Observation of Euglena's Chemotaxis

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Abstract

Our two goals are to reveal whether *Euglena gracilis* has chemotaxis and to examine how strongly they react with various materials. We conducted an experiment using agar. As a result, we found that *E.gracilis* showed a reaction to glucose and ethanol. In order to get a more accurate result, we performed another experiment using a micro channel which is made of polydimethylsiloxane (PDMS), and observed the response with a microscope. *E.gracilis* shows a positive chemotaxis in response to carbon dioxide, and showed a negative chemotaxis in response to ammonia and 1.0mol/l ethanol.

1. Introduction

Euglena gracilis is known for its phototaxis, and scientists have studied them extensively. However, there are few papers that reveal the details of chemotaxis. Therefore, we decided to examine how strongly *E.gracilis* react with other chemicals.

Phototaxis means movement in response to light while chemotaxis means movement in response to chemicals. There are two kinds of chemotaxis.One is a positive taxis and the other is a negative taxis. When organisms approach chemicals, it is called a positive chemotaxis, and when organisms flee from chemicals, it is called a negative chemotaxis.

In our experiments, we used *E.gracilis* which we had bought two years before from *Shimadzu Rika Corporation.* We found that *E.gracilis* gathered around the rice as organic matter when we put rice into a plastic bottle including *E.gracilis*, so we hypothesized that *E.gracilis* has chemotaxis. We started our experiments using polysaccharide and amino acid because for human beings, they are necessary to live.

We hypothesized that *E.gracilis* shows a positive taxis to carbon dioxide, glucose and monosodium glutamate. *E.gracilis* has chloroplasts and we thought that it requires carbon dioxide to photosynthesize. Moreover, glucose is needed an energy source as respiratory substrate, and monosodium glutamate is needed as for protein synthesis. That was why we thought *E.gracilis* would show positive reaction to these substances. In contrast, we thought *E.gracilis* would show a negative reaction to harmful substances. In addition, we chose high concentration ethanol to compare the results of our experiments with that of previous research.

2. Methods

2.1. Experiment 1

We made agar which included one of the target chemicals, put it in the middle of a petri dish and poured *E.gracilis* around it. An hour later, we observed how *E.gracilis* reacted with a concentration gradient of the chemical. We used two methods to do experiments.
One was time-lapse photography and the other was measuring the density of *E.gracilis* by spectrometer. We set two plastic partitions into the petri dish (Fig.1) just before we measured the density. We conducted experiments with glucose, ethanol, glutamate, and sodium hydrogen carbonate. We conducted these experiments under red light in a dark room in order to prevent *E.gracilis* from showing phototaxis.

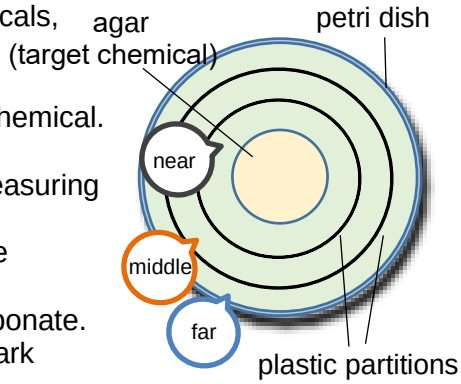

Fig.1 Apparatus of experiments 1

2.2 Experiment 2

We set two agar (one with a chemical and one without) on the petri dish. One hour later, we set a ladder-like plastic partition into the petri dish, and we measured the density of *E.gracilis* in each part. We can prevent *E.gracilis'* tendency to move to the edge of petri dish by using this apparatus. In this experiment, we examined the chemotaxis toward sodium hydrogen carbonate, ethanol, and glucose.

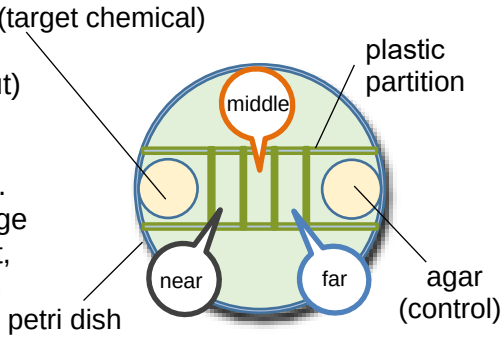

Fig.2 Apparatus of experiment 2

2.3 Experiment 3

We used a micro channel as a new device in our experiments to observe the chemotaxis more clearly. It is made of polydimethylsiloxane (PDMS). PDMS is a kind of silicone which small molecules can go through because it is porous. The microchannels are clear, so we can observe the movement of *E.gracilis* directly. We passed chemicals through the thin tube, and made a round space called micro aquarium where they could move freely (Fig.3). Chemicals were injected into one channel, and pure water or air was put into the other channel for comparison. Then, we tried to observe the chemotaxis of the *E.gracilis* by putting them into the micro aquarium. In addition, we collected the sample *E.gracilis* from a culture solution following the steps below.

Step 1. Centrifuge culture solution using centrifugal machine (the gravitational acceleration is 50G)
Step 2. Discard the supernatant and add pure water to replace the culture solution.
Step 3. Centrifuge again.
Step 4. Collect the *E.gracilis* that have precipitated.
Step 5. Using a micropipette, pour an appropriate amount of this sample into the micro aquarium shown in Fig.3.
Step 6. Place the slide glass on the micro channel in the state of step 5.
Step 7. Cover the light source of the microscope with a red film and observe with red light.

We used pure water (step 2) so that the *E.gracilis* were not influenced by the substances included in culture solution. We observed *E.gracilis* under the red light (step 6) in order to prevent it from showing phototaxis.

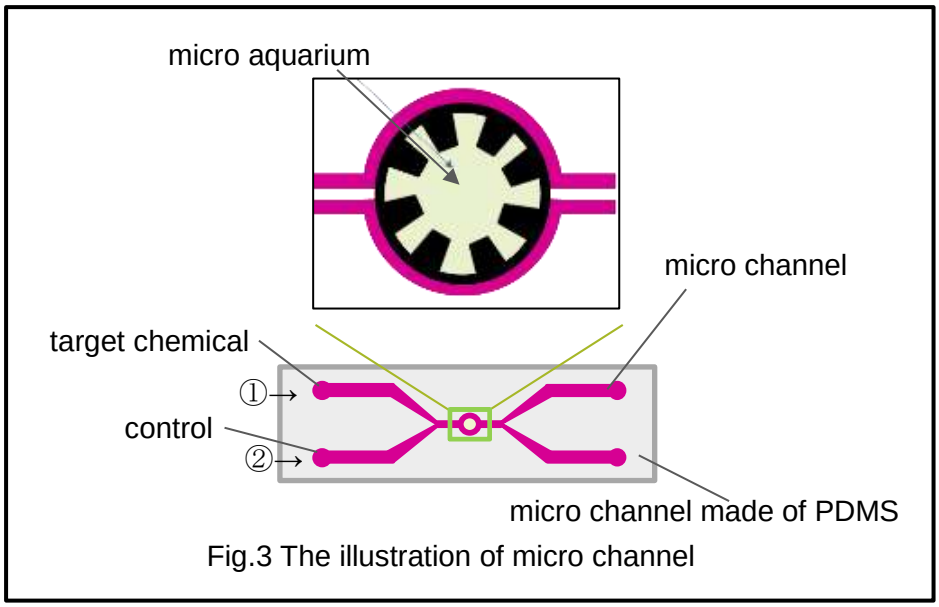

Fig.3 The illustration of micro channel

3. Results

3.1. Experiment 1

As a result of taking time-lapse photography, we were able to observe that *E.gracilis* gathered around the agar once, and then separated from it afterward. The density showed that *E.gracilis* tended to go to the edges of petri dishes.

3.2. Experiment 2

Fig.4 shows the result of experiment 2. In experiment 2, unlike the former experiment, *E.gracilis* didn't show any reaction to the control agar, so at the time we found that this experiment was reliable. In this experiment, *E.gracilis* tended to approach the agar which contained 1.0mol/l ethanol, and to move away from 2.0mol/l ethanol, 0.1mol/l glucose.

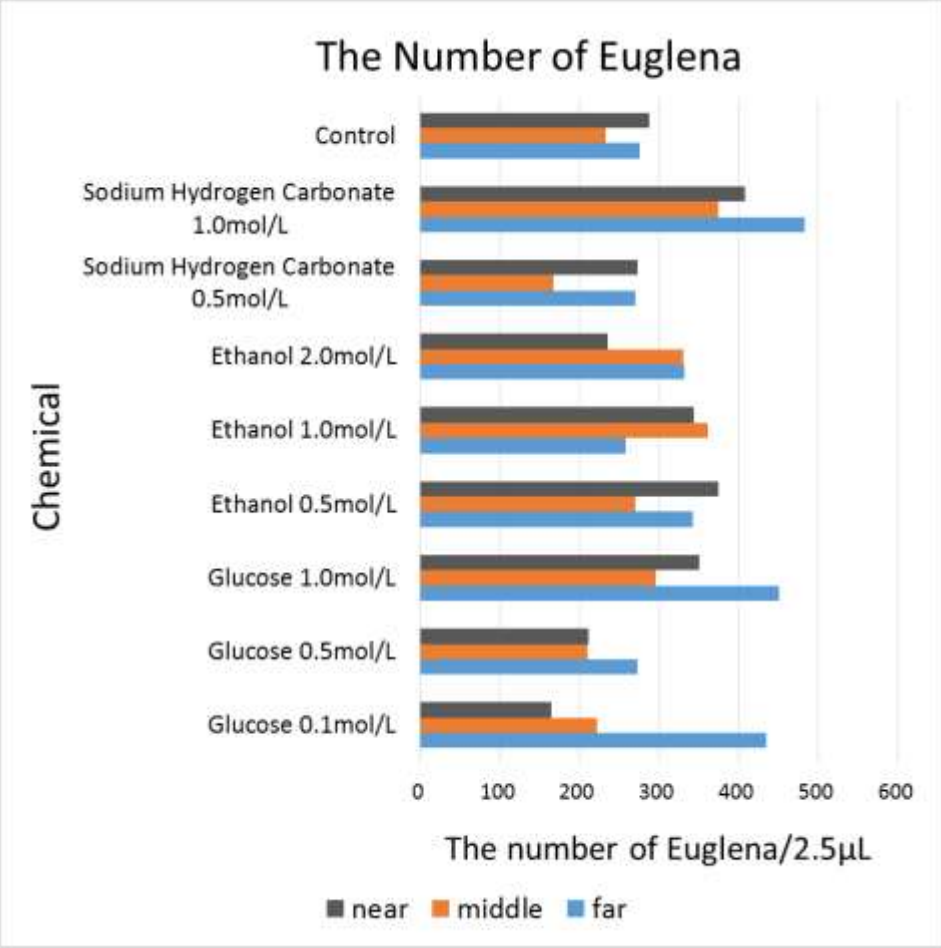

Fig.4 The results of experiment 2

3.3. Experiment 3

The pictures below show the actual experimental results. The round part in these images is a micro aquarium, and the black particles are *E.gracilis*. The target chemical is coming out from the lower channel, and the pure water or air is coming out from the upper channel for comparison.

E.gracilis showed negative chemotaxis for 0.1mol/l ethanol, which is consistent with the previous research. No changes were seen using 0.1mol/l sodium glutamate. They approached carbon dioxide. *E.gracilis* showed weak negative chemotaxis to 50% ammonia and strong

negative chemotaxis to 100% ammonia. In addition, almost all *E.gracilis* after the ammonia experiment were dead.

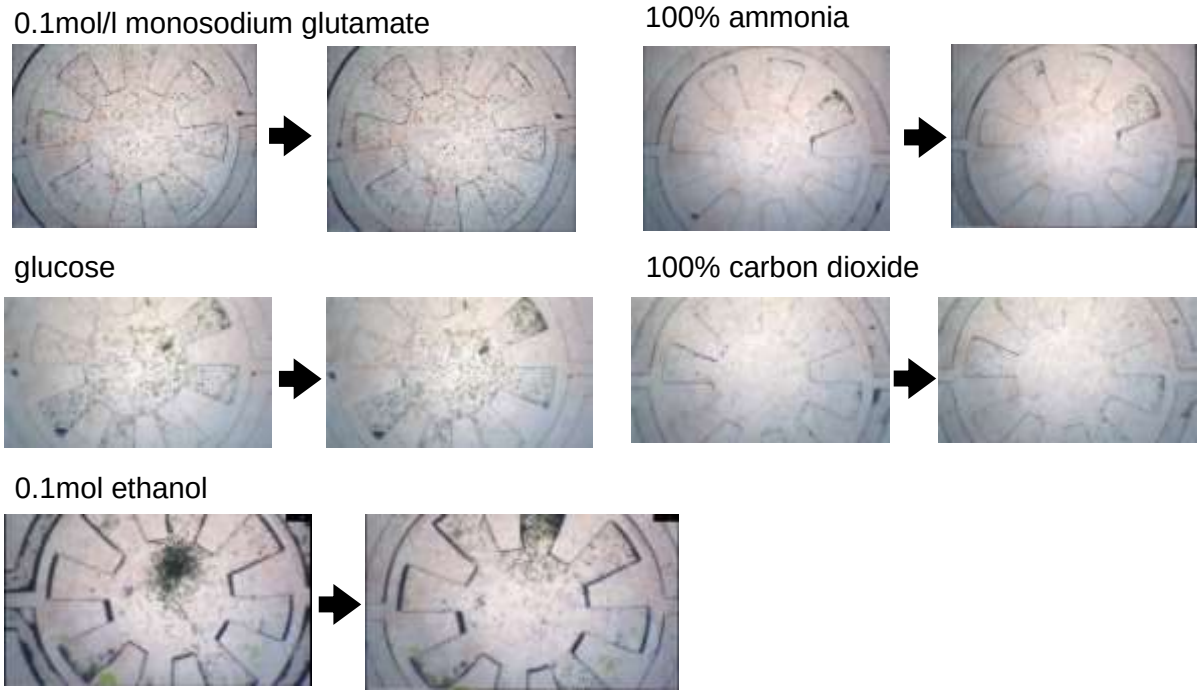

Fig.5 The photographs of experiment 3(target chemicals flow into the lower micro channel)

3.4. Comprehensive results

E.gracilis showed negative chemotaxis to high concentration carbon dioxide and high concentration ammonia and did not show chemotaxis in response to monosodium glutamate. In the experiment using monosodium glutamate, *E.gracilis* moved at random.

4. Discussion

4.1. Experiment 1

We considered this behavior a result of equalization in concentration. We observed that *E.gracilis* tended to go to the edges of petri dishes. At first glance, it looked like negative taxis. However, this was not reliable. They showed this behavior because *E.gracilis* are influenced by the minute water current and they can only go straight. The reproducibility of this experiment was not sufficient, so we decided that it is inconclusive.

4.2. Experiment 2

This result did not correspond to the previous research, which was conducted by Mr. Ozasa and his team at Institute of Physical and Chemical Research. Moreover, the relationship between one chemical and its concentration was not what we had expected. The overall results of central parts is seen to be low (Fig.4). we could not explain why the number of *E.gracilis* that reside in the middle part is small. Therefore we could not detect chemotaxis in this experiment. In this experiment with agar, it was difficult to take the difference of density and dissolving speed of each chemicals into account. Moreover, it is difficult to make the agar which contains ethanol because it is volatile. Thus we conclude that this experiment was not reliable.

4.3. Experiment 3

It was confirmed by Mr.Ozasa and his team at Institute of Physical and Chemical Research that *E.gracilis* shows negative reaction to carbon dioxide at concentration above 15%. However, in our research, *E.gracilis* showed a positive chemotaxis to carbon dioxide. We thought it was because the amount of carbon dioxide was too little to make concentration gradient. We also conducted an experiment with glucose, but the result was inconclusive because we could not reproduce the result. As for ammonia, we can hypothesize that at a certain concentration, *E.gracilis* will not show any chemotaxis to ammonia.

4.4. Comprehensive discussion

E.gracilis has three layers of chloroplasts. The previous research about the evolution of *E.gracilis* by Shinichiro Maruyama states that *E.gracilis* was originally a heterotrophic monad, but some unicellular green algae went inside it and they lived together. Heterotrophic means that an organism is dependent on nutrition. Because of this heterotrophy, we expected that *E.gracilis* would show positive chemotaxis in response to glucose and sodium glutamate because they are respiratory substrates, and repeated the experiment many times. Finally, we ended up using a micro channel (PDMS), which is very difficult to use. We tried to make *E.gracilis* heterotrophic by depriving it of fluid fertilizer or rice. However, it was difficult keep them healthy. On top of that, in the third experiment, there is a possibility that we failed in making concentration gradient because the molecular weight of glucose and sodium glutamate is too high to dissolve.

There are differences between the agar device and micro channel. The amount of chemical is less than that of *E.gracilis* in the agar experiments. We could not make the concentration gradient on the micro aquarium when we conducted experiments about glucose's chemotaxis or sodium glutamate. Our *E.gracilis* was raised in a culture which contained a little rice and liquid fertilizer. We think that due to this, *E.gracilis* did not show positive chemotaxis because it did not need more nutrition.

5. Conclusion

E.gracilis showed a negative chemotaxis in response to high concentrations of ethanol and low concentrations of glucose. A positive taxis was observed in response to carbon dioxide. Nothing happened in reaction to sodium glutamate. The results of the experiment with ethanol corresponded to the previous research, but that of carbon dioxide did not. We estimate that it is because we could not flow gas constantly throughout the experiment. As for glucose and sodium glutamate, the results are contrary to our hypothesis. We think it was because their molecular weight are relatively large.

We had a lot of difficulties in our experiments. First of all, we had to establish the culture method to keep *E.gracilis* healthy and pure as well as heterotrophic. On top of that, we had to find how to quantify the number of *E.gracilis* using a micro channel. We are going to plan to count the number of *E.gracilis* by dividing the recording into smaller sections. During the experiments using a micro channel, we need to keep the target chemicals flowing in the tube. We also need to exchange the target chemicals to control one and learn whether the results are reliable after the experiment. Moreover, we must conduct more experiments to gain the enough data as well as consider the influence of pH and osmotic pressure.

Acknowledgements

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7. Key word

Euglena, chemotaxis, micro channel, PDMS