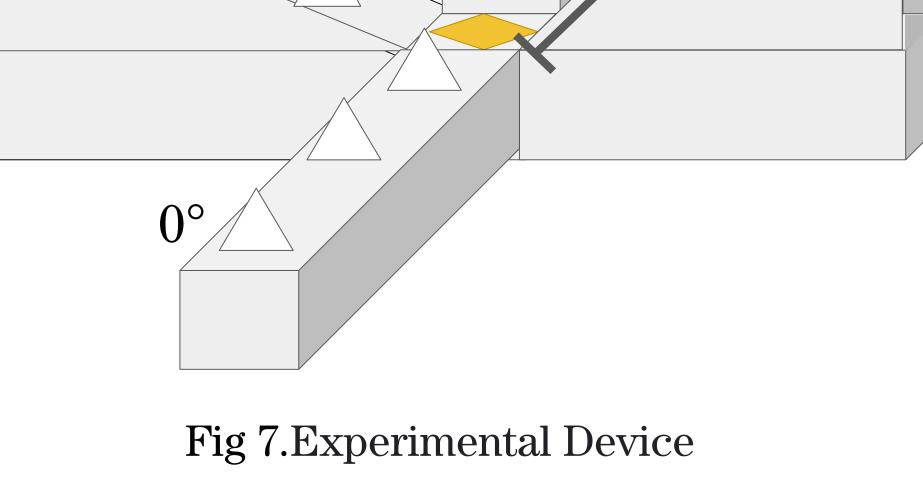


Fig 8.Actual Photo

*Physarum* was surrounded by  $0^{\circ}$ ,  $22.5^{\circ}$ ,  $45^{\circ}$ , and  $90^{\circ}$ inclinations agar as the figures above show.

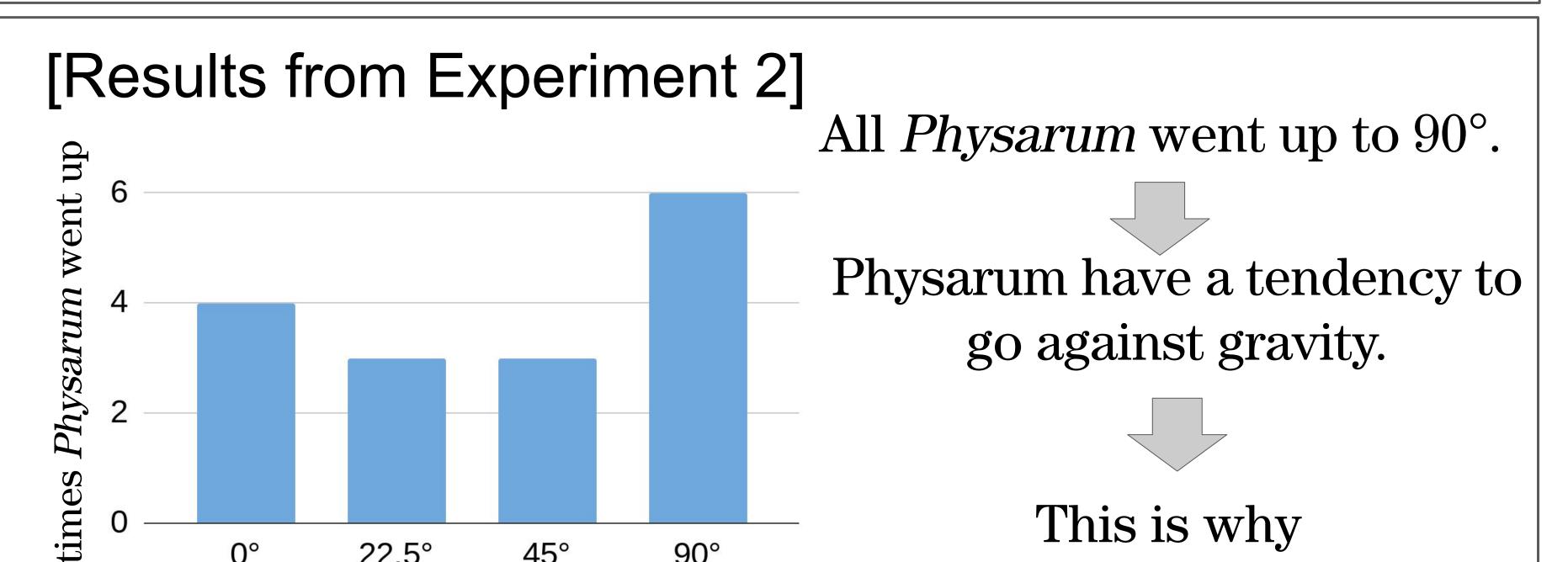




Fig 1.From above

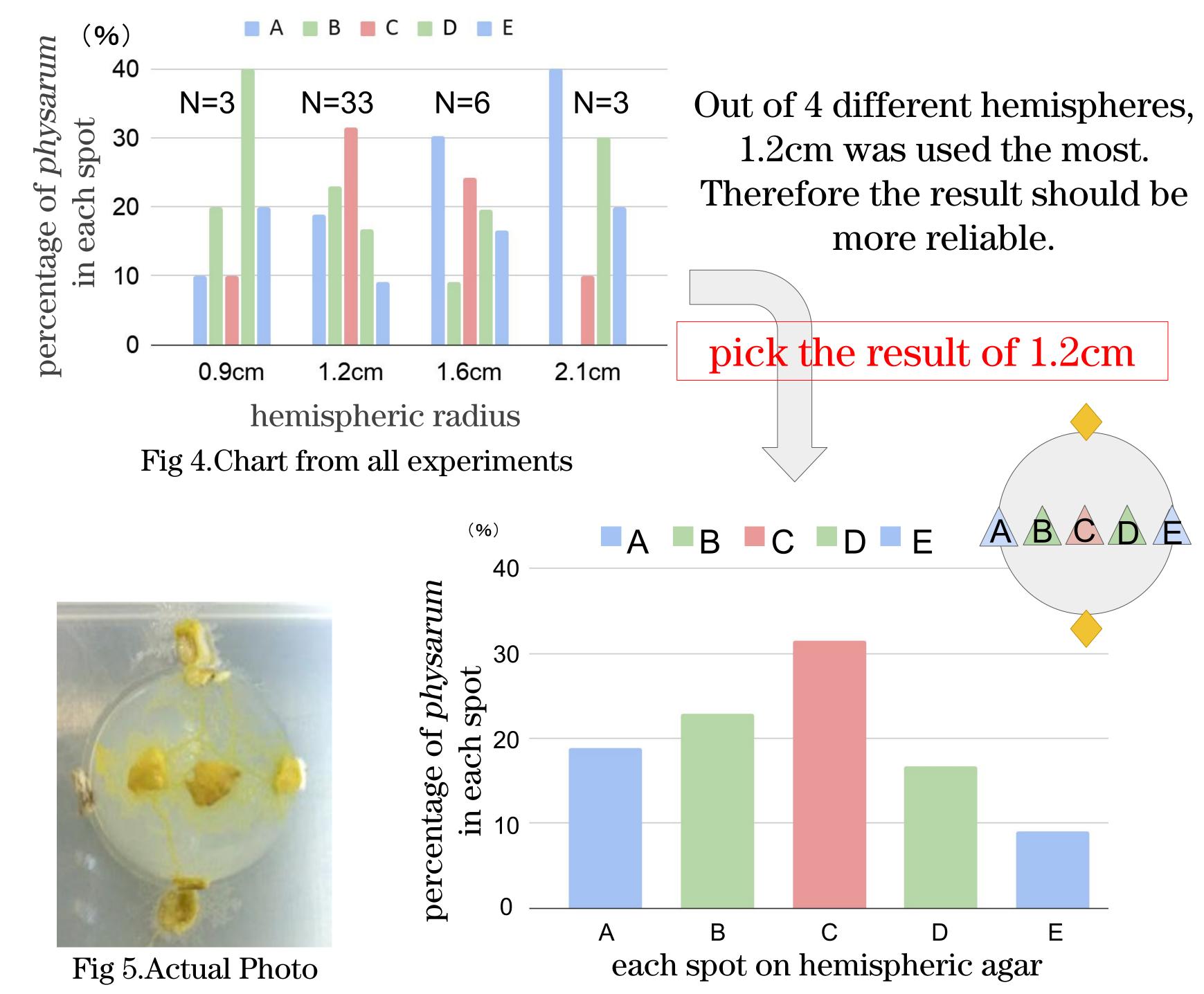
Fig 2.From side

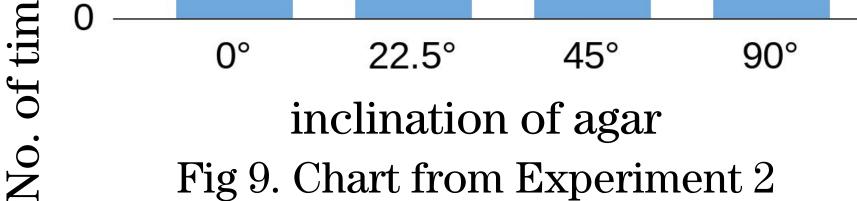
Fig 3.Actual Photo

*Physarum* and oatmeals were put on hemispheric agar as the figures and the photo above show.

The length between *Physarum* and oatmeal is the same, no matter which path they take, because it is a hemispheric agar. Therefore only gravity affected *Physarum*'s activity.

[Results from Experiment.1]





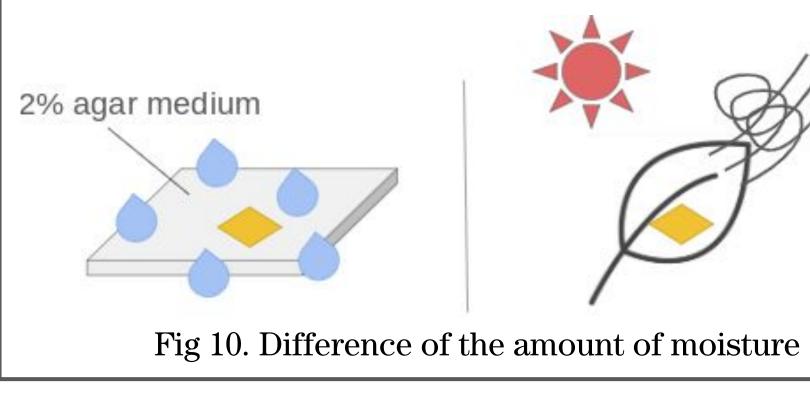
This is why *Physarum* can not solve 3D mazes in the shortest path.

# IV. Discussion

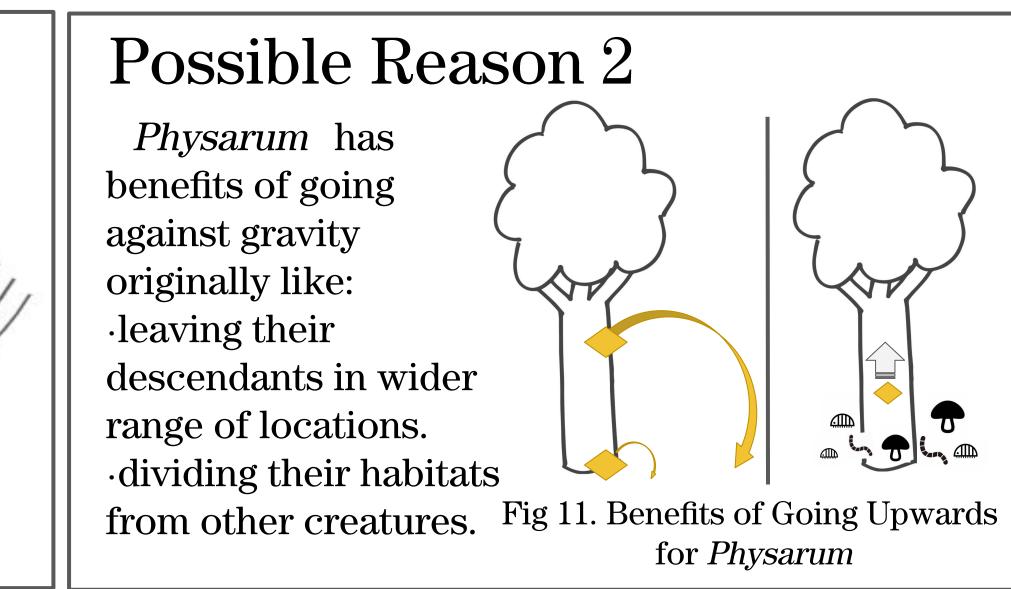
There are 2 possible reasons why *Physarum* went against gravity in this research.

### Possible Reason 1

We could not reconstruct the natural environment in our experiments.



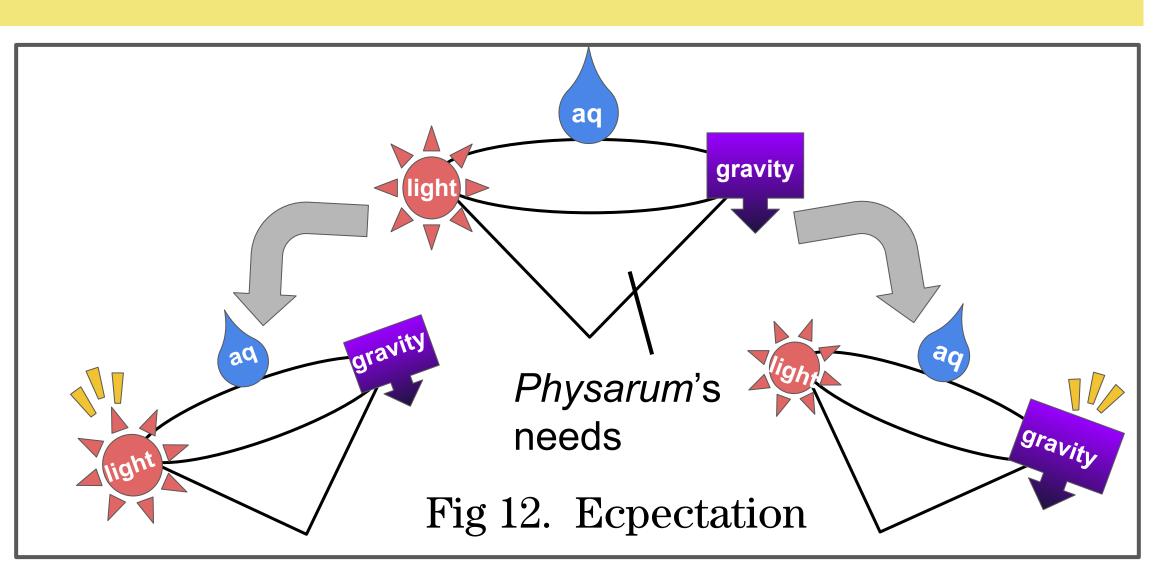




### Fig 6.Chart from 1.2cm experiments

70% of *Physarum* went towards the top. The tendency for *Physarum* to go upwards was seen.

As a result from two experiments, Physarum can not solve 3D mazes in the shortest path because of the tendency of going against gravity. We expect that moisture,



light, and gravity affect *Physarum*'s activity and *Physarum* chooses the most necessary factor of three depending on their needs.

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### The research for Rust -Catalytic action of Titanium dioxide-

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### Abstract

The goal of this project is to elucidate the effect of photocatalysis of Titanium dioxide (TiO<sub>2</sub>) on the oxidation of iron. We conducted three experiments: the first used TiO<sub>2</sub>, Calcium carbonate (CaCO<sub>3</sub>), and Calcium sulfate (CaSO<sub>4</sub>) as variables for the type of solute in the coating solution; the second took as a variable the amount of moisture in the environment for static storage after coating; and the third used Methylene blue. The first experiment showed that TiO<sub>2</sub> with sunlight rusted best. From the second experiment, we found that those in a vapor-liquid equilibrium environment rusted best. In addition, experiments on the oxidizing power of TiO<sub>2</sub> using Methylene blue showed that the dye was best decomposed with TiO<sub>2</sub> and ultraviolet light.

#### 研究の概要

酸化チタンの光触媒作用が鉄の酸化に与える影響を解明することを目的とする。コーティング溶液の溶 質の種類を変数として,酸化チタン,炭酸カルシウム,硫酸カルシウムを用いた実験,コーティング後の 静置環境の水分量を変数とした実験,またメチレンブルーを用いた吸光度測定実験を行った。すると,酸 化チタン溶液を鉄に滴下し日光に当てたものと,気液平衡の環境のものが最もよくさびるという結果が得 られた。さらに,メチレンブルーを用いて酸化チタンの酸化力について実験した結果,酸化チタンありの 上で紫外線ありが最もよく色素を分解した。

#### Introduction

TiO<sub>2</sub>, which has photocatalytic properties, is used in various applications such as antimicrobials and is attracting attention because it does not emit toxic substances. There have been no previous studies on TiO<sub>2</sub> and rust, so we investigated whether the oxidizing power of TiO<sub>2</sub> also affects metals. We found that TiO<sub>2</sub> has the effect of accelerating the oxidation of metals.

<Principle of Rust Formation>

 $H_2O$  loses electrons to holes to generate OH radicals. We decided to investigate whether  $TiO_2$  can rust (oxidize) metals by conducting

experiments to see whether OH radicals and holes have enough oxidizing power to oxidize iron. In this experiment, we used iron because it rusts relatively easily and it was easy to confirm the change. In addition, to evaluate the oxidizing power of the  $TiO_2$  used in the experiments. Experiments were also carried out using the redox indicator Methylene blue to assess the oxidizing power of the  $TiO_2$  used in the experiments. It is already known that  $TiO_2$  is a photocatalytic material, which is excited by UV irradiation. The electrons emitted by the excitation react with water to produce OH radicals, which are known to have strong oxidizing power and high reactivity.

Decomposition occurs when OH radicals deprive organic substances of electrons. We hypothesize that this strong oxidizing force can rust metals.

Method and Results

[Experiment 1]

Experiment using TiO<sub>2</sub>, CaCO<sub>3</sub> and CaSO<sub>4</sub>

< Objective >

To investigate whether  $TiO_2$  has an effect on the formation of rust on iron.

<Hypothesis>

 $TiO_2$  rusts metals the most.

<Method>

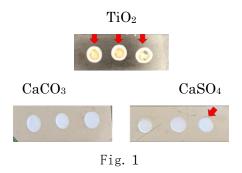
1. grind the reagent in a mortar. Also, file the iron to remove rust from the iron to be used.

2. mix one of TiO<sub>2</sub>, CaCO<sub>3</sub> or CaSO<sub>4</sub>  $\cdot$  H<sub>2</sub>O (3.0 g) with water (8.11 g). The resulting suspension is used as the coating solution and is dropped onto the iron using a pipette while stirring with a stirrer.

The sample was exposed to sunlight for approximately one day.

 $\langle \text{Results} \rangle$ 

Among TiO<sub>2</sub>, CaCO<sub>3</sub>, and CaSO<sub>4</sub>, TiO<sub>2</sub> rusted iron the most. The discolored area in the center is rust. The arrow points to that.



[Experiment 2]

Experiment using the moisture content of the static environment as a variable

< Objective >

To investigate the effect of water content on  $TiO_2$  rust formation.

<Hypothesis>

The higher the moisture content, the more rusting of iron will occur.

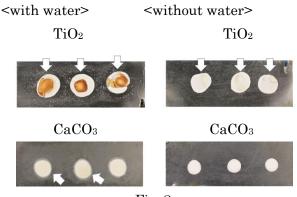
 $<\!{
m Method}\!>$ 

1. Prepare samples of  $TiO_2$  and  $CaCO_3$  using the method of Experiment 1.

2. In the first case, the samples were placed in a box lined with damp paper towels and the box was closed with plastic wrap to create a vaporliquid equilibrium environment; in the second case, the samples were placed in a petri dish and left in an indoor environment without plastic wrap in the sunlight. The rusting process was observed with a time-lapse video.

<Result>

Figure 2 shows that when the  $TiO_2$  samples were left in a vapor-liquid equilibrium environment, the highest amount of rusting occurred on iron. It was also found that the time taken for rust to form was approximately 30 minutes from the time-plus video.





### [Experiment 3]

Detection of iron(II) and iron(III) ions using potassium iron(III) hexacyanide and potassium iron(II) hexacyanide as indicators.

< Objective >

To investigate whether iron(II) and iron(III) ions are included in the components of rust generated in experiments 1 and 2.

<Hypothesis>

The rust generated in Experiments 1 and 2 contains Fe(II) and Fe(III) ions.

<Method>

1. Collect the rust.

2. Add hydrochloric acid to dissolve the collected rust.

3. Add drops of potassium iron(III) hexacyanide and potassium iron(II) hexacyanide, respectively.4. Observe the color change.

 $<\!\mathrm{Results}\!>$ 

Figures 3 and 4 show that the rust to which potassium iron(III) hexacyanide acid was added produced dark blue precipitates in part. Rust to which potassium iron(II) hexacyanide was added showed a large amount of dark blue precipitation.

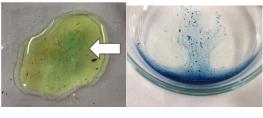


Fig.3



### [Experiment 4]

Absorbance measurement experiment using Methylene blue

<Objective>

To investigate whether photocatalysis of  ${\rm Ti}O_2$  deprive electrons or not.

<Hypothesis>

The absorbance of Methylene blue decreases due to the photocatalysis of  ${\rm TiO}_2$ .

### <Methods>

1. Prepare samples of  $TiO_2$  (1.0 g) mixed with distilled water and samples to which nothing was added.

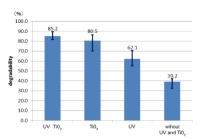
2. Leave the prepared samples in a UV chamber (253.7 nm) or in a dark room for 1 hour, and then add Methylene blue solution

(2.7 x 10<sup>5</sup>mol/L 10mL).

3. centrifugation (micro mini centrifuge, SiGMA Micro mini centrifuge, SiGMA, speed 10000 rpm x 4 min) to precipitate the  $TiO_2$  and measure the absorbance (664.2 nm) of the supernatant solution.

 $<\!\mathrm{Results}\!>$ 

In the Methylene blue experiment, the absorbance of the sample with  $TiO_2$  was lower than that of the sample without. The absorbance of the sample with  $TiO_2$  added and irradiated with UV light was lower than that of the sample without UV light.

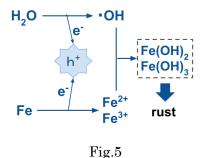


Graph 1. Result from Experiment 4

### Discussion/Hypothesis

The  $TiO_2$  samples did not show much difference regardless of whether they were irradiated with or without UV light. The reason for this is considered to be the possibility that  $TiO_2$  is excited in a short time even by fluorescent light, since the sample without UV irradiation was not exposed to fluorescent light in the experiment, but was slightly exposed to light during movement. The process of rust generation is shown in Fig.5. Considering that iron does not rust easily when dry, water is likely to be involved.

The holes created by the excitation of  $TiO_2$  by UV light take electrons from water, resulting in the formation of hydroxyl radicals. They also take electrons from iron, resulting in the formation of iron ions. The reaction between these iron ions and hydroxyl radicals causes rust. Some of the iron ions react with the electrons emitted by the excitation and return to iron.



### Conclusion

 $TiO_2$  has photocatalytic properties. It also exhibits oxidizing power when exposed to ultraviolet and indoor light, which can rust iron. In the future, we will conduct similar experiments with other metals to determine if our hypothesis is correct. Further experiments will be conducted to increase the accuracy of the results. We also plan to apply this technology to copper greening, aluminum passivation, and the use of iron rust.

### Acknowledgements

We would like to thank the teachers in and outside Tsuyama High School who guided us in carrying out this research, and those who supported this research.

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### The research for Rust $\sim$ Catalytic action of Titanium dioxide $\sim$

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### I. Research Objective

The purpose of this study is to elucidate the effect of photocatalysis of Titanium dioxide( $TiO_2$ ), which is used in house coatings, on the oxidation of Iron. Although there had been some research on the photocatalytic action of TiO<sub>2</sub>, there is no research to study the relationship between TiO<sub>2</sub> and rust, so we devised our own experiment.

### II. Basic knowledge

started forming rust after 30 minutes.

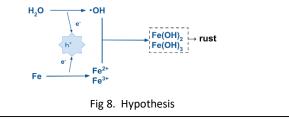
Processes by which TiO<sub>2</sub> degrades bacteria. TiO<sub>2</sub> is excited by UV light and emits electrons. Then, holes are created in TiO<sub>2</sub>, which deprive water molecules of electrons. The electron-deprived water molecules become OH radicals which have strong oxidizing power and deprive bacteria of electrons. V. Experiment3III. Experiment(1) [Expt.3] Detection of Fe<sup>2+</sup> and Fe<sup>3+</sup> [Expt.1] Rust-promoting control experiments Collect the rust on TiO<sub>2</sub>. Add drops of  $K_3$ [Fe(CN)<sub>6</sub>] with TiO<sub>2</sub>. and  $K_4$ [Fe(CN)<sub>6</sub>], respectively. Observe the color Grind 3.0 g of  $TiO_2$ , change.  $CaCO_3$ , or  $CaSO_4 \cdot 2H_2O$ [Result from Expt.3] in a mortar and then pour in 8.11g water. solution drip method Second, coat the iron Fig2. Solution drip method sheet with TiO<sub>2</sub>, CaCO<sub>3</sub> and CaSO₄using solution drip method.  $Fe^{2+}(K_{3}[Fe(CN)_{6}])$  $Fe^{3+}(K_{4}[Fe(CN)_{6}])$ Third, expose the samples to UV light for a day, Fig7. Detection of Fe<sup>2+</sup> and Fe<sup>3+</sup> after that, we observed them. TiO, [Result from Expt.1]  $TiO_2$  rusts the iron the best. The discolored area in the CaCO. CaSO, dark blue precipitation. center is rust. The arrow points to that area. Fig3. Result of Expt.1 **VI**. Hypothesis IV. Experiment (2) [Expt.2] Humidity of static environment H<sub>2</sub>O OH We used TiO<sub>2</sub> and Fe(OH)<sub>2</sub> CaCO<sub>3</sub>.Also,we – rust Fe(OH)<sub>3</sub> compared them with and without water. CaCO The images were Fig 8. Hypothesis filmed overnight in Fig4. Environment of Expt.2 time-lapse. References [Result from Expt.2] 2001) Fig5. Result of Expt.2 Fig6. Samples after 30 minutes 岡技科大工)村上能規,太田 育樹,遠藤 健史,野坂 篤子,野  $TiO_2$  with water rusts iron the best. Also, the  $TiO_2$ 坂 芳雄 without water was not very rusty. Also, rust

Fig1. The process of generation

### holes by TiO<sub>2</sub>'s excitation

Rust to which  $K_3[Fe(CN)_6]$  was added produced dark blue precipitates in part. Rust to which  $K_4$ [Fe(CN)<sub>6</sub>] was added showed a large amount of

The process of rust generation is shown in Fig 8.



1) 光触媒反応における OH ラジカルの生成と寄与 野坂芳雄 2) 様々な化学物質の利用による酸化チタンの酸化能力の変化 (千葉県立船橋高等学校理数科課題研究 2017 年 理数科 3 年) 3)活性酸素種の反応の新展開 沢木泰彦(オレオサイエンス 4)酸化チタン光触媒で生成する OH ラジカルの生成機構(長

5)酸化チタンの光触媒反応を利用した抗菌作用(無機マテリ

アル1999)

### Factors Influencing the Growth of Ice Stalagmites

Ayu Moriyasu Mei Terasaka Rikuto Tsuneto Supervisors Hiroaki Minami Yuya Sasayama

#### Abstract

Ice stalagmites form naturally when drops from a cave or other structure gradually drip and freeze to the ground. Because of the difficulty of creating them artificially, research on them has not proceeded. This study examined the factors influencing the growth of ice stalagmites by creating them with different solutions, and also clarified their crystal grains and orientation. As a result, ice stalagmites made from electrolyte solutions containing ions in which those with small ionic radius were hydrated grew the longest. Furthermore, we discussed the process of ice stalagmites formation by measuring the concentration, observing the crystal grains and taking videos of it in the forming process.

### I 研究の概要

氷筍とは滴り落ちた水滴が上向きに凍って柱状にできる氷塊のことである。ただ人工的に作成することが難しく研究はあまり進んでいない。本研究では氷筍の成長に影響を与える要因の考察を行い、さらに 氷筍の結晶粒および結晶方位の解明を行った。その結果、電解質水溶液のうちイオン半径の小さいもの同 士が水和している溶液で作成した氷筍が最も長く伸びることがわかった。また濃度の滴定、結晶粒の観察、 形成過程の撮影により氷筍の成長過程を説明することができた。

### II Introduction

Ice stalagmites are sometimes used to create slippery ice for skating rinks. Today, with global warming, the amount of ice that can be produced within the natural environment is likely to be limited. This study aims to explore the unknowns and the factors influencing the growth, which can be applied to the more efficient creation of skating rinks and ice sculptures as art objects.

In a previous study in junior high school, it was possible to create artificial ice stalagmites, and the experiments showed that the length of the ice stalagmites formed differently depending on the kind of solutions (Moriyasu, 2021). However, the experiments lacked reproducibility because they were conducted in an environment affected by temperature. And also, mass percentage was used instead of molar concentration to determine concentration. Therefore, we conducted this study using improved apparatus and the molar concentration as a method for indicating density.

#### III Method

The experimental apparatus is shown in Fig. 1. We stacked two polystyrene boxes and placed them on top of the freezer with the lid open. An intravenous tubing with a plastic bottle for pouring the solutions attached ran from the top of the boxes into the freezer. The solution was dripped onto the tray in the freezer through the intravenous tubing at 4-second intervals. In the top box, the solution was chilled in ice water to maintain the constant temperature at 4.5 to 5.5  $^{\circ}$ C.

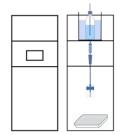


Fig. 1 the experimental apparatus

A straw was used to keep the amount of air entering the bottle the same, which leads to keeping the drop rate constant. The second box had a hole in its side to roll up or down the roller of the roller clamp, which allowed us to control the drop rate. The temperature in the freezer was kept at -22 °C and continued dropping for 4 hours. The experiments were conducted with 5 kinds of aqueous solutions of sodium chloride, sodium hydrogen carbonate, potassium nitrate, acetic acid, and urea of 0.4 mol/L.

### **IV** Experiments

### 1. Experiment 1

From the previous study, it was hypothesized that solutes in the solutions that are bonded by intermolecular forces will form longer ice stalagmites than those that are ionically bonded. So, this experiment examined the differences in the length of ice stalagmites by changing their solutions to create them. We conducted this 3 times in each solution, and measured the length.

### 2. Experiment 2

In the course of experiment 1, as the ice stalagmites created with the acetic acid solution started to melt, the smell was felt to be lighter than the original solution. So, it was considered that the concentration might differ depending on the location inside them. Hence, in this experiment, we cut the ice stalagmites horizontally into 3 or 4 pieces and measured their concentration after they melted. Concentrations were measured by the second stage of double indicator titration of sodium carbonate for sodium hydrogen carbonate solutions, and the Mohr's method for sodium chloride solutions.

#### 3. Experiment 3

Experiment 3 examined the crystal grains and orientation of the ice stalagmites. The piece of ice stalagmites, the ones cut horizontally into three and the others vertically into two, were observed using polarizing plates and LED light.

### V Results

### 1. Result from Experiment 1

Ice stalagmites made from sodium chloride solution, of which the solute is an electrolyte and ionic radius of both hydrated anion and cation in it are small in size, grew the longest. Even if those are made from solutions in which the solute is an electrolyte, they didn't grow so long (an electrolyte, such as sodium hydrogen carbonate and potassium nitrate, the ionic radius of the hydrated anion in the solution is big in size and the hydrated cation is small in size). Also, those made from solutions with large ionic radius of hydrated molecules in them, such as urea solution, showed the shortest length (Fig. 2).

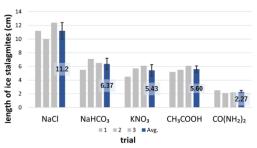
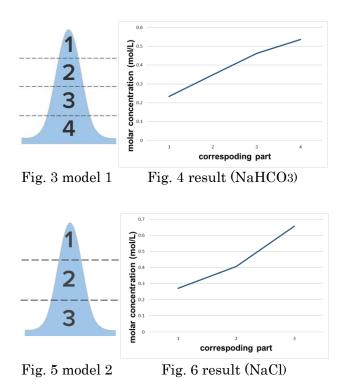


Fig. 2 the length in different solutions

#### 2. Result from Experiment 2

The pieces of the ice stalagmite are numbered like the models (Fig. 3 and 5). Fig. 4 and 6 indicates that the bottom part of the ice stalagmite has a higher concentration and the top part has the lowest concentration. Therefore, in both ice stalagmites made from electrolyte and non-electrolyte aqueous solutions, the bottom is denser than the top.



### 3. Result from Experiment 3

The pieces of the ice stalagmite are numbered like Fig. 7. According to Fig. 8, 9 and 10, it can be seen that the inner crystals of the ice stalagmite are fine-grained, and the outer ones are coarsegrained. This is a different result from the previous study that showed the top of the ice stalagmite to be a single crystal (Takahashi, Oishi, & Matsuhashi, 2012). Moreover, Fig. 11 shows that the inner crystals are aligned roughly vertically which is the direction of growth of the ice stalagmite.

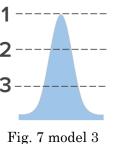




Fig. 8 crystal grains (1)









Fig. 11 crystal orientation

However, the ice stalagmite which we observed had been stored in the freezer for several days after it was formed, so there was a possibility that recrystallization had occurred. Therefore, we observed the crystals of the ice stalagmite just after making it.

According to Fig. 13, 14 and 15, they are composed of alternating stacks of fine-grained crystals and roughly vertically aligned ones, constituting a state of rhythmic layering.

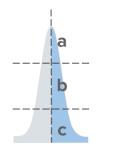




Fig. 12 model 4 Fig. 13 crystal grains (a)





Fig. 14 crystal grains (b) Fig. 15 crystal grains (c)

### VI Discussion

### 1. Discussion of experiment 1

It is considered that if the hydrated anions and cations in a solution are small, the ions can get into the gaps between the water molecules, and the solution form long ice stalagmites. In the case that hydrated ions in a solution are various sizes, ice stalagmites created with that don't grow so long. In contrast, in the case that hydrated molecules in a solution are large, it is difficult for crystals to pile up equally and the ones created with it grow only a little

### 2. Discussion of experiments 2 and 3

The process of ice stalagmites forming is considered as follows. The ice is formed as a base at first and then a hole is made by dripping solution at the top. Next, a bowlshaped part is formed in the center of the top, and the solution accumulates there and gradually freezes. When the solution overflows, it flows down the sides. In the process of flowing, water as a solvent gradually freezes first, and then the concentration of the solution, which is still liquid, increases as it flows and freezes, too. Because of this, the bottom part of the ice stalagmite becomes denser, and finally, the bowled shaped part is covered. Then a hole is made at the top again, and continues growing like that. This process occurs cyclic to form ice stalagmites (Fig. 16).

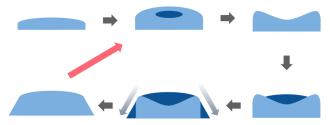


Fig. 16 the process of ice stalagmites formation

### VII Conclusion

① The size of the ion radius of hydrated ions and molecules in a solution affects the length of ice stalagmites.

② The process of ice stalagmites formation is cyclical.

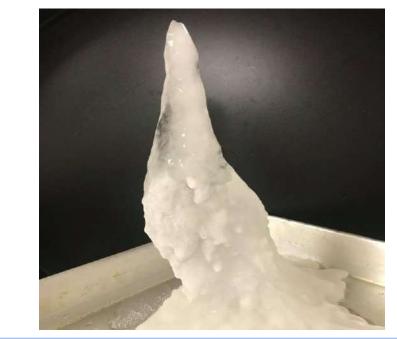
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# Factors Influencing the Growth of Ice Stalagmites

Okayama Prefectural Tsuyama High School Science and Mathematics Course Ayu Moriyasu Mei Terasaka Rikuto Tsuneto

# I. Introduction



In Tsuyama Junior High School, one of us, Ayu Moriyasu, could make it artificially.



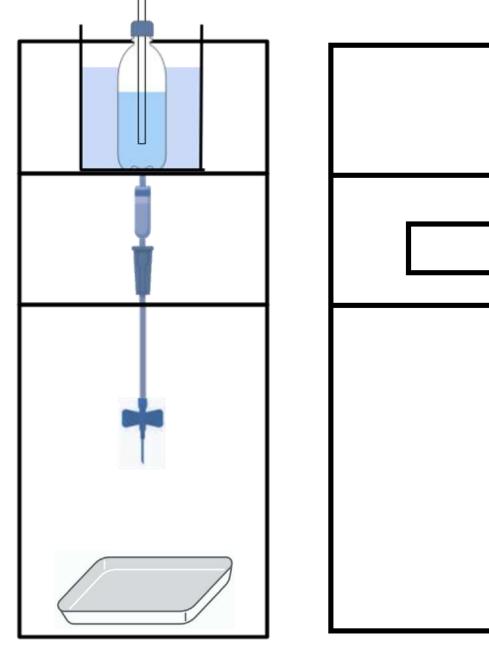
### II. Method

The experiment apparatus used IV tubing to maintain the drop rate. The duration of the experiments was 4 hours. The freezer was kept at -22°C.

### 

### [Experiment 3]

We examined <u>the crystal grains and their</u> <u>orientation</u>. [Experiment 3: Result]



(1) The solution was chilled in ice water. It was kept between  $4.5^{\circ}$ C and  $5.5^{\circ}$ C.

We <u>used a straw to keep the drop</u> rate constant.

(2) We made a hole to allow us to roll up or down the roller of the roller clamp.

Figure 1.

experimental apparatus

③ In the freezer, drops from the IV tubing dripped onto the tray.

**Solutions** All the solutions were unified at 0.4 mol/L.

3

- Sodium Chloride (NaCl)
- Sodium Hydrogen Carbonate (NaHCO<sub>3</sub>)



Figure 4. horizontal section



Figure 5. vertical section



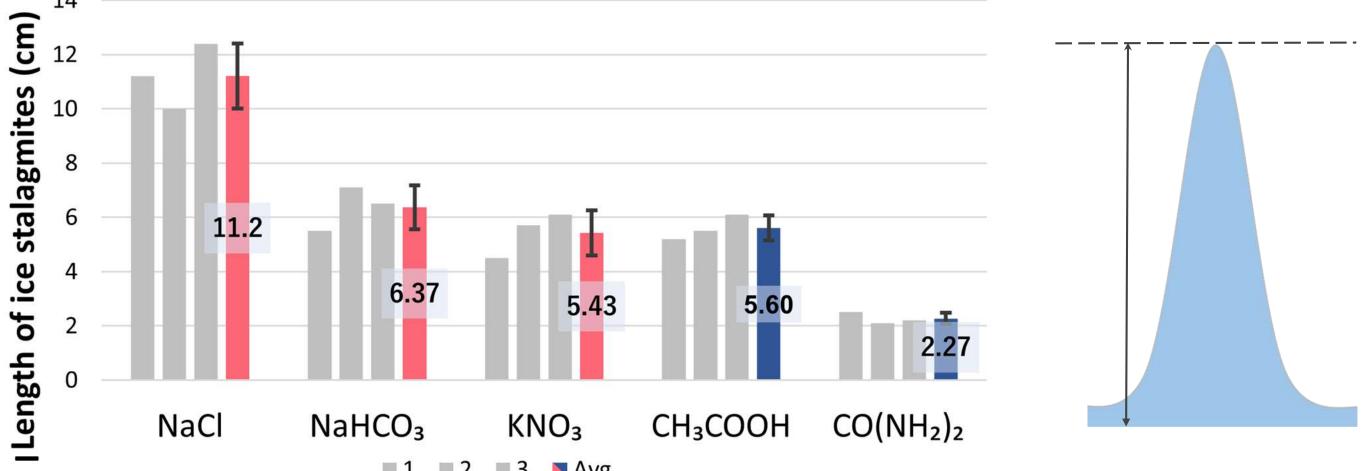




- Potassium Nitrate (KNO<sub>3</sub>)
- Acetic Acid (CH<sub>3</sub>COOH)
- Urea  $(CO(NH_2)_2)$

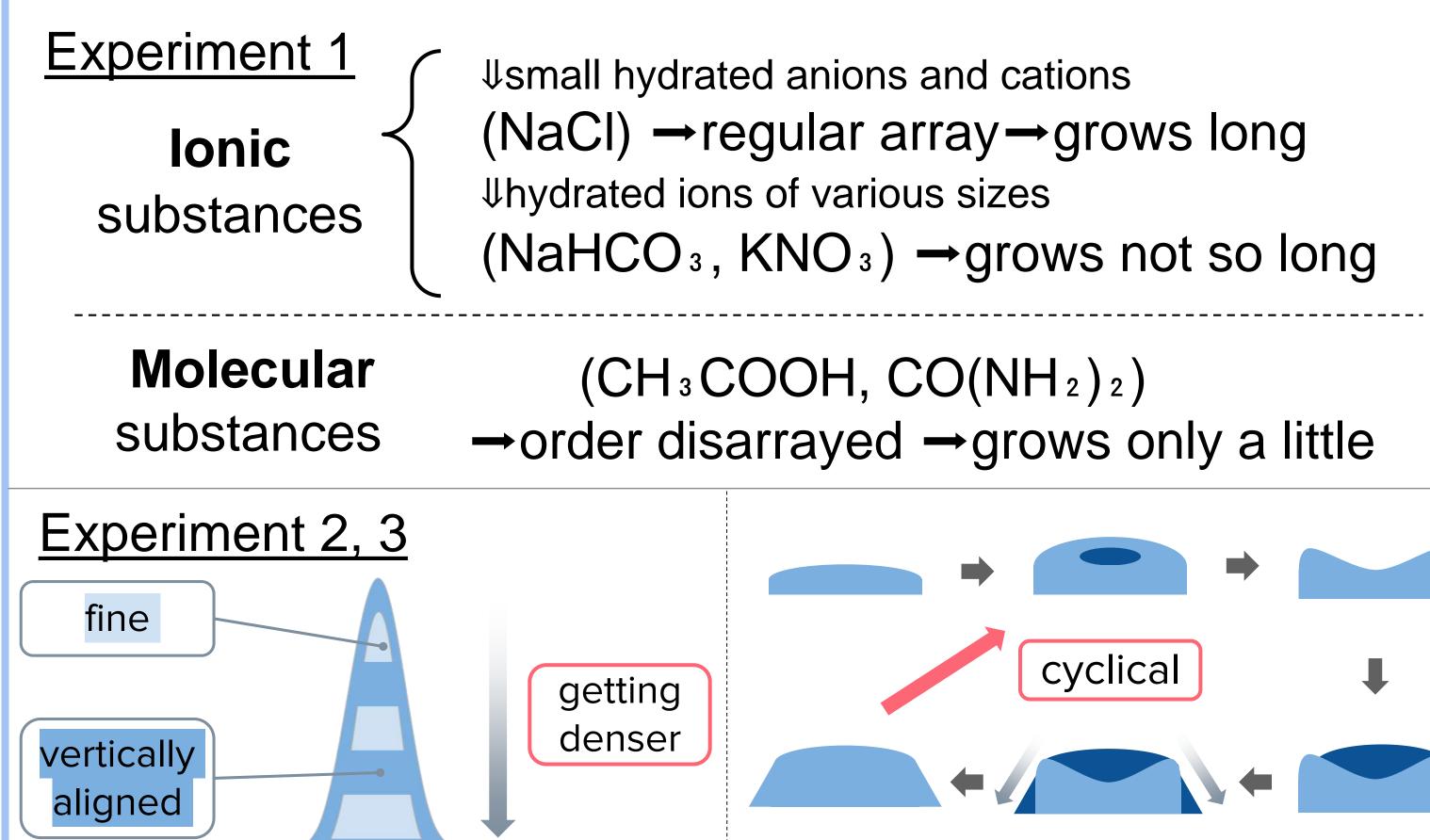
# III. Experiment

【Experiment 1】 We examined the differences in <u>length</u>.
[Experiment 1: Result]





### **IV**. Discussion



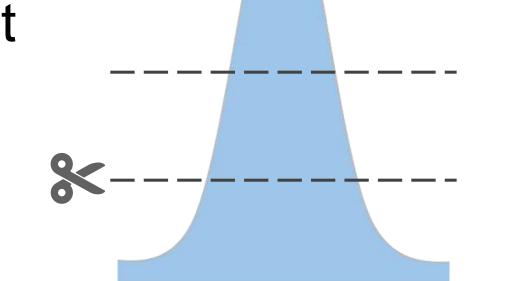
trial Figure 2. length in different solutions

### [Experiment 2]

We measured <u>the distribution of concentration</u> inside the ice stalagmites.

In the course of our experiments, we considered that the concentration might differ depending on the location.

Cut them horizontally



## V. Conclusion

• The size of the ion radius of hydrated ions and molecules in a solution affects the length.

The formation process of ice stalagmites is cyclical.

### **VI**. References

1)森安歩友.課題研究2021論文集,津山中学校,氷筍の成長と水溶液の 種類・pH・表面張力の関係,2021,17-19 2)高橋忠司・大石かおる・松橋美穂.埼玉学園大学・川口短期大学機関 リポジトリ,氷筍を使ったチンダル像の作成,2012.9,145-153

### Factors that Affect the Formation of Fruiting Bodies of Slime Mold, Physarum

China Ishio, Ayumu Otani, Mao Kamiya, Haruhi Takahashi, Kanon Nakamura Supervisor: Takashi Yamamoto

### Abstract

Physarum polycephalum is a unicellular, multinucleate, and eukaryotic slime mold. In this study, we focused on the process that a deformed body transforms into fruiting bodies and conducted experiments under conditions that can occur in the natural environment. From the results of our experiments, we considered that moisture content is most closely related to the formation of fruiting bodies and light such as sunlight and infrared light don't affect the formation. That means light conditions evaporate the moisture contained in the slime mold or agar and affect the formation of fruiting bodies indirectly.

### 1. 研究の概要

モジホコリ(以下、粘菌と呼ぶ)とは単細胞多核の真性粘菌である。粘菌の生活環における変形体から 子実体へと変化する条件について、先行研究を調べたところ、明確な条件は明らかにされていなかった。 そこで、本研究では変形体から子実体への変化の条件に着目し、自然環境で起こり得る変化を実験条件と して研究を行った。その結果、変形体に含まれる水分量が子実体の形成に最も関係し、先行研究で示され ている日光や赤外線などの光条件は粘菌が乾燥するための二次的な要因であることが示唆された。

### 2. Introduction

In Tsuyama High School, research on physarum polycephalum has been conducted, and the studies revealed many things about physarum polycephalum. However, our seniors had difficulty culturing the deformed body (fig.1). Actually, the research on the appropriate culture environment of physarum polycephalum has not progressed.

physarum polycephalum Recently. has attracted attention in many fields due to its unique characteristics. If we can bring physarum polycephalum to their life stages at the timing we them. the research need on physarum polycephalum will proceed more efficiently. So we began investigating the growth process and the factors.

The life cycle of physarum polycephalum (fig.3) is already known, but research on the factors that affect the transition for each stage has not progressed. Also, in the previous studies, experiments were conducted with a single condition, which means there are no experiments that combined more than two factors. In the natural environment, there is no case that only a single condition is related to the changes. So we thought we needed to conduct experiments under complex conditions. In this study, we especially focused on the fruiting bodies (fig.2), and studied the factors that affect transition from the deformed body to the fruiting bodies.



fig. 3 Life cycle

### 3. Experiments

In order to clarify the conditions under which slime mold transforms from a deformed body into a fruiting body, we conducted experiments under combined conditions that can occur in the natural environment.

① Experiment 1 humidity & feed

 $\langle Purpose \rangle$ 

Based on previous research by Public Hakodate Future University System Information Science Training(2011) showing that the condition of no feeding is related to fruiting body formation, an experiment will be conducted to see if fruit bodies form after adding the condition of dryness.

 $\langle Assumption \rangle$ 

We considered that the most likely transformation into fruiting bodies occurs in the case of drying and no feeding.

Because slime mold grow mainly in forests and require moisture for their growth, they store nutrients in the form of deformable bodies during the humid season. Therefore, the season of low humidity is a life-threatening season for slime mold, during which they may spend time in the preparatory stage of producing offspring called fruiting bodies.

 $\langle Methods \rangle$ 

Cut out 1.5cm x 1.5cm pieces of slime mold (on agar medium) in deformed body condition with and without feeding and place them in the center of a glass petri dish.

- Put the petri dishes in an auto-desiccator for 3 days. (fig.4)
- The humidity in the auto-desiccator is kept at 20% to 30%, and in the clean bench in the bioroom at 50% to 60%.
- · Each room is shaded from light.



fig. 4 in an auto-desiccator

 $\langle \text{Results} \rangle$ 

When it was not fed, 8 of the 15 individuals transformed into fruiting bodies. When it was fed, 2 of the 15 individuals transformed into fruiting bodies. It took 3 days to transform. Also, some individuals changed into dormant sclerotia.

### ② Experiment 2 light & feed

### $\langle Purpose \rangle$

Since experiment 1 was conducted entirely under conditions of darkness, we now considered the possibility that light is involved in the formation of fruiting bodies.

 $\langle Assumption \rangle$ 

We considered that slime mold prefer dark places, such as tree hollows and the shadows of fallen leaves, where moisture is high. However, fruiting bodies are packed with spores to produce offspring and need to spread spores over a wide area to avoid critical situations, so a deformed body would transform into fruiting bodies in open areas, such as fields in sunlight, rather than in closed areas such as tree cavities. Therefore we considered that the most likely transformation into fruiting bodies occurs in the case of light and no feeding.

 $\langle Method \rangle$ 

Cut out 1.5cm x 1.5cm pieces of slime mold (on agar medium) in deformed body condition with and without feeding and place them in the center of a glass petri dish.

- Expose the petri dish to white light or sunlight for three days.
- The amount of water fed to the physarum polycephalum and the temperature of the room were kept constant.
- $\cdot$  Each room was shaded from light.

 $\langle \text{Result} \rangle$ 

When it was exposed to direct sunlight, 9 of the 20 individuals transformed into fruiting bodies. It took 3 days to transform. However, it didn't transform when it was exposed to white light.

### ③ Experiment 3 light & feed

 $\langle Purpose \rangle$ 

From the result of experiment 2, we considered the possibility that physarum polycephalum transforms into fruiting bodies by detecting invisible light such as ultraviolet light and infrared light. Also, we found the previous study by Ota(1967) that showed blue light has a great effect on the formation of fruiting bodies. On the other hand, the previous study by Horiuchi(1997) showed light in the red family affects the formation. Considering these things. we conducted experiments that used visible/invisible light in the blue family (short wavelength light) and visible/invisible light in the red family (long wavelength light).

### $\langle Assumption \rangle$

From the result of experiment 2, which shows

that the deformed body doesn't transform into fruiting bodies when it is exposed to white light, we considered physarum polycephalum to be affected by the invisible light contained in sunlight and transform into fruiting bodies. So we made an assumption that the deformed body transforms into fruiting bodies when it is exposed to ultraviolet light.

### $\langle Method \rangle$

Cut out 1.5cm x 1.5cm pieces of slime mold (on agar medium) in deformed body condition with and without feeding.

- Place slime mold on the case which we prepared for this experiment.
- The empty part of the case is filled with agar medium in order to prevent the deformed body from moving from the area where the light isn't applied.
- Place the case in the equipment which irradiates light.
- The equipment is placed in a cardboard box to make sure that the experiment is not affected by light from outside.

 $\langle \text{Result} \rangle$ 

No matter which light was applied to the deformed body, it didn't transform into fruiting bodies.

### 4. Discussion

From experiment 1, we found that the deformed body is more likely to transform into fruiting bodies under dry, no-feed, and dark conditions. But from experiment 2, which resulted in transforming into fruiting bodies regardless of feed when it is exposed to sunlight, sunlight is considered to be the condition for transformation to fruiting bodies. And when comparing the difference between white light and sunlight, we considered that the invisible light, infrared and ultraviolet rays, are related to the change to fruiting bodies because white light does not contain invisible light but sunlight contains rays from short to long wavelengths, regardless of whether they are visible or invisible.

However, experiment 3 suggested that the wavelength of light had no effect on the formation of the fruiting bodies. Then, when the fruiting bodies formed in experiment 2, we focused on the water droplets inside of the petri dish, suggesting that changes in the amount of water in the medium or in the slime mold may have caused them to transform into fruiting bodies. In experiment 2, some individuals transformed into fruiting bodies while others transformed into dormant sclerotia. We consider that the difference between the conditions for transforming into fruiting bodies and dormant sclerotia is the difference in the rate of dehydration. We considered that a rapid due to exposure to dehydration light or other factors causes the formation of fruiting bodies, while on the other hand gradual dehydration causes the transition to the mycorrhizal state.

### 5. Conclusion

From experiment 1, the most transformation to fruiting bodies occurred under the conditions of drying and no feeding. From experiments 2 and 3, it was not possible to confirm whether light was involved in the transformation to fruiting bodies(fig.5).

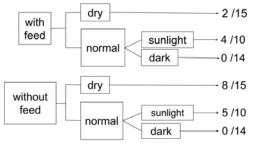


fig. 5 logic tree of these experiments and number of times a deformed body transformed into fruiting bodies

### 6. Outlook

The present study suggests that water content is related to the transformation from deformed bodies to fruiting bodies. However, since the results of the light experiment in experiment 3 were contrary to those of the previous study, it is necessary to conduct the experiment again by changing the illuminance, wavelength, and other parameters. In addition, the relationship between moisture content and temperature should also be investigated in the experiment, since the most significant change in moisture content occurred when the fruit was exposed to direct sunlight.

### 7. Special Thanks

We would like to thank Professors Masanori Kuwamori and Junichi Kuriwaki, professors of the Department of Nutrition at Mimasaka University Junior College, for their guidance in conducting this research.

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### Factors that Affect the Formation of Fruiting Bodies of Slime Mold, Physarum

Tsuyama High School: Haruhi Takahashi, China Ishio, Ayumu Otani, Mao Kamiya, Kanon Nakamura Supervisor : Takashi Yamamoto

### 1.Abstract

Physarum polycephalum is a unicellular, multinucleate, and eukaryotic slime mold. In this study, we focused on the process that a deformed body transforms into fruiting bodies and conducted experiments under conditions that can occur in the natural environment. From the results , we considered that amount of water has the greatest effect on the formation of fruiting bodies and light conditions is one of condition for drying.

### 2. Introduction

It is not clear what factors affect the formation of fruiting bodies. We conducted experiments identify the factors.



picture 1, 2: slime mold

### 3. Research Content

Experiment 1: humidity and feed $\langle Assumption \rangle$ no-feed and dry  $\rightarrow$  transform the most $\langle Method \rangle$ 

 $\times 2$ 

①dry/feed ②dry/no-feed ③wet/feed ④wet/no-feed /



in an auto-desicctor

picture 3.

<Result>

### chart1: result of experiment1

	dry	wet
feed	2 / 15	0 / 14
no-feed	8 / 15	0 / 14

became fruiting bodies a total of 10 / 58 times. Experiment <u>2</u>: sunlight

 $\label{eq:assumption} $$ (Assumption) sunlight and no-feed $$ \rightarrow transform the most $$ (Method) put petri dishes in direct sunlight $$$ 

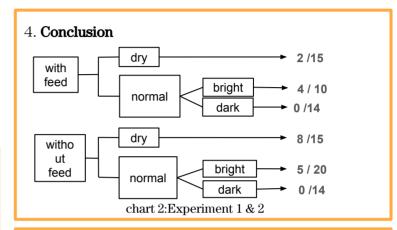
(Results) Deformed body transforms into fruiting bodies. It took 3days to transform.



picture 4: Experiment 2

became fruiting bodies a total of **9 / 40 times** <u>Experiment 3</u>: light and feed (blue light • UV • red light • infrared light)

Assumption:  $UV \rightarrow transform$  the most Method: put petri dishes in these types of lights Results: Everything exposed to any type of light did not transform into fruiting bodies.



### 5. Discussion

Experiment 3 suggested that light had no effect on the formation of the fruiting bodies. Then, when the fruiting bodies formed in Experiment 2, water droplets were observed on the inside of the petri dish, suggesting that changes in the amount of water in the medium or in the slime mold may have caused them to turn into fruiting bodies.



picture 5:experiment 2

picture 6: life cycle

### Special Thanks

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### Characterization of yeasts isolated from flowers in Tsuyama High School

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### Abstract

We tried to collect yeast from flower petals and hypocotyls from the grounds of Tsuyama High School. And we were able to collect about 25 strains of yeasts. We observed the characteristics of each yeast such as size, colony, division rate of yeast and so on. We also tested if the yeast has an alcohol fermentation capacity, and then we found yeast isolated from scilla perviana has weak one.

#### 1. 研究の概要

津山高校の敷地内に生息する花の花弁や胚珠から酵母の採取を試み,およそ 25 株の酵母の採取に成功した。 そして採取した酵母の大きさやコロニーの特徴,分裂速度等の酵母の特性を観察した。また,アルコール発酵能 の有無を検証し、オオツルボから弱い発酵能力を持つ株を発見することができた。

### 2. Objective

We were interested in breads or alcohol made with flower yeasts and we wanted to make original food of Tsuyama high school.

So we started this research. As we continued our research, we found that flower yeasts are surrounded by mystery and it was more difficult to find flower yeasts than we had imagined, especially finding fermentative yeast.

However, we also found that yeasts have diversity, their characters and abilities are different for each colony, and we were interested in it. So, we decided to characterize 4 kinds of yeasts that we collected from Tsuyama High School.

### 3. Hypothesis

We thought there are many yeasts from flowers that can be used in foods like fermenting nectar.

### 4. Method

We attempted to isolate yeast from flowers as follows.

- ① We collecte plant flowers and broke them down into petals, stamens, pistils, and sepals.
- ② We soaked them in a 5% glucose solution and allow them to stand for about one day.
- ③ We applied to an agar medium containing chloramphenicol to eliminate bacterial colonies.
- ④ We incubated those agar mediums at 37 degrees for several days.
- (5) Through observation of collected colonies using a microscope, we incubated likely yeast of them by judging from their shape and structure, using agar medium or liquid culture medium.

### 5. Experiments

We conducted experiments to know and categorize the characteristics of yeasts.

We experimented dry yeast in the same way as the other yeasts for comparison.

- (1) We observed the each colony of flower yeast with the naked eye, and that colony were observed under a microscope.
- 2 We measured cell size of fungal colonies that appear to be yeast using a micrometer.
- ③ We observed the increase in each yeast population through a cell counter.
- ④ We tested if each yeast has fermentation capacity through a Kühne's fermentation tube and Durham tube, and observed the fermentation liquid.

### 6. Result

We were able to collect 25 kinds of what can be considered yeasts. Among them, we focused our observations and measurements on yeasts obtained from scilla peruviana, banana, morning glorie, and pomegranate. In banana, pomegranate and scilla peruviana, we observed they were circular, low convex, and whitish colony masses. In morning glory, we observed they were filamentous and dark green. (fig.1)



banana



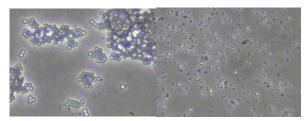


scilla peruviana morning glory fig. 1 view of each yeast colony

Each cell image obtained by observing the colony under a microscope is shown below. (fig.2)

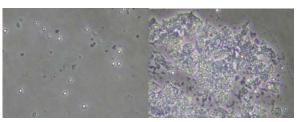
In scilla peruviana and pomegranate, most of the yeasts we collected ranged in size from about 2.5 to 5.0 micrometers. In yeasts collected from banana and morning glory, their ranges were about 5.0 micrometers.

Microscope examination of yeasts show that scilla peruviana and pomegranate yeasts were cohesive.



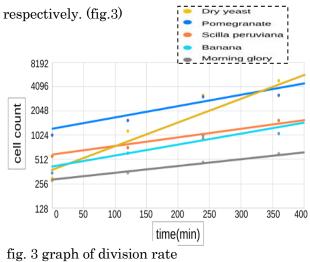
scilla peruviana

banana



morning glory pomegranate fig. 2 photographs of yeast

The division rates for dry yeast, banana, pomegranate, scilla perviana, and morning glory were 100, 205, 210, 290, and 355 minutes,



(vertical axis : cell count / horizontal axis : time[m]) We found that yeast collected from scilla peruviana have the fermentation capacity through Kühne's fermentation tube test and Durham tube test. Its fermentation speed is not as fast as dry yeast. In some additional experiments after that, there were times when no fermentation ability was observed.

(fig. 4-1, 4-2)



fig. 4-1 scilla peruviana yeast showing fermentation capacity in a Kühne's fermentation tube test



fig. 4-2 scilla peruviana yeast showing fermentation capacity in a Durham tube test (left : scilla peruviana, right : dry yeast)

The results showed that the banana yeast culture turned yellow. It did not have a smell.

Furthermore, the liquid surface of the banana culture had a film on it. (fig. 5)

We confirmed that yeasts obtained from scilla perviana and pomegranate have flocculation property.



scilaperuviana

banana





Morning glory

pomegranate

fig. 5 experiments in a Kühne's fermentation tube

### 7. Consideration

The organisms collected in this study had a unique size, shape, and structure. These three characteristics fit the definition of yeast.

So we considered them to be yeasts.

Especially, banana yeast is considered membrane yeast because it formed a membrane.

The division rates we collected are about twice in banana and pomegranate, about 3.5 times in morning glory, as slow as dry yeast.

We attempted to collect yeast from about 90 different flowers, but we could only identify what we thought was yeast from about 25 colonies. So we found that it is difficult to collect yeast from nature.

Also very few flower yeasts have fermentation capacity, and we found that flower yeasts that can be used for food products are very rare. Fermentative yeast hiding in flowers may have a small population because it does not offer many advantages to flowers.

### 8. Future Prospects

In the future, we would like to find more yeasts with fermentation ability in flowers.

Also, fermentation ability of the yeast collected from the scilla peruviana varied greatly depending on the conditions and the time of the experiment. According to previous research, the fermentation ability of yeast changes depending on the stage in the yeast's life cycle. We would like to investigate this as well.

In the cultures of the morning glory and banana, we observed a small amount of bubbles, but we couldn't have concluded that the bubbles derived from yeast fermantation yet. We would like to find out whether the generation of bubbles is due to fermentation or whether there are other causes.

### 9. Acknowledgement

We want to express our thanks to Prof. Hatano、Prof. Kuwamori、Prof. Kuriwaki. We appreciate your advice. Thank you very much.

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### Characterization of yeasts isolated from flowers in Tsuyama High School

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### Purpose

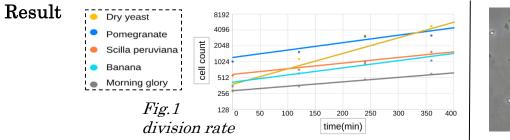
We were interested in breads or alcohol made with flower yeasts. We also found that yeasts have diversity, their characters and abilities are different for each colony, and we were interested in it. In this study, we conducted some experiments using yeast we collected. And also we characterized them by comparing their result and search for usefulness.

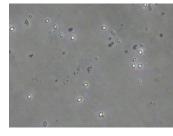
### Method ①

- 1. Collected plant flowers and broke them down into petals, stamens, pistils, and sepals.
- 2. Soaked them in a 5% glucose solution and allowed them to stand for about one day.
- 3. Applied that solution to an agar medium containing chloramphenicol.
- 4. Incubated that agar medium at 37 degrees for several days. Observed the formed colonies under a microscope.

### Method 2

- 1. Tested if yeasts have fermentation capacity through Kühne's fermentation tube test and Durham tube test.
- 2. Observed each colony of flower yeast that we had collected.
- 3. Measured the division rate using a cell counting plate and the cell size of fungal colonies that appear to be yeast.





*Fig.2 yeast of morning glory* 

Tig.s major axis, colony, and termentation potential of various yeasts						
Compare Items						
✓ yeast	banana	scilla peruviana	morning glory	pomegranate	dry yeast	
major axis (µm)	5	2.5~5	5	2.5~5	5~7.5	
	circular,	circular,		circular,		
	low convex,	low convex,	filamentous and	low convex,	—	
colony	and whitish	and whitish	dark green	and whitish		
fermentation or not	×	Δ	×	×	0	

Fig.3 major axis, colony, and fermentation potential of various yeasts

The number of plants : succeed to collect yeast / all attempts = 25/90

### Consideration

- The organisms had a unique size, shape, and structure.  $\Rightarrow$  We considered them to be yeast.
- Very few flower yeasts have fermentation capacity, and we found that flower yeasts that can be used for food products are very rare.  $\Rightarrow$  Do they not offer many advantages to flowers?

### **Future Prospects**

We found that yeast of scilla peruviana had the fermentation capacity through the tests in the Kühne's fermentation tube and the Durham tube, and then it varied greatly depending on the conditions and the time of the experiment. We would like to investigate this as well.

Also, we would like to isolate yeasts with fermentation capacity.