2020 年度
SSH 生徒課題研究集録
(抜粋)

2021 年 3 月

立命館高等学校
スーパーサイエンスコース
SSG クラス

課題研究テーマ一覧

研究番号	20SS043	研究について	□個人　■グループ
研究テーマ	The benefits of three types of herbs　～3 種類のハーブの成分による効能～		

A：研究目的（Purpose）

ハーブによる人への利益は漢方薬というように知られているが、植物への利益は知られていないため、私たちはハーブ成分による植物への影響に焦点をおいた。

B：研究方法（Materials & Methods）

0. 8 種類の溶液(スイートバジル、ローズマリー、ディル、ホワイトクローバーの 4 種類を水またはエタノールを用いて成分を抽出した。それぞれ、無機溶媒と有機溶媒とした)を作成した。
1. 植物の発芽前の種と発芽後の植物をそれぞれのシャーレに準備し、10 種類の溶液を加えた。
2. 発芽後の植物に一度水を抜き、萎れさせた後、3 種類のハーブ有機溶媒溶液と水をそれぞれ加えた。
3. 作ったハーブ溶液を使用し、大腸菌の増殖を抑制できるかを調べた。

C：研究結果（Result）

1. ハーブ溶液は植物の発芽を抑制し、成長においては促進した（図 1）。
2. ハーブ溶液は植物の復活力を強く持った。
3. 4 種類のハーブには増殖抑制効果が見られた。特にディル溶液の効果が大きかった。

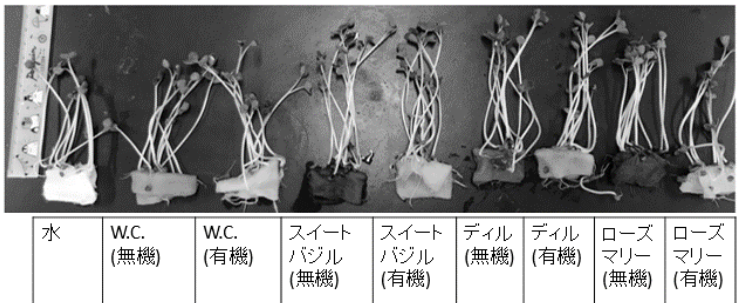


図 1. 溶液のよる 3 日間でのかいわれ大根の成長の結果

D：考察（Discussion）

ハーブは、植物の発芽において成長ホルモンを抑制する作用があり、植物の成長においては肥料や栄養分としての効果があると考えられる。

E：今後の展望（Future Direction of Research）

今までは全ての実験をシャーレ(コットン)を用いて行っていたが、今後は土で使用し、できるだけ自然に近い条件下で実験を行いたい。

F：参考文献（References）

Borrás-Linares, Isabel, et al. “Rosmarinus Officinalis Leaves as a Natural Source of Bioactive Compounds.” *International Journal of Molecular Sciences*, MDPI, 10 Nov. 2014, www.ncbi.nlm.nih.gov/pmc/articles/PMC4264185/.

1. 序論

ハーブは漢方薬など芳香を持つ薬として使われている。ヒトへの効果は実証されているが、植物への効果はまだ知られていないため、ハーブを用いることにより、植物に利益を持つ薬を開発することを目的に研究を行った。現在植物を育てるために多くの農薬が使用され、それらが人体や環境に害を与えている。しかしこの研究が成功すれば、自然由来の農薬に置き換えることができ、人体や環境に害を与えずに植物を育てることができると考えた。本研究ではスイートバジル、ローズマリー、ディルの 3 種類のハーブを使用し、植物への成長促進作用、発芽抑制作用、復活度、除菌作用に焦点を当て様々な実験を行った。

2. 方法

実験 (準備作業)

実験を行う前に 3 種類のハーブと、ホワイトクローバーから、それぞれ水溶性抽出物と脂溶性抽出物に分けて、合計 8 種類の溶液を作成した。

材料

電子測り機、ビーカー、スイートバジル、ローズマリー、ディル、ホワイトクローバー、イオン交換水、薬さじ、乳棒、乳鉢、漏斗、キムワイプ、エタノール、ガラスシャーレ、ミクロスパーテル、コニカルチューブ、ホールピペット

方法

水溶性化合物の抽出

(1) 1 g 測り、熱湯の入ったビーカーにて 1 日ふやかした。
(2) ふやけた葉っぱを取り出し、水を適量加えて入れて乳鉢ですりつぶした。
(3) (2)でできた溶液を濾過して溶液を得た（以下、無機溶媒液と呼ぶ)。

脂溶性化合物の抽出

(1) 1 g 測り、熱湯の入ったビーカーにて一日ふやかした。
(2) ふやけた葉っぱを取り出し、エタノールを適量加えて乳鉢ですりつぶした。
(3) (2)でできた溶液を濾過し、できた溶液をガラスシャーレに入れ、一日乾燥させた。
(4) 乾燥後、ガラスシャーレについた表面の粉をミクロスパーテルで削って集めた。
(5) (4)でのガラスシャーレに再びエタノールを加えて、ミクロスパーテルで混ぜた後、その溶液を 2 ml チューブで保存し、蓋を開けて乾燥させた。
(6) (4)または(5)でできた粉 100 mg あたり 1 ml の濃度になるように水で希釈した。（以下、有機溶媒溶液と呼ぶ)。

表 1 本実験で用いる 10 種類の溶液

無機溶媒	スイートバジル	ローズマリー	ディル	ホワイトクローバー	水
有機溶媒	スイートバジル	ローズマリー	ディル	ホワイトクローバー	エタノール

実験 1-1

植物の発芽率について、10 種類の溶液を使用し、ハーブ液と他の溶液の場合を比較した。

材料

10 種類の溶液、シャーレ、コットンシート、かいわれ大根の種、ホワイトクローバーの種

方法

(1) かいわれ大根の種を 1 シャーレにつき 10 粒ずつコットンの上にのせたものを 10 セット(10 シャーレ)分用意した。
(2) 10 種類の溶液でそれぞれ 1 種類ずつ浸した。
(3) これら 10 種類を 6 日間毎日観察した。
※同じ手順でホワイトクローバーの種でも行った。

表 2　10 種類の溶液の配置表 (実験 1、2 同様)

(無機溶媒)	ホワイトクローバー	ディル	スイートバジル	ローズマリー	水
(有機溶媒)	ホワイトクローバー	ディル	スイートバジル	ローズマリー	エタノール

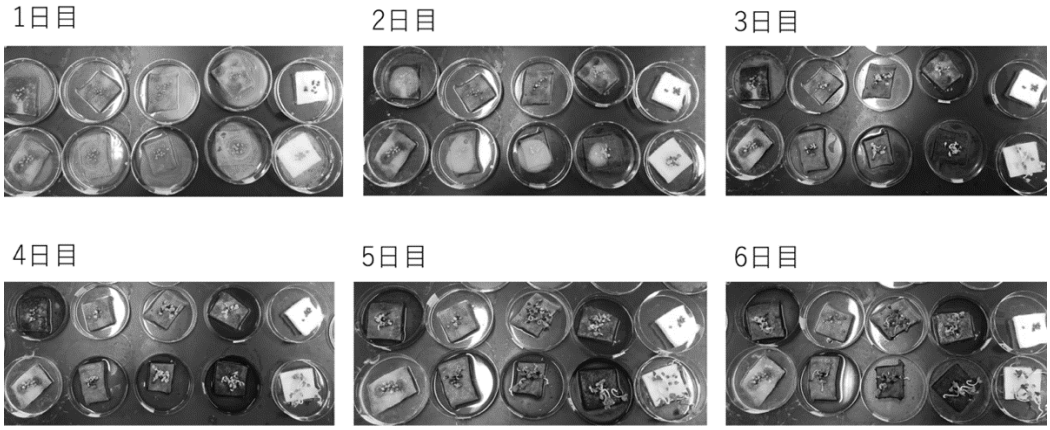


図 1　6 日間観察したかいわれ大根の発芽の様子

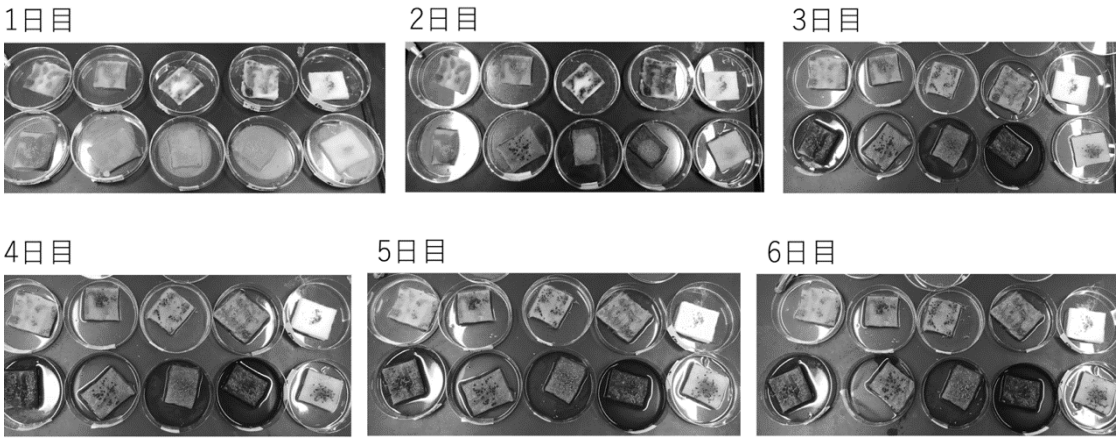


図 2　6 日間観察したホワイトクローバーの発芽の様子

実験 1-2

植物の発芽後の成長率について、10 種類の溶液を使用し、ハーブ液と他の溶液の場合を比較した。

材料

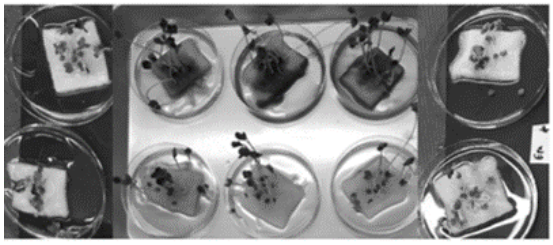
10 種類の溶液、シャーレ、コットンシート、種(かいわれ大根とホワイトクローバー)

方法

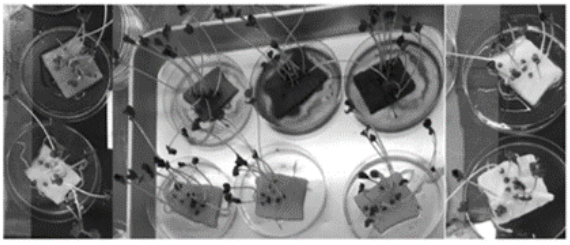
(1) かいわれ大根の種を 1 シャーレにつき 10 粒ずつコットンの上にのせたものを 10 セット(10 シャーレ)分用意し、10 セット全てを水で発芽するまで育てる。
(2) 発芽後、10 種類の溶液でそれぞれ 1 種類ずつ浸す。
(3) これら 10 種類を発芽してから 3 日間毎日観察を行った。

※同じ手順でホワイトクローバーの種でも行った。

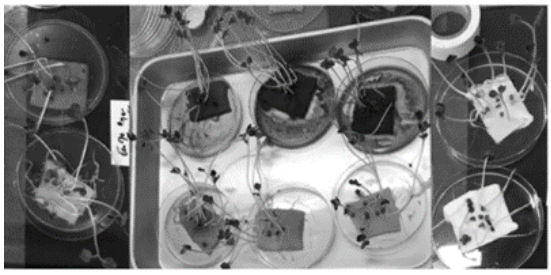
1日目



2日目



3日目

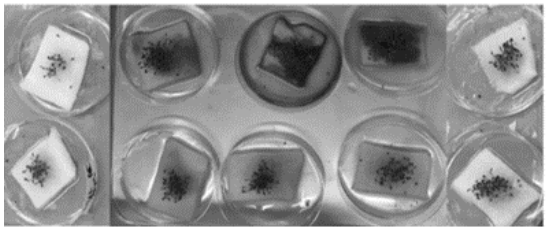


10種類の溶液の配置表

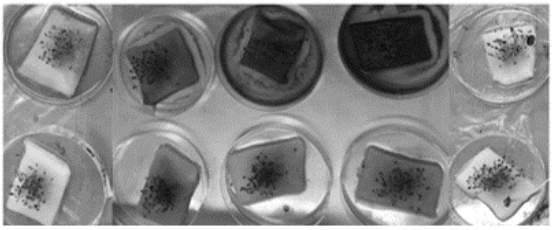
無機溶媒	ホワイトクローバー	ディル	スイートバジル	ローズマリー	水
有機溶媒	ホワイトクローバー	ディル	スイートバジル	ローズマリー	エタノール

図３　３日間観察したかいわれ大根の成長の様子

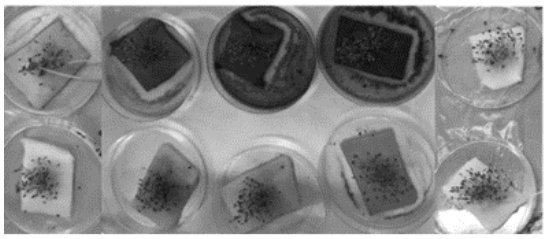
1日目



2日目



3日目



10種類の溶液の配置表

無機溶媒	ホワイトクローバー	ディル	スイートバジル	ローズマリー	水
有機溶媒	ホワイトクローバー	ディル	スイートバジル	ローズマリー	エタノール

図４　３日間観察したホワイトクローバーの成長の様子

実験 2

植物の回復力について、4 種類の溶液を使用し、ハーブ液と水の場合を比較した。

材料

4 種類の溶液(有機溶媒の 3 種類のハーブ溶液、水)、シャーレ、コットンシート、かいわれ大根の種

方法

(1) かいわれ大根の種を 1 シャーレにつき 10 粒ずつコットンの上にのせたものを 4 セット(4 シャーレ分)用意し、4 セット全てを水で発芽するまで育てた。
(2) 4 セット全ての水を抜き、一日かけて植物を萎れさせた。
(3) その後、4 種類の溶液でそれぞれ 1 種類ずつ浸した。
(4) これらを 3 の過程終了後 12 時間ごとで 1 日観察した。

実験 3

ハーブに除菌効果があるかどうか調べるため、3 種類のハーブ溶液が大腸菌の生育を抑制できるか否かについて実験を行った。

材料

3 種類のハーブ溶液・シャーレ・大腸菌・LB 培地

方法

(1) LB 培地を作成した。
(2) ハーブ溶液を 100 mg あたり水 1 ml を原液として、1/10、1/100 の濃度となるように希釈した。
(3) (2)での溶液と大腸菌培養液を混ぜた。
(4) (3)を LB 培地に塗布し、数日間観察した。

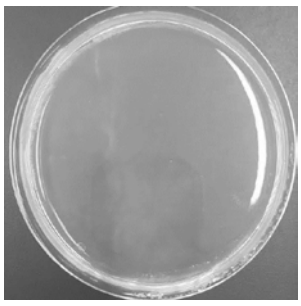


図 5 過程 1 での LB 培地

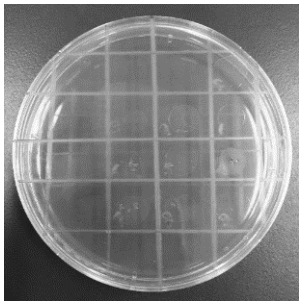
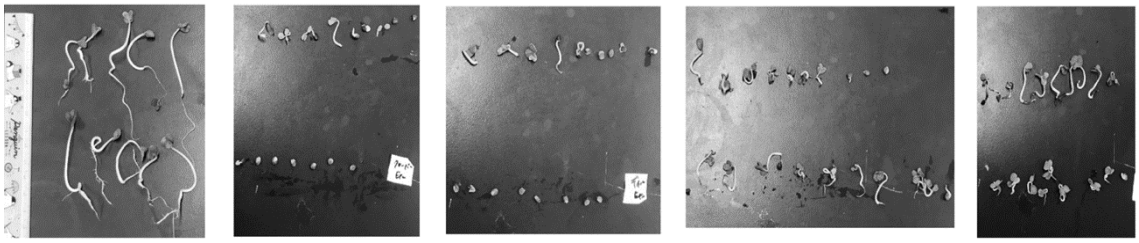


図 6 過程 4 終了後の LB 培地

3. 結果

結果 1-1

植物の発芽において、ハーブ溶液とホワイトクローバーの溶液は抑制効果を持った。特にディル溶液、ホワイトクローバー溶液では大きく伸長抑制が見られた。



水　　ホワイトクローバー　ディル　　スイートバジル　　ローズマリー

図 7 各溶液によるかいわれ大根の種子の発芽率の結果、及び水で育てたものとの比較
※上の種子は無機溶媒溶液をかけたもので、下の種子は有機溶媒溶液をかけたものである。

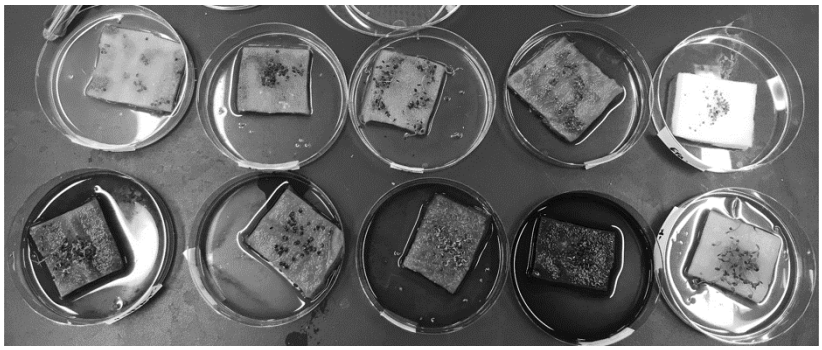


図 8 最終日の 6 日目の各溶液によるホワイトクローバーの発芽率の結果

結果 1-2

植物の成長において、ハーブ溶液は水やホワイトクローバーの溶液よりも促進効果を持った。特にディルでは大きく伸長促進が見られた。

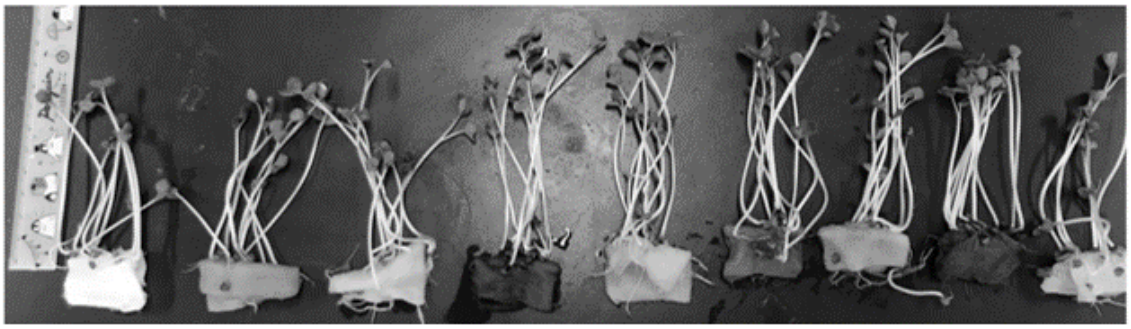


図 9 各溶液のよる 3 日間でのかいわれ大根の成長の結果

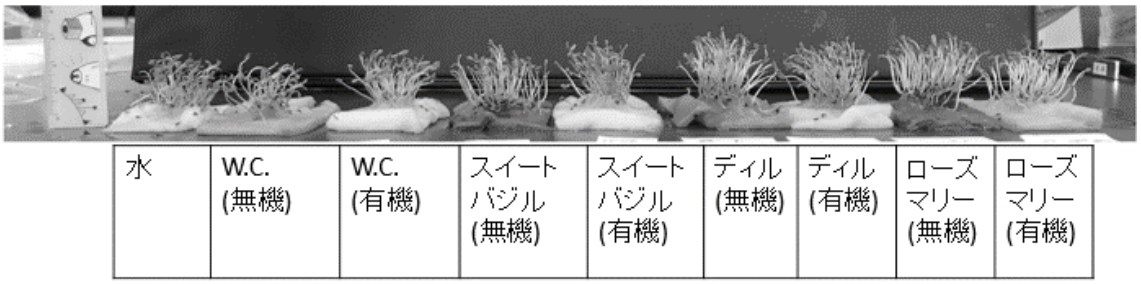


図 10 各溶液による 3 日間でのホワイトクローバーの成長の結果

結果 2

ハーブ溶液で浸した植物は水で浸した植物よりも健康的に回復していた。特にスイートバジル、ローズマリーで浸された植物よりも健康的だった。

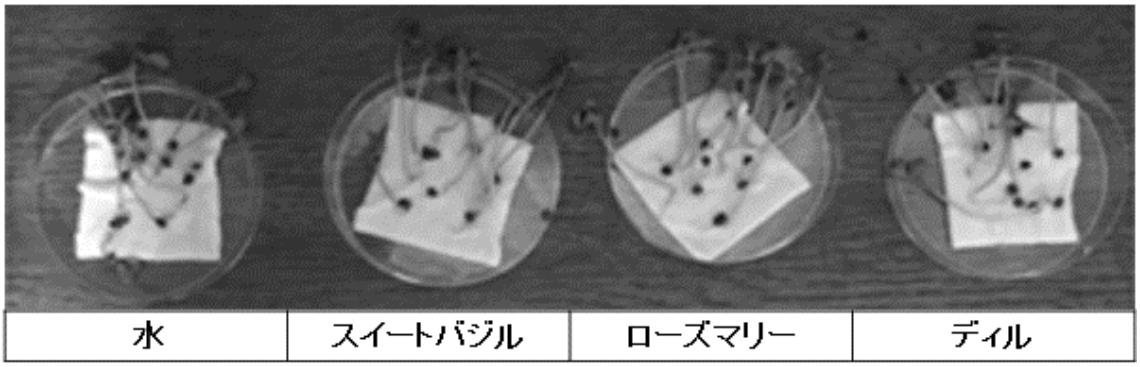


図 11 水を抜いて一日後の植物の様子

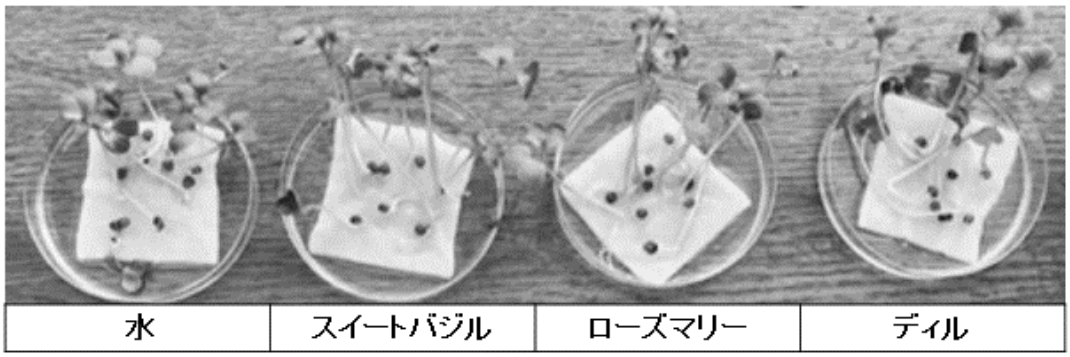


図 12 各溶液で浸した 12 時間後の植物の様子

図 13 各溶液で浸した 24 時間後の植物の様子

結果 3

ハーブにはわずかに除菌効果があることがわかった。特にディルでは顕著に見られた。

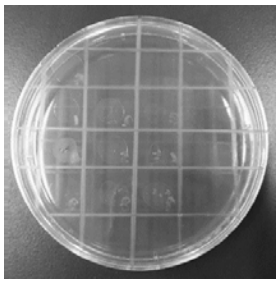
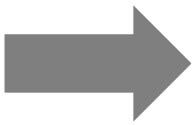


図 14 過程 4 終了後の LB 培地



ローズマリー→
バジル→
ディル→

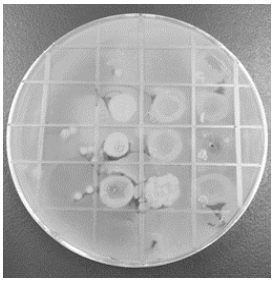


図 15 過程 4 終了から数日後の LB 培地の様子

表 4 実験 1~3 の総結果

	水	エタノール	スイートバジル	ディル	ローズマリー	ホワイトクローバー
発芽(かいわれ大根)	○	×	Δ	Δ	Δ	×
発芽(クローバー)	○	×	Δ	Δ	Δ	Δ
成長(かいわれ大根)	○	/	◎	◎	○	○
成長(クローバー)	○	/	○	◎	◎	○
復活度	○	/	◎	○	◎	/
除菌	/	/	○	◎	○	/

※ ◎; 水よりも促進効果を持った ○; 水程度の効果を持った

Δ; 水よりも少し抑制効果を持った ×; 水よりもかなりの抑制効果を持った

/; 実験を行わなかった

4. 考察

実験 1-1 よりハーブ溶液とホワイトクローバー溶液は植物の発芽に欠かせないジベレリンという植物ホルモンを抑制する成分を持つと考えられる。ハーブに限らず、植物の葉の成分は他の植物の発芽率を成長させると考えられる。

実験 1-2 よりハーブが肥料としての役目を果たし、より伸長成長を促進したのだと考えられる。

実験 2 よりハーブに含まれる有機物(ビタミンなど)が栄養分の役割を果たしたのだと考えられる。

実験 3 より 3 種類のハーブには除菌効果があることがわかった。そのため、溶液中での最近の繁殖が抑えられたことが、植物の成長に良い影響を与えたと考えられる。また、3 種類のうちディルの効果が最も大きかった。

5. 展望

実験 1-2 と 2 において、シャーレ培地から土培地への変更と植物の種類の変更をして再度実験を行いたい。また、3 種類のハーブ溶液はそれぞれ違うメリットが見られたので、混ぜ合わせた溶液を用いて、植物により利益が多い溶液を発見していきたい。最終的に、植物の成長を促進させ、短い期間で効率的に植物を育てるためのハーブ溶液を作成したい。

6. 参考文献

・Borrás-Linares, Isabel, et al. “Rosmarinus Officinalis Leaves as a Natural Source of Bioactive Compounds.” *International Journal of Molecular Sciences*, MDPI, 10 Nov. 2014, www.ncbi.nlm.nih.gov/pmc/articles/PMC4264185/.

・“Chemical Compounds in Herbs & Spices.” *Compound Interest*, 15 Mar. 2015, www.compoundchem.com/2014/03/13/chemical-compounds-in-herbs-spices/.

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The Benefits of Three Types of Herbs

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JSSF 2020
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Abstract

We focused on the positive effects on plants using herbs. We used sweet basil, rosemary and dill in our experiments. First, we made two types of solutions which were inorganic and organic solutions. 8 solutions (inorganic and organic solvents created from sweet basil, rosemary, dill, and white clover) were created. The solutions made from white clover were made as common plants to use as controls to compare with herbs. In Experiment 1, we found herbs have the ability to prevent germination and in contrast they have another ability to promote the growth after germination. In Experiment 2, herbs helped recovering plants become healthier than those grown with water. In Experiment 3, herb solutions were successful in sterilizing bacteria. In the future, we want to conduct experiments by mixing the solutions. Eventually, we want to create a solution from herbs that can help plants with disease recover.

Keywords: herb, inorganic, organic, germination, promote, growth, sterilizing, bacteria, recover

Introduction

Herbs have many effects like sterilization, weeding, antiseptic and insect repellents. Moreover, herbs are aromatic, so they have sedation and excitement effects. So, we were interested in using herbs in our research. We used three types of herbs which are sweet basil, dill, and rosemary. Sweet basil has flat and large leaves and it contains lots of calcium and vitamin K. Dill has slender stems and is often eaten with salmon. It contains primarily vitamin C and manganese. Rosemary has tough stems and good flavor. It is rich in iron, calcium, and vitamin B-6. Now, medicines made from herbs for helping humans are widespread. However, no medicine for helping plants has been discovered yet. Our hypothesis is herbs have benefits on plants. Our purpose is to create a solution which can be used as a natural agricultural chemical from herbs.

Materials and Method

Materials 0

Before carrying out my experiments, we made 8 kinds of solutions which are inorganic and organic solvents derived from sweet basil, rosemary, dill, and white clover. We used an electronic weighing instrument, beaker, ion exchange water, dispensing spoon, pestle, mortar, funnel, Kimwipe, ethanol, glass petri dish, micro-spatula, conical tube, and transfer pipette.

Method 0

The production method of inorganic solutions:

(1) 1g of each herb and the white clover were soaked in hot water for one day.

(2) The leaves were collected and crushed in water using a mortar and pestle.

(3) The liquid was filtrated out and diluted with water.

The production method of organic solutions:

(1) 1g of each herb and the white clover was soaked in hot water for one day.

(2) Leaves were collected and crushed in ethanol using a mortar and pestle.

(3) They were dried in glass petri dishes.

(4) After a few days, the residue was collected used to make a powder.

(5) The rest of powder attached to the glass petri dishes was dissolved by ethanol to eliminate lipids and these solutions was put into conical tubes and dried.

(6) After drying, powders (made in method 4) and solutions (made in method 5) were diluted by water.

❖ The concentration was constant at 100 mg/ml.

Table 1. The 10 kinds of solutions used in experiments

Inorganic	Sweet basil	Rosemary	Dill	White clover	Water
Organic	Sweet basil	Rosemary	Dill	White clover	Ethanol

Materials 1-1

We researched the difference in the germination rate using herb solutions and other solutions. We used the 10 kinds of solutions, petri dishes, cotton sheets, radish sprout seeds and white clover seeds.

Method 1-1

10 radish seeds on each cotton were put in each petri dish and soaked in the 10 solutions (Table 1). They were then observed daily for 6 days. The experiment was repeated using white clover seeds.

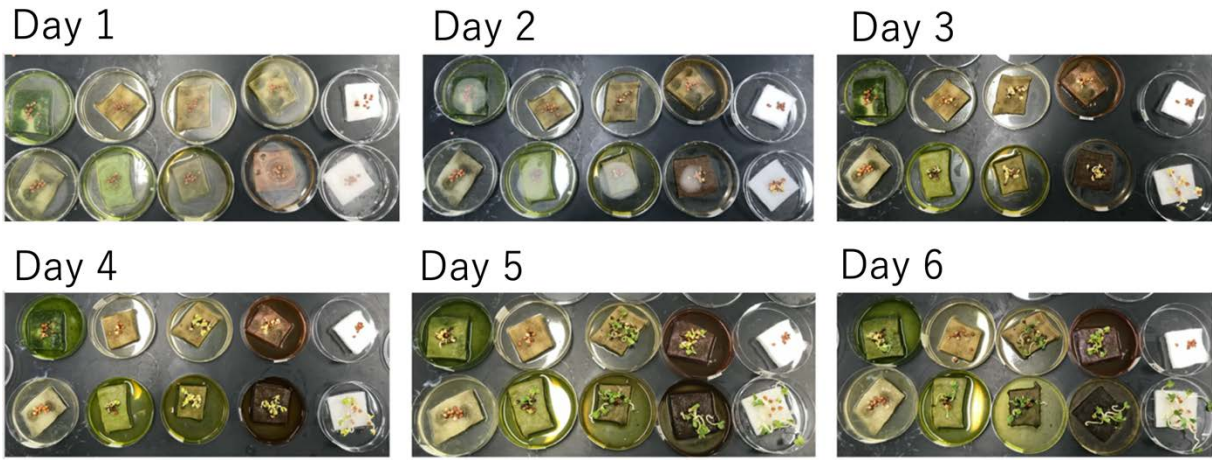


Fig.1 Observation of the germination of radish sprouts from day 1 to day 6. Petri dishes are arranged as shown in Table 1 above.

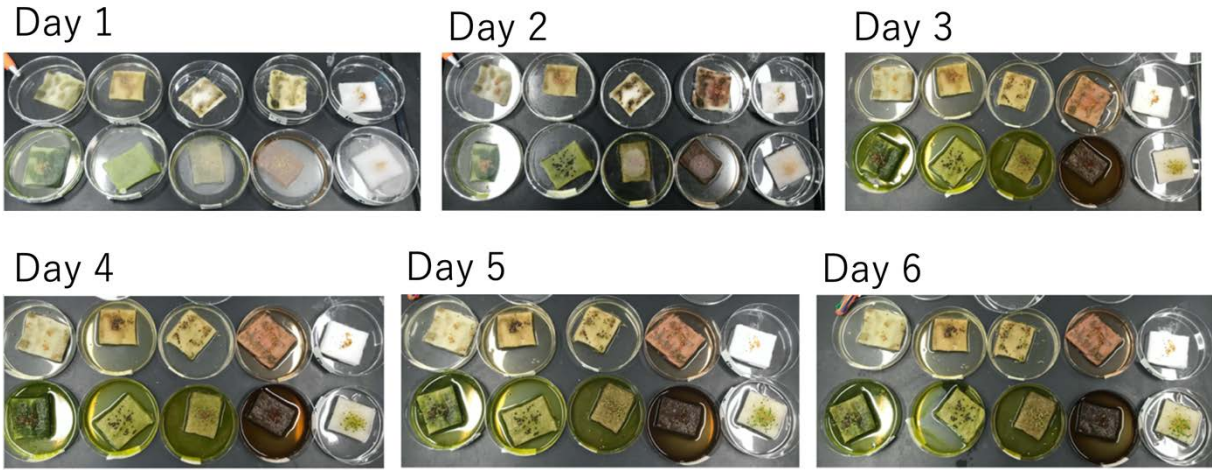


Fig.2 Observation of the germination of white clover from day 1 to day 6. Petri dishes are arranged as shown in Table 1 above.

Materials 1-2

We researched the difference in growth using different herb and other solutions using the same materials as in experiment 1-1.

Method 1-2

10 pieces of cotton with 10 radish seeds on each were prepared. They were grown until they germinated. After germination, sprouts were divided into separate petri dishes and soaked with 10 kinds of solutions (Table 1). Their growth was then observed over 3 days. The experiment was repeated with white clover seeds.

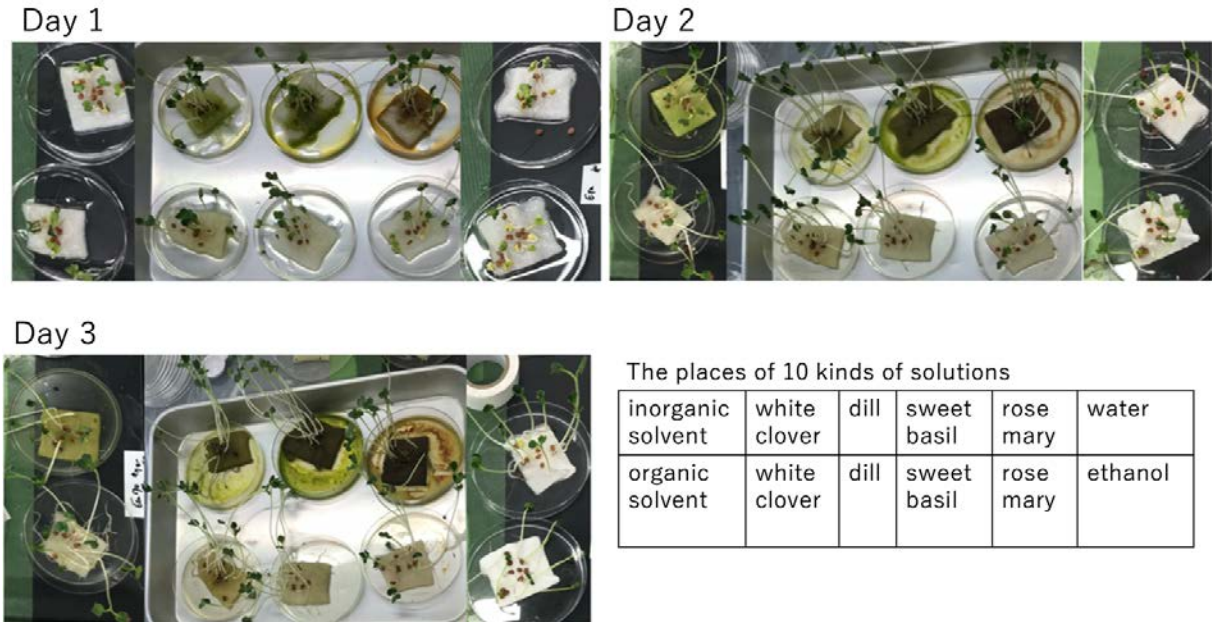


Fig.3 Observation of the growth of radish sprouts from day 1 to day 3.

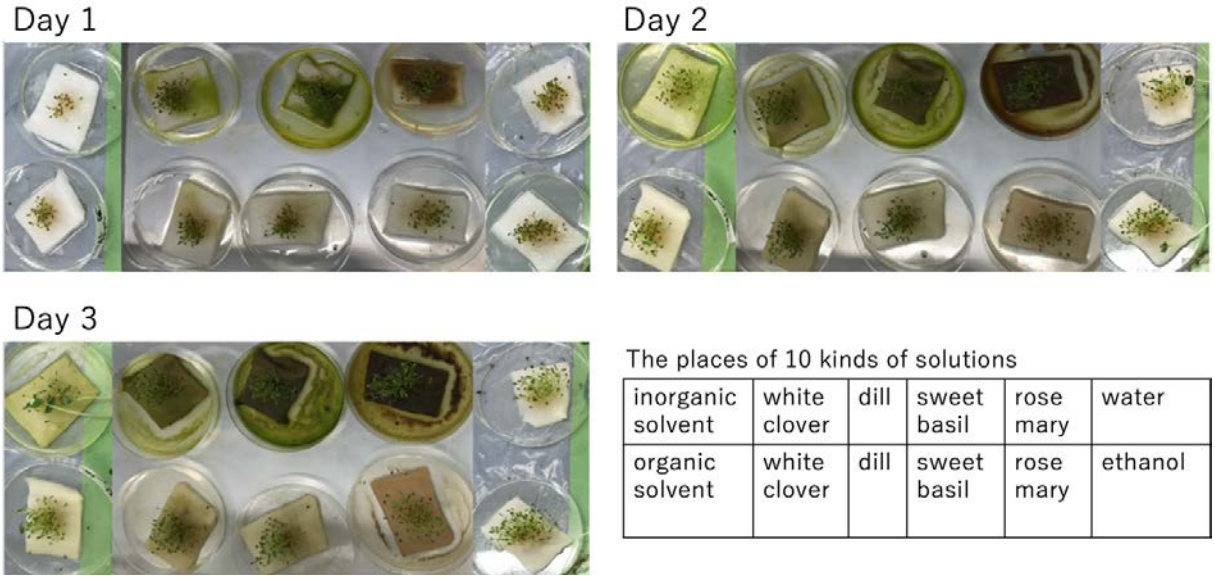


Fig.4 Observation of the growth of white clover from day 1 to day 3.

Materials 2

We researched the difference in the recovery power while using herb solutions and water. We used the organic solutions of 3 kinds of herbs, water, petri dishes, cotton sheets and radish sprouts.

Method 2

4 pieces of cotton with 10 radish seeds were prepared in separate petri dishes. They were grown with water until they germinated. After germination, the water was removed causing sprouts to wilt. After 1 day without water, they were soaked with 4 kinds of solutions (water, sweet basil, rosemary, and dill). The herb solutions were diluted at 250 µl of undiluted solution / 20 ml of water. The recovery power of each solution was observed for 1 day every 12 hours.

Materials 3

We checked if the bacteria could be sanitized by the herb solution. 3 kinds of herbs powder, LB medium (Trypton, NaCl, ager, Yeast extract), E. coli, petri dishes and pipettes were used.

Method 3

First, LB mediums were made. Herb solutions were diluted to 1/10 and 1/100 (The concentration of undiluted. solution was constant at 100 mg/ml). E. coli and herb solutions were then mixed. They were put on the mediums. After a few days, they were incubated at 37C° and observed.

Fig.5 LB medium before adding solutions.

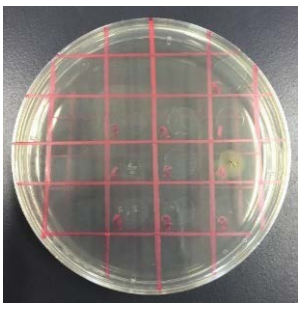


Fig.6 LB medium after adding solutions in a grid formation.

Data Analysis

Result 1-1

Herbs and clovers had greater suppression effects than water on the germination of plants. Dill and clover were found to have especially strong suppression effects. Also, herbs had a positive effect on the growth of plants after germination. It was thought that herbs inhibited Gibberellin, a plant hormone, during germination. It is thought the growth was due to the effect of fertilizer which was used in one of the growth conditions.

water white clover dill sweet basil rosemary

Fig.7 Comparison of germination rates of radish seeds in the 10 solutions. Top: inorganic solution. Bottom: organic solution.

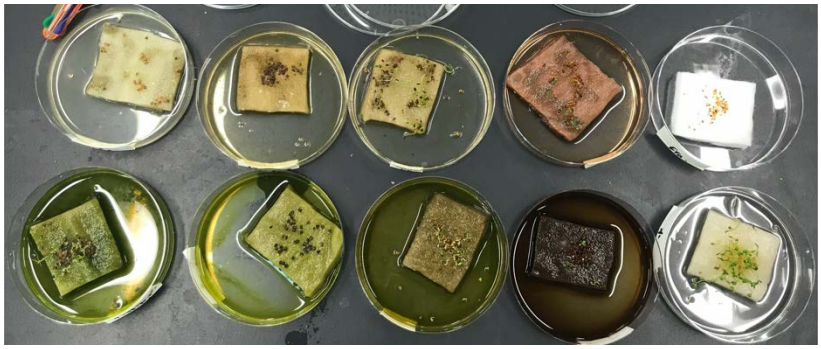


Fig.8 Germination rate of white clover seeds in the 10 solutions. From left to right: sweet basil, rosemary, dill, white clover, water (top) / ethanol (bottom). Top: organic. Bottom: inorganic.

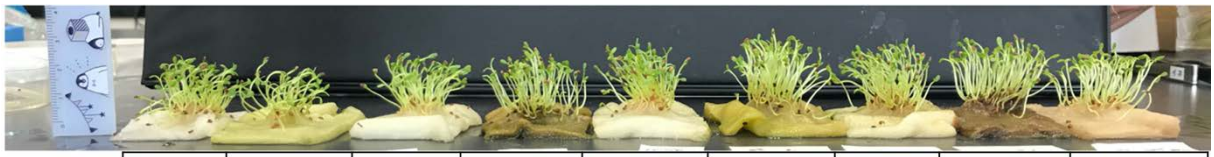
Result 1-2

The radish plants had a successful recovery from their weak condition by using 4 types of solutions. Furthermore, the plants were healthier when herbs were used

more than water. Especially, sweet basil and rosemary had a positive effect on the plants. It was thought that herbs had some organic nutrients like vitamins that helped the plants to recover quicker.

water	white clover (Inorg.)	white clover (Org.)	sweet basil (Inorg.)	sweet basil (Org.)	dill (Inorg.)	dill (Org.)	rose mary (Inorg.)	rose mary (Org.)

Fig.9 Growth of radish sprouts in the 10 solutions.



water	White clover (Inorg.)	White clover (Org.)	Sweet basil (Inorg.)	Sweet basil (Org.)	Dill (Inorg.)	Dill (Org.)	Rose mary (Inorg.)	Rose mary (Org.)

Fig.10 Growth of white clover in the 10 solutions.

Result 2

Radish sprouts successfully recovered from their weak conditions by using 4 solutions. Furthermore, herbs demonstrated greater recovery power than water. Especially, sweet basil and rosemary had benefits

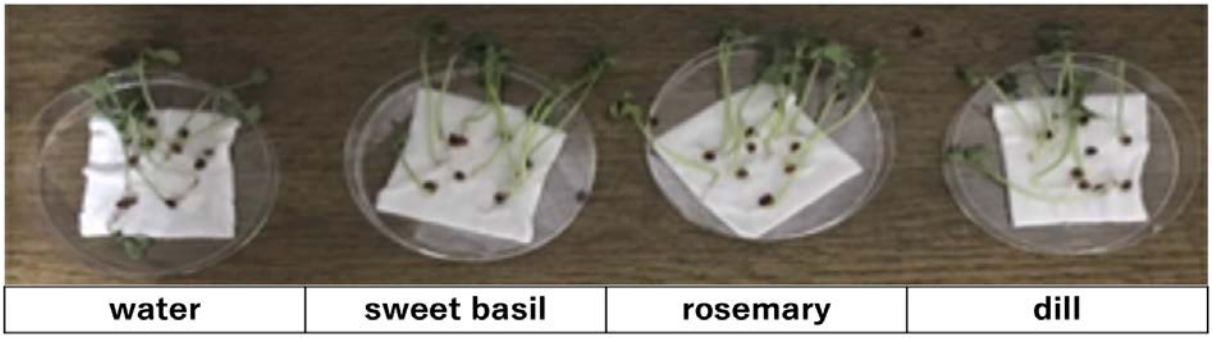


Fig.11 Radish sprouts when water was removed.

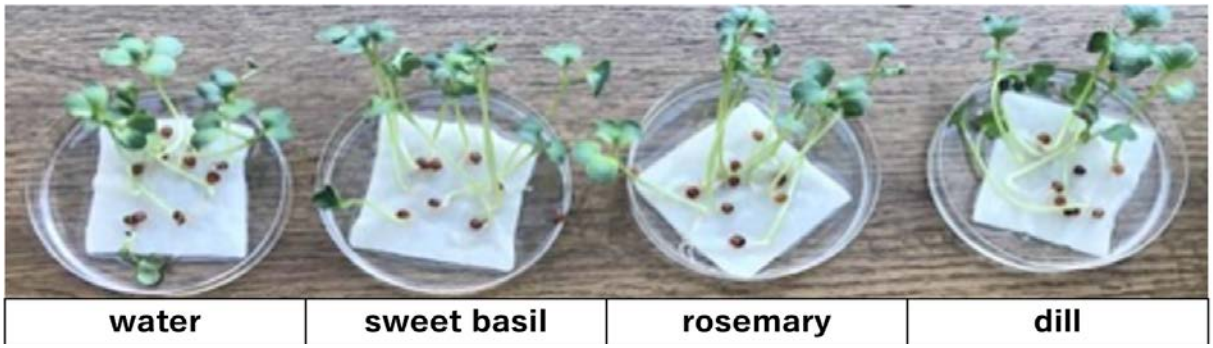


Fig.12 12 hours after adding solutions.

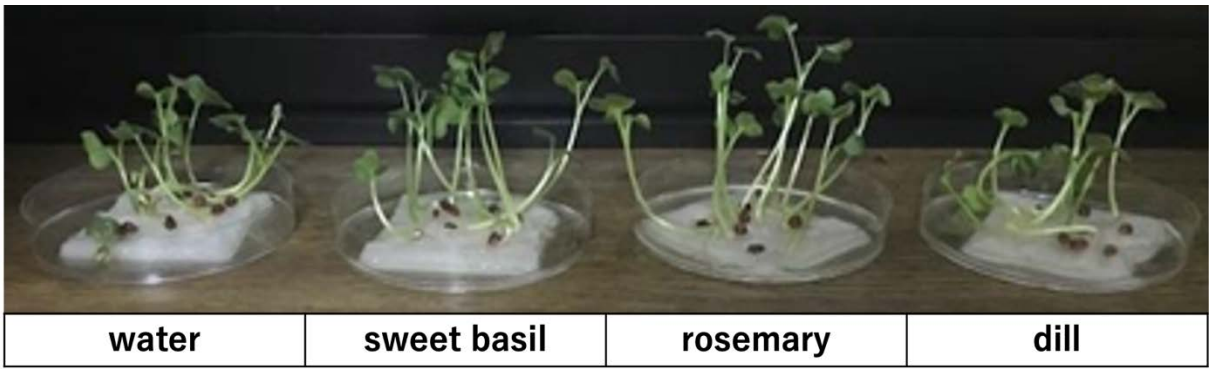


Fig.13 24 hours after adding solutions.

Result 3

3 kind of herbs could suppress the bacteria, so they had antibacterial effects. It was thought that herbs have special properties. The condition was revealed when the bacteria was removed. For these reasons, the content of the solution will be removed to remove substances like protein and glucose.

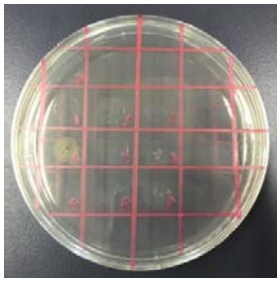
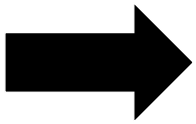


Fig 14 LB After preparation



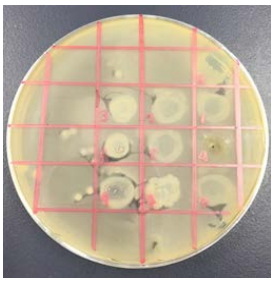


Fig 15 LB medium after incubation

Table 2. Summary of the results from experiment 1 to 3

	Water	Ethanol	Sweet basil	Dill	Rosemary	White clover
Ex.1 Germination (radish)	○	×	△	△	△	×
Ex.1 Germination (clover)	○	×	△	△	△	△
Ex.1 Growth (radish)	○	/	◎	◎	○	○
Ex.1 Growth (clover)	○	/	○	◎	◎	○
Ex.2 Recovering	○	/	◎	○	◎	/
Ex.3 Sterilization	/	/	○	◎	○	/

◎;greater promotion than water ○;same as water △;less promotion than water
×;greater prevention than water /;did not do

Discussion

Ⅰ ”Why did herbs suppress the germination rate and promote growth?”

It was thought that herbs inhibited Gibberellin, a plant hormone in germination. Also, we thought the growth was due to benefit of fertilizer which was used in one of the growth conditions.

Ⅱ “Why could the herbs help plants become healthier?”

It was thought that herbs had some organic nutrients like vitamins that helped the plants to recover healthier.

Ⅲ “Why could the herbs suppress bacteria?”

It was thought that herbs have special properties. The condition was revealed. when the bacteria was removed. For these reasons, the content of the solution will be removed to remove substances like protein and glucose.

Conclusion

In Experiment 1, herbs and white clovers had a greater suppression effect on plants. Also, they promoted growth after plant germination. It was found that dill had a better effect on the plants.

In Experiment 1, both inorganic and organic solutions were used but in Experiment 2, we used only organic solutions. Herbs were found to help the plants to recover faster. In particularly, sweet basil and rosemary were the most effective. Dill was shown to be more effective when an inorganic property was used. On the other hand, sweet basil and rosemary were more effective when an organic property was used. Clover had some effect, but it was found that herbs were more effective.

In experiment 3, we found the 3 herbs had anti-bacterial effects. However, the bacteria elimination which we could observe was minimal, so we want to try again.

Ideas for Future Research

Dill was found to be the most effective. Thus, it is recommended to do more experiments using dill. Also experiment 2 and 3 should be repeated using mixed herb solutions such as sweet basil and dill, dill and rosemary and rosemary and sweet basil. In experiments 1 and 2, only radishes and white clover were used, so I want to use other plants like flowers and do the same experiments. The growing environment should also be changed from cotton to soil to simulate actual conditions for growing plants. Lastly, sterilization of real molds attached to real plants will be carried out.

Acknowledgements

We would like to express our very great appreciation to my research supervisor, Dr. Mie Ichikawa for her advice and assistance in many times. In fact, she suggested us to use herbs when I was worried about our research theme.

We would also like to express our gratitude to all the teachers, especially, Mr. Joseph Greenleaf, Ms. Ann Flanagan, and Ms. Nanako Takeda for their assistance during the writing process and corrections in English.

Finally, we would like to extend our thanks to our families for their support and advice and our classmates for motivating us.

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Ritsumeikan High School

The Benefits of 3 Types of Herbs

Abstract

Herbs have many benefits. It is said that herbs are useful plants for sterilization, weeding, antiseptics and insect repellents. Moreover, herbs are aromatic so they have sedation and excitement effects. So, we were interested in using herbs in our research. Although it is already known that medicine is made from herbs to help humans, medicine for helping plants has not been discovered yet. In our research, we focused on the benefit of herbs on plants using herbs. In our experiments, we used Sweet Basil, Rosemary and Dill as herbs. Before carrying out our experiments, we made two kinds of solutions which were inorganic and organic solutions. In Experiment 1, we found herbs have the ability to prevent the germination and in contrast they have another ability to promote the growth after germination. In Experiment 2, herbs helped plants to recover. Then, the length of each root was soaked in an herb solution longer than water. In Experiment 3, the dill solution was successful to sterilize bacteria. Eventually, we want to create a new herb solution that can help plants to recover from disease as our future plan.

Fig. 1 3 types of herbs

Preparation

Two kinds of solutions which are inorganic and organic solvent by using water or EtOH were made.

○Inorganic solvent (used in Ex.1)

1. 1g of three types of herbs and white clover were measured and soaked in hot water.
2. Then, they were crushed.
3. Liquid was filtrated from them.

○Organic solvent (used in Ex.2,3)

1. 1g of three kinds of herbs were measured and soaked in hot water.
2. They were crushed in ethanol.
3. They were dried in glass petri dishes.
4. After a few days, they were collected and made into a powder.
5. The rest of the powder was dissolved by ethanol to eliminate lipids; these solutions were put into conical tubes and dried.
6. After drying, these solutions and powders were diluted by water.

※The concentration was constant at 100 mg/ml.

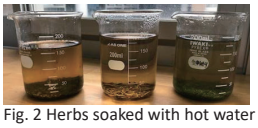
Fig. 2 Herbs soaked with hot water


Fig. 3 Crushed with EtOH

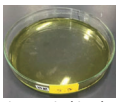
Fig. 4 Dried in glass petri dish


Fig. 5 Herb powder

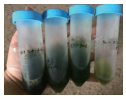
Fig. 6 Inorganic solutions

Experiment 1

In this experiment, we researched the germination rate and growth using 10 solutions.

Materials

- 10 solutions (with and without EtOH) Rosemary water, White clover water, Dill water, Sweet basil water
- Petri dish ・Cotton sheet
- White clover and radish seeds

Method 1 (Germination rate)

1. Cotton was put with 10 radish seeds in each petri dish.
2. They were soaked with 10 different kinds of solutions.
3. The same experiment was conducted using white clover.
4. Both were observed daily for 6 days.

Method 2 (Growth)

1. 10 pieces of cotton with 10 radish seeds on each were prepared and grown until germinated.
2. The same experiment was conducted using white clover.
3. After germination, they were divided into petri dishes one by one and were soaked in 10 different kinds of solutions.
4. The growth of each was observed after 3 days.

Above; organic solution

Table 1 Organic and Inorganic Solutions

W.C.	Dill	Basil	Rosemary	EtOH
W.C.	Dill	Basil	Rosemary	Water

Below; inorganic solution

W.C.; white clover

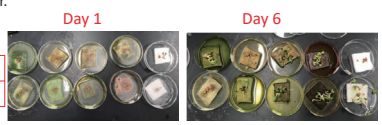

Fig. 7 Observation of radish sprouts on Day 1 (left) and Day 6 (right)

Result 1 & Discussion 1

Herbs and clovers had greater suppression effects than water on germination of plants. Especially, dill and clover were found to have strong suppression effects. Also, herbs had a positive effect on the growth of plants after germination.

Fig. 8 The germination rate using 10 types of solutions

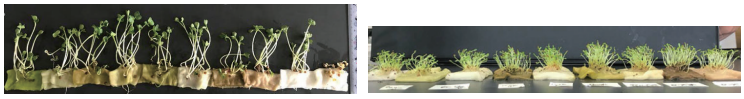

Fig. 9 The growth rate of radishes (left) and white clover (right) in 10 different types of solutions

Ⅰ. "Why did herbs suppress the germination rate and promote growth?"

It was thought that herbs inhibited Gibberellin, a plant hormone in germination. Also, we thought the growth was due to the effect of fertilizer which was used in one of the growth conditions.

Experiment 2

In this experiment, three types of herbs and water were used in each petri dish. How much the plant recovered was measured.

Materials

- 3 types of organic herb solutions and water ・radish seeds ・Petri dish ・Cotton sheet


Fig. 11 Left - Diluted solution
Right - Water solution

Table 2 4 types of solutions

Water	Sweet basil	Rose mary	Dill

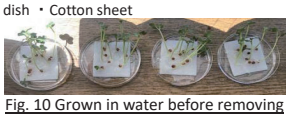
Fig. 10 Grown in water before removing

Method

1. 4 pieces of cotton with 10 radish seeds were prepared in each petri dish and grown until they germinated with water.
2. After that, water was removed; they died.
3. They were soaked in 4 types of solutions. The herb solution was diluted as 250 µl of undiluted solution / 20 ml of water.
4. How much the plants recovered was measured.

Result 2 & Discussion 2

The radish plants had a successful recovery from their weak condition by using 4 types of solutions. Furthermore, the plants were healthier when herbs were used more than water. Especially, sweet basil and rosemary had a better effect on the plants.


Fig. 12 Radishes when water was removed


Fig. 13 12 hours after giving each solution

Table 2 4 types of solutions

Water	Sweet basil	Rose mary	Dill


Fig. 14 24 hours after giving each solution

Ⅱ. Why could the herbs help plants become healthier?

It was thought that herbs had some organic nutrients like vitamins that helped the plants to recover quicker.

Experiment 3

It was thought that these 3 kinds of herbs have anti-bacterial effects. Bacteria was tested to see if it could be sanitized by them.

Materials

- 3 kinds of herb powder
- LB medium (Trypton, NaCl, ager, Yeast extract)
- *E.coli* ・Petri dish ・pipette

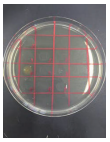
Fig. 15 LB medium

Method

1. LB medium was made.
2. Herb solutions were diluted (1/10 and 1/100).
3. *E.coli* and herb solutions were mixed.
4. They were put on the medium.
5. After a few days, they were incubated at 37C° and observed.

Result 3 & Discussion 3

As a result, 3 kind of herbs could suppress the bacteria. So they had antibacterial effects. It was thought that herbs have special properties. The condition was revealed when the bacteria was removed. For these reasons, the content of the solution will be removed to remove substances like protein and glucose.

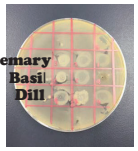

Fig. 16 LB medium

Summary

		Water	Ethanol	Basil	Dill	Rosemary	Clover
Exp.1→	Germination (radish)	○	×	△	△	△	×
Exp.1→	Germination (clover)	○	×	△	△	△	△
Exp.1→	Growth (radish)	○	/	◎	◎	○	○
Exp.1→	Growth (clover)	○	/	○	◎	◎	○
Exp.2→	Health of the plant	○	/	◎	○	◎	/
Exp.3→							

◎ ; greater promotion than water
○ ; same as water
△ ; less promotion than water
× ; greater prevention than water
／ ; did not do

By Experiment 1, herbs and clover had a greater suppression effect on plants and they promoted growth after plant germination. It was found that dill had a better effect on the plants. By Experiment 2, herbs helped the plants to recover faster. In particular, sweet basil and rosemary were the most effective. In Experiment 1, we used inorganic solutions but in Experiment 2, we used organic solutions. Therefore, dill had was more effective when an inorganic property was used. On the other hand, sweet basil and rosemary was more effective when an organic property was used according to the results. Clover had some effect but it was found that herbs were more effective. By experiment 3, 3 kinds of herbs had anti-bacterial effects especially, dill. Therefore, we are going to do more experiments using dill.

Future Plan

We found dill was the most effective. Thus, we are going to do more experiments using dill. In Experiment 1 and 2 we used only radishes so we want to use other plants like flowers and do the same experiments. In addition, we will change the environment from cotton to soil. Furthermore, we will carry out sterilizing real molds which are attached with real plants.

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研究番号	20SS044	研究について	■個人　□グループ
研究テーマ	The time dependent change of redox reaction under various conditions　～さまざまな条件下における信号反応の時間の変化～		

A：研究目的（Purpose）

酸化還元反応が色の変化でわかる信号反応を用いて、どのような条件下で酸化還元反応の反応速度が変化するのを知り、その際反応に規則性があるかどうかを調べた。

B：研究方法（Materials & Methods）

水酸化ナトリウム、グルコース、インジゴカーミンを使用してできる信号反応を用いて行った。この研究では、使用する機械を変更して溶液の攪拌方法を変化させたり、使用した薬品の濃度を 2 倍もしくは 1/2 倍にした際の、信号反応の色が変わる時間を計測し、反応速度がどのように変化しているのか、また規則性があるのかを研究した。

C：研究結果（Result）

水酸化ナトリウムの新旧によって、反応速度に大きな変化があることが分かった。溶液の混ぜ方を変化させると赤→緑のパートが、濃度を変化させると緑→赤のパートに大きな変化が出ることが分かった。還元性のある糖はもちろん色が変化したが、還元性を持たない糖でも一部の色が変化した。

D：考察（Discussion）

溶液を混ぜたときは、混ぜ方によってボトルの上部の空気との混ざり方が変化して、攪拌している赤→緑の色の変化の際に、大きな色の変化が出たのだと考えた。濃度を変化させたときは、回転方法は同じだったので赤→緑の部分で顕著な時間の変化は見られなかったが、濃度によって還元反応のスピードが変化して、緑→赤の部分で色が変化したのだと考えた。今回使用したスクロースのような、還元性が無い二糖を使用した場合は、長期間の使用で、何らかの形で還元性が出てきたのではないかと考えた。

E：今後の展望（Future Direction of Research）

今後は、化学反応に影響がある温度変化を行い、酸化還元反応の反応速度の変化を見たい。さらに、新たな二糖を用いて、還元性が無い糖がどのような影響を及ぼすのかを調べたい。

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1. 序章

信号反応とは、酸化還元反応を用いて色を変化させる反応であり、インジゴカーミンによって、酸化還元反応が可視化して分かる反応である。この反応は、水酸化ナトリウム、グルコース、インジゴカーミンをイオン交換水に溶かして、攪拌させると色が変わる。薬品をイオン交換水に溶かした段階では緑色であるが、そのまま放置すると、赤色を通して黄色に変化する。その後に攪拌させると、黄色の溶液が赤色を通して緑色に変化し、再び放置すると、同じように赤色を通して黄色に変化する。この反応では、グルコースがグルコキシドイオン、グルコン酸イオンに変化して、インジゴカーミンの還元剤となっている（ref.1）。私は、所属していたサイエンス部でこの実験を行っていたが、その際に薬品の量などの条件を同じにしていても反応時間に大きな差が出ることが多くあった。そこで、反応速度や反応時間が実験条件によってどのような影響を受けるのか、またその影響に規則性があるのかについて研究することにした。

2. 方法

2-0. 共通事項

信号反応の色の変化は攪拌させた後の黄色→赤色（YR）、赤色→緑色（RG）、放置した後の緑色→赤色（GR）、赤色→黄色（RY）の時間を計測した。

2-1-1. 使用した試薬

・水酸化ナトリウム 5.0 g
・グルコース 3.0 g
・インジゴカーミン 0.05 g
・イオン交換水 250 ml

2-1-2. 使用した器具

・300 ml ビーカー
・300 ml 集気びん
・300 ml メスシリンダー
・300 ml 丸底フラスコ
・薬さじ
・ガラス棒
・プラスチックペトリ皿
・駒込ピペット
・パラフィルム

2-2. 水酸化ナトリウムの量の検討

水酸化ナトリウムの量を、標準の 5.0 g から、2 倍の 10.0 g、半分の 2.5 g に変更して行った。この実験ではまず 300 ml ビーカーにイオン交換水と試薬をすべて入れ、試薬が完全に溶け切ったところで、丸底フラスコに移し替えて、両手で振った。

2-3. 溶液の攪拌方法の検討

手で行うと攪拌具合が統一できないため、シェーカー（図 1）とスターラー（図 2）を使用し、容器を攪拌させた場合と、溶液自体を攪拌させた場合の時間の違いを調べた。どちらも、溶液が漏れないように、また外気と中の溶液の接触を避けるために、パラフィルムを巻いた。

図 1 使用したシェーカー

2-3-1. シェーカーの検討

3 つの攪拌方法（横、回転、八の字）をそれぞれ比較した。攪拌速度は、横と回転が 125 rpm、130 rpm、140 rpm、150 rpm、175 rpm、200 rpm で、八の字はスターラーの設定により、95 rpm、105 rpm、110 rpm、120 rpm の条件下で行った。

図 2 使用したスターラー

2-3-2. スターラーの検討

溶液中にスターラーバー（回転子）を入れ、1000 rpm、1350 rpm、1700 rpm の速度で回転させた。

2-4. スターラーを用いた水酸化ナトリウムの量の検討

正確なデータを得るために、2-2 の実験をスターラーを用いて行った。回転速度は最大の 1700 rpm に設定して行った。

2-5. 糖の種類の検討

グルコースを他の糖に変えて行った。使用した糖は、同じ単糖のガラクトース、フルクトース、還元性を持たない二糖のスクロースを用いた。この実験から、薬品の混ぜ方を変えた。濃度をなるべく同じにするため、個々のビーカーで行う実験回数分の薬品を溶かし、それを 300 ml の三角フラスコに移し替えて攪拌させた（図 3）。

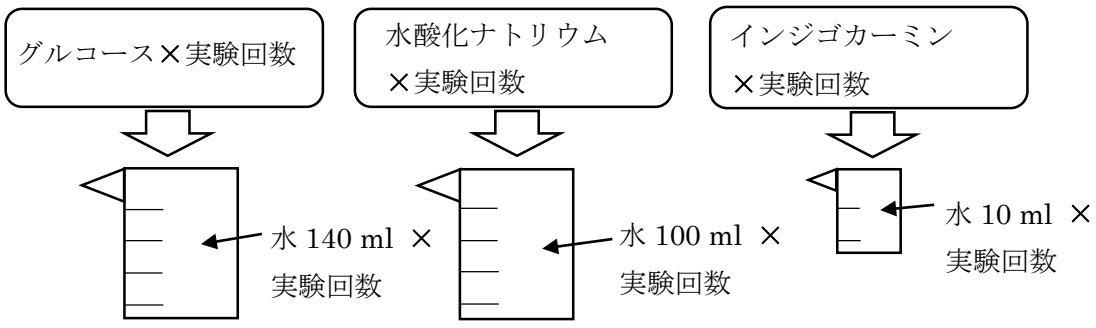


図 3 糖の種類を変える実験での薬品の混ぜ方

3. 結果・考察

3-1.水酸化ナトリウムの量の検討(2-2)

表 1.水酸化ナトリウムの量を変化させた際の時間の変化

	元の量 YR	2倍 YR	半分 YR	元の量 RG	2倍 RG	半分 RG	(s)
1回目	0.71	0.60	0.32	9.10	8.43	6.00	
2回目	0.80	1.36	0.91	3.45	7.06	3.94	
平均	0.76	0.98	0.62	6.28	7.75	4.97	
標準偏差	0.05	0.38	0.30	2.83	0.69	1.03	

	元の量 GR	2倍 GR	半分 GR	元の量 RY	2倍 RY	半分 RY	(s)
1回目	79.92	83.90	184.54	419.05	124.73	154.20	
2回目	143.23	35.19	162.97	85.88	79.33	101.03	
平均	111.58	59.55	173.76	252.47	102.03	127.62	
標準偏差	31.66	24.36	10.79	166.59	22.70	26.59	

YR では、攪拌を始めてすぐの変化だったこともあり、3 つのパターンに大きな変化は見られなかった。しかし、RY の元の量の値などで、1 回目と 2 回目で約 5 分ものずれが生じた。他のところでも、1 回目と 2 回目で時間に大きな差が出たところが多数あった。考えられることとして、1 回目に使用した水酸化ナトリウムは、長い間使用していた古いものであったので、蓋の開閉などにより水酸化ナトリウムが外気と触れて、一部が別の物質に変化したのだと考えた。2 回目に使用した水酸化ナトリウムは、この実験が初めての使用であり、外気との影響を受けていない純粋な水酸化ナトリウムだった。この違いが、約 5 分間の時間の差を生み出したのだと考えた。

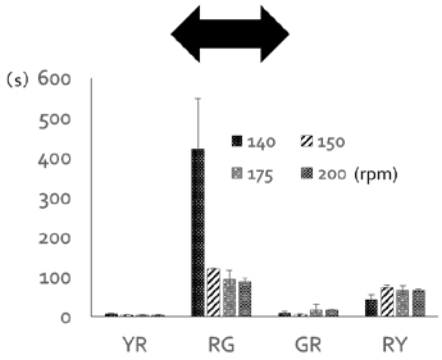
3-2. 溶液の攪拌方法の検討

3-2-1. シェーカーを用いた結果(2-3-1)

回転させた時は、遠心力によって溶液がボトルの上部の空気とうまく混ざらなかったので、色が変化しなかった。

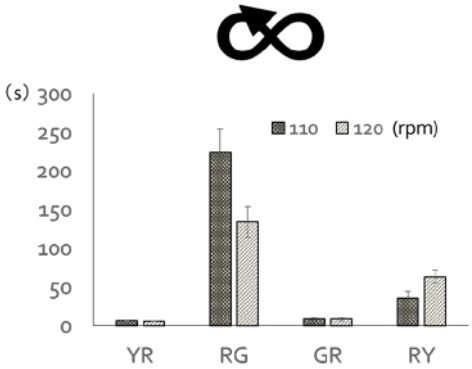
変化なし

図 4.シェーカーを用いた際の時間の変化～回転～



横に攪拌させた場合は、125 rpm、130 rpm で色が変化しなかった。また、RG の部分で、140 rpm と 150 rpm~200 rpm の間で約 5 分の差が生じた。

図 5.シェーカーを用いた際の時間の変化～横に攪拌～



八の字に攪拌させた場合は、95 rpm、105 rpm で色が変化しなかった。横回転と同じく、RG の部分で 110 rpm と 120 rpm の間で約 2 分の差が出た。

図 6.シェーカーを用いた際の時間の変化～八の字に攪拌～

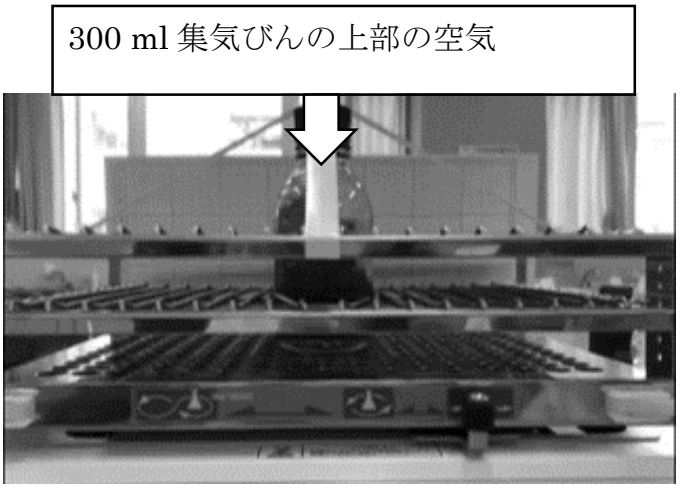


図 7.集気びんの上部の空気

攪拌速度が遅くなるにつれて RG の部分で色の変化まで長く時間がかかっていることが分かった。攪拌速度を遅くすると、ボトルの上部にある空気（図 7）と混ざりにくく、そこで酸化反応のスピードが遅くなっているのだと考えた。

図 8.スターラーで攪拌した際に生じる渦

スターラーを用いた際は、シェーカーを用いた際と比べて色が変化する時間が短かった。スターラーはシェーカーと違い、スターラーバーで攪拌した際に生じる渦（図 9）が酸化に大きく影響していることが分かった。その証拠に、シェーカーでは集気びんの上部の溶液から色が変化していったが、スターラーは、渦のでき初めである溶液の下部から色が変化していった。

3-2-2 スターラーを用いた結果(2-3-2)

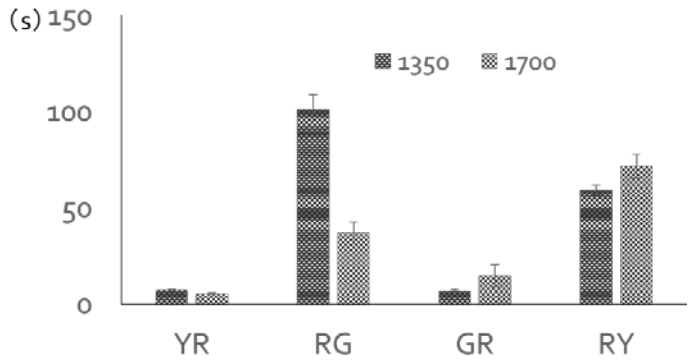


図 9.スターラーを用いた際の時間の変化

1000 rpm で攪拌した際は、色が変化しなかった。スターラーではシェーカーと同じく RG で 1350 rpm と 1700 rpm で約 1 分半の差が出た。シェーカーと同じく、攪拌速度を遅くすると、回転によって生じる渦の大きさが小さくなり、溶液全体が空気と触れ合うまでに時間が

かかっていることが原因だと考えた。また、シェーカーよりも色の変化の時間が短い理由として、生じた渦と溶液が触れる表面積が、シェーカーよりも大きく、酸化反応がシェーカーよりも早く行われていたことが要因だと考えた。

3-3.水酸化ナトリウムの量（スターラーを用いた場合　2-4）

表 2.水酸化ナトリウムの量を変化させた際の時間の変化（スターラーで攪拌）

	元の量 YR	2倍 YR	半分 YR	元の量 RG	2倍 RG	半分 RG	(s)
1回目	5.34	6.18	5.99	36.18	41.9	27.26	
2回目	5.48	6.27	4.68	24.5	47.12	25.85	
3回目	5.12	5.73	5.65	28.74	42.02	21.45	
平均	5.31	6.06	5.44	29.81	43.68	24.85	
標準偏差	0.15	0.24	0.56	4.83	2.43	2.47	

	元の量 GR	2倍 GR	半分 GR	元の量 RY	2倍 RY	半分 RY	(s)
1回目	13.43	4.43	33.27	63.15	41.69	77.28	
2回目	34.34	5.13	47.17	74.31	52.28	79.33	
3回目	30.19	7.24	65.7	90.75	66.35	83.21	
平均	25.99	5.6	48.71	76.07	53.44	79.94	
標準偏差	9.04	1.19	13.28	11.34	10.10	2.46	

4 つの色の変化のパターンの中で、GR が一番時間の変化に差が生じた。2 倍が一番時間が短く、半分が一番時間が長いという結果になった。GR では還元反応が起こっているので、水酸化ナトリウムの濃度は、還元反応に大きな影響を与えていることが分かった。2 倍にしたものは、一番水酸化ナトリウム濃度が高いので、一番時間がかからず、半分にしたものは、一番水酸化ナトリウム濃度が低いので、還元に時間がかかったのだと考えた。

3-4. 糖の種類(2-5)

表 3.糖の種類を変化させた場合の時間の変化（グルコースとガラクトース）

	グルコース YR	ガラクトース YR	グルコース RG	ガラクトース RG	グルコース GR	ガラクトース GR	グルコース RY	ガラクトース RY	(s)
1回目	3.88	5.18	25.77	7.59	102.73	133.10	174.81	60.44	
2回目	4.34	4.61	20.78	9.93	40.85	137.05	184.56	77.4	
3回目	3.96	4.39	23.11	10.40	29.70	148.7	190.54	102.07	
平均	4.06	4.73	23.22	9.31	57.76	139.62	183.30	79.97	
標準偏差	0.20	0.33	2.04	1.23	32.12	6.62	6.48	17.09	

ガラクトースは、グルコースと同様に色が変化した。しかし、RG と GR で両方の糖には大きな変化が見られた。ガラクトースとグルコースの違いは、糖の構造なので（Ref.2）、この時間の変化の違いは、糖の構造によるものだと考えた。

フルクトースは、溶液を作成して黄色にした後、攪拌させても赤色までしか色が変わらな

かった。また、攪拌をやめると、スターラーバーが回り終わる前に黄色に戻った。グルコースやガラクトースは、赤色から黄色に戻るまでに時間がかかったが、フルクトースは瞬間で色が元に戻った。フルクトースは、アルデヒド基を持っていないが、アルカリ条件下では還元性を示す（Ref.2）。この実験で使用した水酸化ナトリウムは強塩基であるので、強塩基条件下にフルクトースがさらされ、酸化反応よりも還元反応の方が強くなってしまったのではないかと考えた。

スクロースは、溶液生成時に緑色から赤色に変化して反応が止まった。スクロースは水溶液中でも還元性を示さないのだが（Ref.3）、今回の実験では緑色から赤色に変化する還元反応が起こった。スクロースは希酸または酵素スクラーゼで加水分解をすると転化して転化糖になり、還元性を示す（Ref.3）。しかし、この実験ではどちらも使用していないので、この要因ではないと考えた。このスクロースは、長い期間の使用による外気との接触で、還元性を持ってしまったのではないかと考えた。しかし、元々還元性を持っていない糖であるので、グルコースやフルクトースのような強い還元性を示さないので、赤色までしか色が変化しなかったのだと考えた。

4. 今後の展望

水酸化ナトリウム濃度は、GR で起こっている還元反応に影響を及ぼした可能性がある。今後、さらに濃度を高くしたり低くした場合に、今回の結果と比べてどのような時間の変化がみられるかを調べる必要がある。

二糖であるスクロースが長い期間の使用による外気との接触により、還元性が生まれて一部のみ色が反応した可能性がある。今後、還元性が無い二糖を用いて色の変化が生じるかを調べ、変化した場合は単糖との時間の変化を見る必要がある。

その他の条件として、温度を変化させた場合の反応速度の変化を調べる必要がある。

5. 参考文献

Ref.1 木村朋恵、長友未希、鈴木俊彰　（2018）「インジゴカルミンを用いる酸化還元反応と化学教材への応用」、p1-2

Ref.2 【単糖類】グルコース・ガラクトース・フルクトースの分類や構造、性質、二糖や多糖との関係性など。TORU AYASE　https://kimika.net/t3tantou.html#i

Ref.3 卜部吉庸 (2009) 「化学I・IIの新研究」、三省堂、p612-613

The time dependent change of redox reaction under various condition

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JSSF 2020
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0. Abstract

This research examines how changes in the amount of NaOH or stirring conditions affects the speed and time of redox reaction using the chemical traffic light experiment. How to mix and change the substances types and amounts were mainly discussed in this research. In most of the experiments, the time and speed changed. The mixing method was found to greatly affect the redox reaction of solutions. These experiments are redox reaction, so having air in the bottle is very important for these experiments. The method by which to mix the solutions so that they would come in contact with air was determined. The substances were affected by the reducibility in these experiments. For example, there are many types of sugar and not all sugars possess reducibility. Furthermore, the amount of substances was very important, because concentration was affected by it. These factors affected both the speed and time redux reactions conducted in this research.

Keywords: chemical traffic light experiment, redox reaction, solutions, reducibility, sugars, concentration, speed, time

1. Introduction

The chemical traffic light experiment is a popular experiment used to demonstrate the chemical clock reaction. In it, the color of the solution changes after being stirred and left in a pattern resembling a traffic light. The version of the experiment focused on in this research uses NaOH, Glucose, and Indigo carmine. When these materials are dissolved in Ion-exchanged water, and mixed, they transform into “Green Indigo carmine” (Indigo carmine is blue when dissolved in water, but it becomes green in alkaline solutions.) It then changes to a red intermediate through which it becomes a reduced yellow indigo carmine. When the reduced yellow indigo carmine is oxidized, it is immediately converted into a red intermediate, and from this intermediate, it transforms back into a green indigo carmine. However, if left for a short time, glucose becomes a reducing agent, and it is converted into a red intermediate, and changes into yellow indigo carmine. Glucose transforms into glucoxide ions with reducing properties in a NaOH solution and oxidizes into gluconate ions. This reaction occurs only in alkaline solutions. In this research, how affect the time when the method of mix in the bottle was changed and how affect the time when the substances of sugar was changed were examined.

2. Method

This research focused on measuring the time it takes for the solution to change from yellow to red (YR), red to green (RG), green to red (GR), and red to yellow (RY).

2-1. Materials

Regents were 5.0 g of NaOH, 3.0 g of glucose, 0.05 g of indigo carmine, and 250 ml of ion exchange water. A 300 ml beaker, 300 ml gas collection bottle, 300 ml graduated cylinder, 300 ml round bottom flask, lab dispensing spoon, glass rod, plastics petri dishes,

pipette, and parafilm were also used.

2-2. The amount of NaOH

Reaction times between using the original amount of NaOH (5.0 g), double the amount (10 g) and half the amount (2.5 g) were measured and compared. The chemicals were mixed into a solution using the beaker – flask method as above. The flask was mixed by hand.

2-3. The method of stirring the solution

A lab shaker (Fig.1) and a magnetic stirrer (Fig.2) were used to mix the solution to compare each method by observing the container and solution. Parafilm was wrapped in the 300 ml gas collection bottle to leak out the solution, and to prevent contents in bottom from coming in contact with the air.

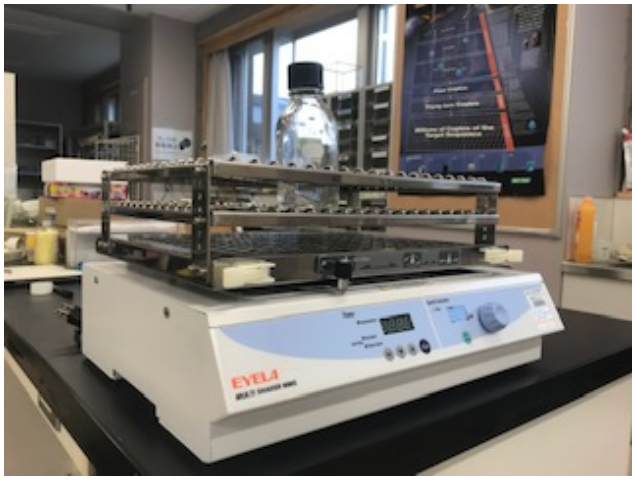


Fig.1 A lab shaker

Fig.2 A magnetic stirrer

2-3-1. Lab shaker

Three different rotation patterns (left-right, circular, figure-eight) were tested at different speeds. The first and second patterns were tested was at speeds of 125 rpm, 135 rpm, 140 rpm, 150 rpm, 175 rpm, 200 rpm. The figure-eight pattern was tested at speeds of 95 rpm, 105 rpm, 110 rpm, 120 rpm. This is because the machine had a max

speed of 120 rpm for the figure-eight setting.

2-3-2. Magnetic stirrer

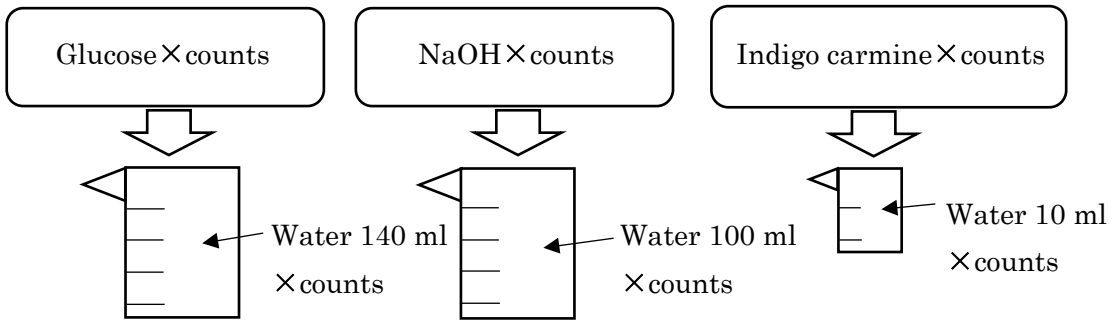
A stirrer bar was inserted into the bottle. Rotation speeds of 1000 rpm, 1350 rpm and 1700 rpm were tested.

2-4. The amount of NaOH

The process was the same as in 2-2 aside from using an electric stirrer instead of stirring by hand. Rotation speed was established at 1700 rpm.

2-5. Type of sugar

Glucose was changed to other sugars to measure the reaction time. Reducibility of sugars was very important in this experiment. Three types of sugar, which were galactose, fructose, and sucrose, were prepared. In this experiment, the way to mix substances was changed. They were first mixed separately to better maintain the concentrations. After each substance was mixed in a 300 ml round bottom flask, they were combined and mixed again. (Fig.3)



※Water indicates Ion-exchanged water

Fig.3 Method of how to mix the substances in experiment 5

3. Result and discussion

3-1. Amount of NaOH

For YR, the original amount of NaOH took the longest followed by double the amount and half the amount. YR was at the beginning of the rotation, so this big a difference

was not expected. For RY, there was a difference of 5 minutes between the solution containing the original amount of NaOH and the doubled / halved amounts. It was believed this is due to the NaOH used in the first trials being old. The second set of trials were done on a different day using new NaOH. This was thought to be the reason for the difference in times. So new NaOH was better than old NaOH.

Table 1. Reaction times between using the original, doubled and half amount of NaOH

	Original amount YR	Doubled YR	Half YR	Original amount RG	Doubled RG	Half RG	(s)
First	0.71	0.60	0.32	9.10	8.43	6.00	
Second	0.80	1.36	0.91	3.45	7.06	3.94	
Avarage	0.76	0.98	0.62	6.28	7.75	4.97	
SD	0.05	0.38	0.30	2.83	0.69	1.03	

	Original amount GR	Doubled GR	Half GR	Original amount RY	Doubled RY	Half RY	(s)
First	79.92	83.90	184.54	419.05	124.73	154.20	
Second	143.23	35.19	162.97	85.88	79.33	101.03	
Avarage	111.58	59.55	173.76	252.47	102.03	127.62	
SD	31.66	24.36	10.79	166.59	22.70	26.59	

3-2. Method of stirring the solution

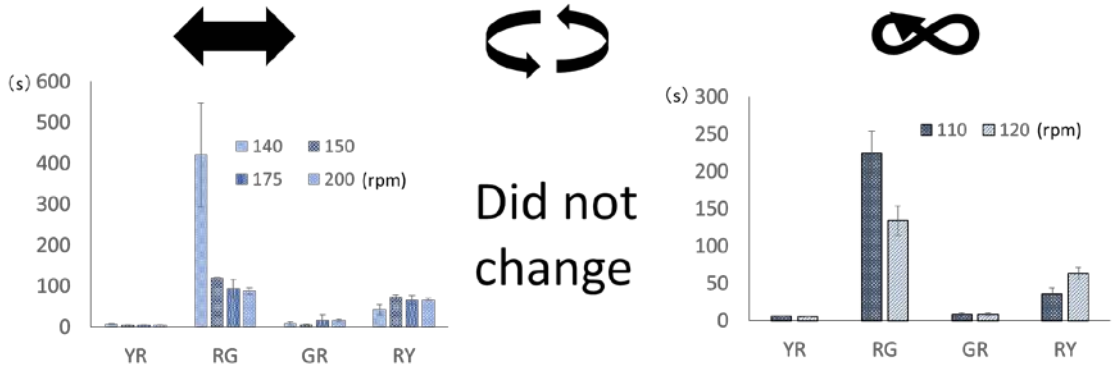


Fig.4 Three different rotation patterns (left-right, middle circular, figure-eight)

The circle pattern did not result in a change of color. That's because centrifugal force was applied in the 300 ml gas collection bottle, so it could not mix with the air in part of the 300 ml gas collection bottle. The left-right pattern at 125 rpm and 135 rpm did not result in a change of color. There was a significant difference between the time it took for the solution to change from red to green at 140 rpm and the other speeds. This

is thought to be because when mixing the solution at 140 rpm, it took longer to reach the oxygen in the air in the bottle (Fig. 5). The figure-eight pattern did result in a change of color at 95 rpm or 105 rpm. There was a difference at RG between 110 rpm and 120 rpm. This difference also is believed to come from a difference in the time taken to reach the oxygen in the bottle.

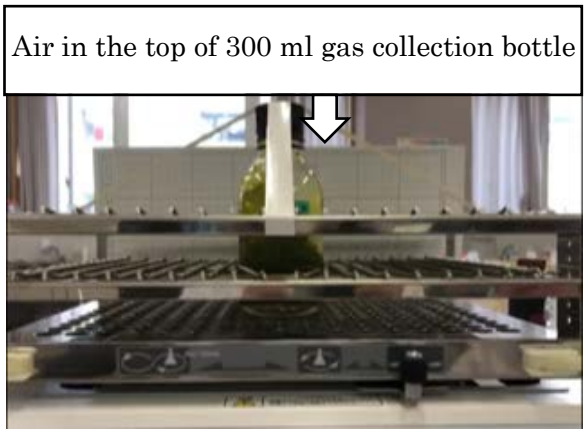


Fig.5 Lab shaker during experiment

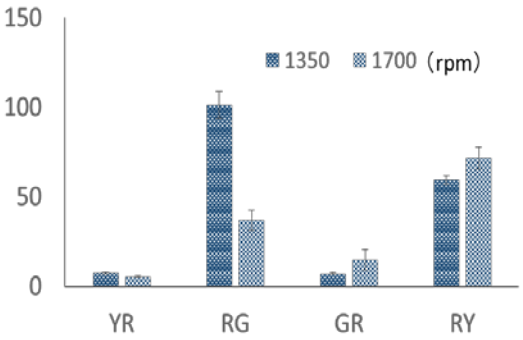


Fig.6 Results of three different rotation speeds

At 1000 rpm the color did not change. At 1350 rpm the color changed from red to green in approximately three minutes, but at 1700 rpm it took approximately one minute. In the shaker, the solution mixed with the air above gas collection bottle, so it changed the color from top to bottom. However, in the stirrer, the air entered the whirlpool inside created by the stirrer bar, so the color changed from the bottom to top. The stirrer was observed to mix the solution more completely, allowing the oxygen to reach the solution more quickly. The surface area touching the air and whirlpool of the solution was bigger than the lab

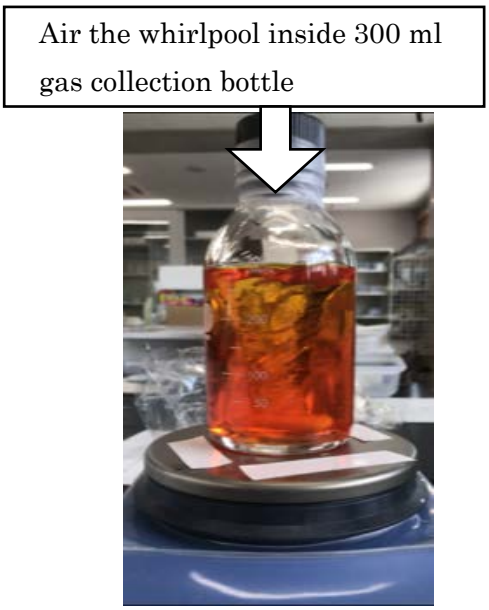


Fig.7 Lab shaker during experiment

shaker, so the oxidation occurred quickly with the magnetic stirrer. The big difference in RG was due to the same reason as using the lab shaker. At the lower rotation speed, it took longer for the oxygen to reach the solution, so it took longer to change color. A larger whirlpool was generated at 1700 rpm, allowing the solution to reach the oxygen more quickly. (Fig.6)

3-3. Amount of NaOH (using a magnetic stirrer)

Table 2. The difference of reaction time in the NaOH using stirrer

	Original amount YR	Doubled YR	Half YR	Original amount RG	Doubled RG	Half RG	(s)
First	5.34	6.18	5.99	36.18	41.9	27.26	
Second	5.48	6.27	4.68	24.5	47.12	25.85	
Third	5.12	5.73	5.65	28.74	42.02	21.45	
Avarage	5.31	6.06	5.44	29.81	43.68	24.85	
SD	0.15	0.24	0.56	4.83	2.43	2.47	

	Original amount GR	Doubled GR	Half GR	Original amount RY	Doubled RY	Half RY	(s)
First	13.43	4.43	33.27	63.15	41.69	77.28	
Second	34.34	5.13	47.17	74.31	52.28	79.33	
Third	30.19	7.24	65.7	90.75	66.35	83.21	
Avarage	25.99	5.6	48.71	76.07	53.44	79.94	
SD	9.04	1.19	13.28	11.34	10.10	2.46	

In GR, there was a big difference between the three patterns. When the amount of NaOH was changed, the speed of reaction changed. This is because the strength was not the same between the three patterns. When the rotation speed was changed, it had a big effect on RG. However, when the amount of NaOH was changed, there was a big difference on GR. The doubled solution was the high-density solution, so it was faster than any other amount of NaOH in GR.

3-4. Type of sugar

Table 3. Reaction times using 2 kinds of sugars

	Glucose YR	Galactose YR	Glucose RG	Galactose RG	Glucose GR	Galactose GR	Glucose RY	Galactose RY	(s)
First	3.88	5.18	25.77	7.59	102.73	133.10	174.81	60.44	
Second	4.34	4.61	20.78	9.93	40.85	137.05	184.56	77.4	
Third	3.96	4.39	23.11	10.40	29.70	148.7	190.54	102.07	
Avarage	4.06	4.73	23.22	9.31	57.76	139.62	183.30	79.97	
SD	0.20	0.33	2.04	1.23	32.12	6.62	6.48	17.09	

The color of galactose changed to a color similar to glucose. However, there was a

big difference between RG and RY. The only difference between the two sugars was the structural formula (Ref.2), so this is thought to be the source of the time difference between that two sugars.

Fructose could change the color until red. Normally, it took longer to shift to RY, but when mixing was stopped, it changed immediately to RY before the stirrer bar stopped. It was found that the ketone group had reducibility, and that speed was faster than glucose. Fructose contains ketone groups which results in reducibility (Ref.2), whereas glucose contains aldehyde groups, leading to this difference between glucose and fructose, because NaOH is a strong base. When fructose was added to the solution, reduction was stronger than oxidation of fructose.

The color of sucrose could not be changed to yellow when mixing the solution. It only changed from green to red in the first change. Sucrose does not normally demonstrate reducibility (Ref.3), because it is a disaccharide, but it did in this experiment. Sucrose become an invert sugar which consisted of glucose and fructose when it was hydrolyzed by dilute acid and enzyme sucrase after that it demonstrated reducibility (Ref.3). However, this experiment did not use either, so this factor did not affect the reducibility. This sucrose had to have demonstrated reducibility for some other reason. However, it did not demonstrate strong reducibility like glucose and fructose because it does not normally demonstrate reducibility. This is the reason it only changed from green to red.

4. Future Plan

There is a possibility that the concentration of NaOH was affected by the reduction which occurred in the GR part. I would like to check by increasing and decreasing the concentration of it and observing how changing the time affects the results.

Because sucrose was able to demonstrate reducibility for some reason, a part of color was changed. In the future, I need to use another disaccharide to check whether it changes the color or not.

Additionally, I need to change the temperature under which the experiment is conducted.

5. References

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The time dependent change of redox reaction under various conditions

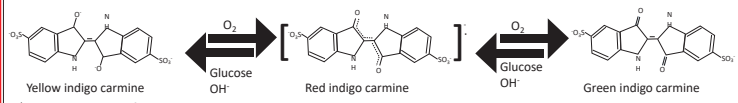
Ritsumeikan High School

Abstract

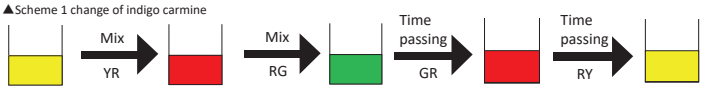
This research examines how changes in the amount of NaOH or stirring conditions affects the speed and time of redox reaction using the chemical traffic light experiment. How to mix and change the substances types and amounts were mainly discussed in this research. In most of the experiments, the time and speed changed. The mixing method was found to greatly affect the redox reaction of solutions. These experiments are redox reaction, so having air in the bottle is very important for these experiments. The method by which to mix the solutions so that they would come in contact with air was determined. The substances were affected by the reducibility in these experiments. For example, there are many types of sugar and not all sugars possess reducibility. Furthermore, the amount of substances was very important, because concentration was affected by it. These factors affected both the speed and time redux reactions conducted in this research.

Introduction

The chemical traffic light experiment is a popular experiment used to demonstrate the chemical clock reaction. In it, the color of the solution changes after being stirred and left in a pattern resembling a traffic light. The version of the experiment focused on in this research uses NaOH, Glucose, and Indigo carmine. When these materials are dissolved in Ion-exchanged water, and mixed, they transform into "Green Indigo carmine" (Indigo carmine is blue when dissolved in water, but it becomes green in alkaline solutions.) It then changes to a red intermediate through which it becomes a reduced yellow indigo carmine. When the reduced yellow indigo carmine is oxidized, it is immediately converted into a red intermediate, and from this intermediate, it transforms back into a green indigo carmine. However, if left for a short time, glucose becomes a reducing agent, and it is converted into a red intermediate, and changes into yellow indigo carmine. This research focused on measuring the time it takes for the solution to change from yellow to red(YR), red to green(RG), green to red(GR), and red to yellow (RY) (Scheme 1) (Fig. 1).

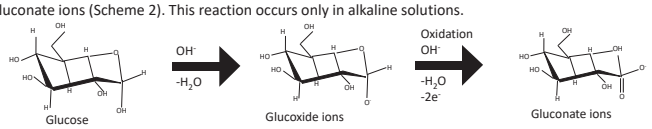


▲Scheme 1 change of indigo carmine



▲Fig. 1 Color change of this reaction

Glucose transforms into glucoxide ions with reducing properties in an NaOH solution and oxidizes into gluconate ions (Scheme 2). This reaction occurs only in alkaline solutions.



▲Scheme 2 Change of glucose

Material

〈Regent〉

NaOH 5.0 g, Glucose 3.0 g, Indigo carmine 0.05 g, Ion exchange water 250 ml

〈Instrument〉

300 ml Beaker, 300 ml Gas collection bottle, 300 ml Graduated cylinder, 300 ml Round bottom flask, Lab dispensing spoon, Glass rod, Plastics petri dishes, Pipette, Parafilm

Experiment 1 ～ Quantity of NaOH ～

Method

Reaction times between using the original amount of NaOH, double the amount and half the amount were measured and compared (Table 1). The chemicals were mixed into a solution using the beaker – flask method as above. The flask was mixed by hand.

Result & Discussion

▼Table 1 Reaction times between using the original, doubled and half amount of NaOH

	Original amount YR	Doubled YR	Half YR	Original amount RG	Doubled RG	Half RG	(s)
First	0.71	0.60	0.32	9.10	8.43	6.00	
Second	0.80	1.36	0.91	3.45	7.06	3.94	
Avarage	0.76	0.98	0.62	6.28	7.75	4.97	
SD	0.05	0.38	0.30	2.83	0.69	1.03	

	Original amount GR	Doubled GR	Half GR	Original amount RY	Doubled RY	Half RY	(s)
First	79.92	83.90	184.54	419.05	124.73	154.20	
Second	143.23	35.19	162.97	85.88	79.33	101.03	
Avarage	111.58	59.55	173.76	252.47	102.03	127.62	
SD	31.66	24.36	10.79	166.59	22.70	26.59	

For YR, the original amount of NaOH took the longest followed by double the amount and half the amount. YR was at the beginning of the rotation, so this big a difference was not expected. For RY, there was a difference of 5 minutes between the solution containing the original amount of NaOH and the doubled / halved amounts. It was believed this is due to the NaOH used in the first trials being old. The second set of trials was done on a different day using new NaOH. This was thought to be the reason for the difference in times, So new NaOH was better than old NaOH.

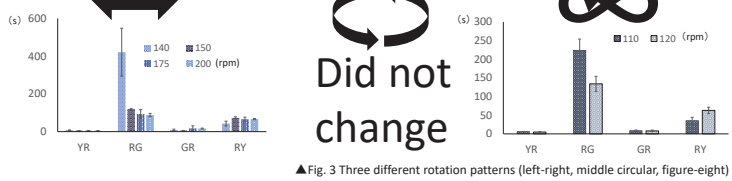
Experiment 2 ～ Comparison mix speed in lab shaker～

Method

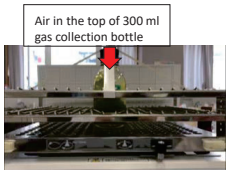
In this experiment, a lab shaker (Fig. 2) was used to mix the solutions (Fig. 3). Three different rotation patterns (left-right, circular, figure-eight) were tested at different speeds. The first and second patterns were tested was at speeds of 125 rpm, 135 rpm, 140 rpm, 150 rpm, 175 rpm, 200 rpm. The figure-eight pattern was tested at speeds of 95 rpm, 105 rpm, 110 rpm, 120 rpm. (This is because the machine had a max speed of 120 rpm for the figure-eight setting.) The chemicals were combined in the same way and amounts as in Experiment 1. Then, they were wrapped in parafilm to seal them off from open air and prevent the solutions from leaking out.

▲Fig. 2 A lab shaker

Result & Discussion



▲Fig. 3 Three different rotation patterns (left-right, middle circular, figure-eight)



▲Fig. 4 A lab shaker during the experiment

The circle pattern did not result in a change of color. That's because centrifugal force was applied in the 300 ml gas collection bottle, so it could not mix the upper part air of 300 ml gas collection bottle. The left-right pattern at 125 rpm and 135 rpm did not result in a change of color. There was a significantly difference between the time it took for the solution to change from red to green at 140 rpm and the other speeds. This is thought to be because when mixing the solution at 140 rpm, it took longer to reach the oxygen in air in the bottle (Fig. 4). The figure-eight pattern did result in a change of color at 95 rpm or 105 rpm. There was a difference at RG between 110 rpm and 120 rpm. This difference also is believed to come from a difference in the time taken to reach the oxygen in the bottle.

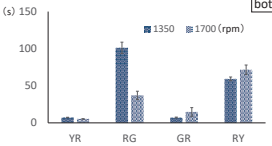
Experiment 3 ～ Comparison mix speed in magnetic stirrer ～

Method

In this experiment, a magnetic stirrer was used to mix the solutions. The preparation of the bottle was the same as in Experiment 2. A stirrer bar was inserted into the bottle. Rotation speeds of 1000 rpm, 1350 rpm and 1700 rpm were tested.

▸Fig. 5 A magnetic stirrer

Result & Discussion



▲Fig. 6 Three different rotation speed graph

◂Fig. 7 A lab shaker during the experiment

At 1000 rpm the color did not change. At 1350 rpm the color changed from red to green in approximately three minutes, but at 1700 rpm it took approximately one minute. In the shaker, the solution mixed with the air above gas collection bottle, so it changed the color from top to bottom. However in the stirrer, the air entered the whirlpool inside created by the stirrer bar, so the color changed from the bottom to top.

The stirrer was observed to more completely mix the solution, allowing the oxygen to reach the solution more quickly. The big difference in RG is due to the same reason as in Experiment 2. At the lower rotation speed it took longer for the oxygen to reach the solution, so it took longer to change color. A larger whirlpool was generated at 1700 rpm, allowing the solution to reach the oxygen more quickly.

Experiment 4～ Quantity of NaOH using stirrer～

Method

Reaction times between using the original amount of NaOH, double the amount and half the amount were measured and compared using a stirrer. The process was the same as in Experiment 1 aside from using an electric stirrer instead of stirring by hand. It is turned 1700 rpm.

Result & Discussion

▼Table 2 The difference of reaction time in the NaOH using stirrer

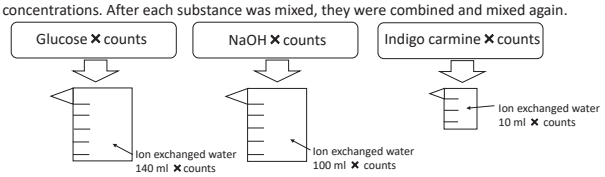
	Original amount YR	Doubled YR	Half YR	Original amount RG	Doubled RG	Half RG	(s)
First	5.34	6.18	5.99	36.18	41.9	27.26	
Second	5.48	6.27	4.68	24.5	47.12	25.85	
Third	5.12	5.73	5.65	28.74	42.02	21.45	
Avarage	5.31	6.06	5.44	29.81	43.68	24.85	
SD	0.15	0.24	0.56	4.83	2.43	2.47	

	Original amount GR	Doubled GR	Half GR	Original amount RY	Doubled RY	Half RY	(s)
First	13.43	4.43	33.27	63.15	41.69	77.28	
Second	34.34	5.13	47.17	74.31	52.28	79.33	
Third	30.19	7.24	65.7	90.75	66.35	83.21	
Avarage	25.99	5.6	48.71	76.07	53.44	79.94	
SD	9.04	1.19	13.28	11.34	10.10	2.46	

In GR, there was a big difference between the three patterns. When the amount of NaOH was changed, the speed of reaction changed. This is because the strength was not the same between the three patterns. When the rotation speed was changed, it had a big effect on RG. However, when the amount of NaOH was changed, there was a big difference on GR. The doubled solution was the densest solution, so it was faster than any other amount of NaOH in GR. That reaction (Scheme 2) became faster by amount of NaOH.

Pre-experiment

From experiment 5, the way to mix substances was changed. They were first mixed separately to better maintain the concentrations. After each substance was mixed, they were combined and mixed again.



▲Fig. 8 Method of how to mix the substances in experiment 5

Experiment 5～ Change the type of sugar～

Method

In this experiment, glucose was changed to other sugars to measure the reaction time. Reducibility of sugars was very important in this experiment. Three types of sugar, which were galactose, fructose and sucrose, were prepared.

Result & Discussion

▼Table 3 Reaction times using 2 kinds of sugars

	Glucose YR	Galactose YR	Glucose RG	Galactose RG	Glucose GR	Galactose GR	Glucose RY	Galactose RY	(s)
First	3.88	5.18	25.77	7.59	102.73	133.10	174.81	60.44	
Second	4.34	4.61	20.78	9.93	40.85	137.05	184.56	77.4	
Third	3.96	4.39	23.11	10.40	29.70	148.7	190.54	102.07	
Avarage	4.06	4.73	23.22	9.31	57.76	139.62	183.30	79.97	
SD	0.20	0.33	2.04	1.23	32.12	6.62	6.48	17.09	

The color of galactose changed to a color similar to glucose. However, there was a big difference between RG and RY. The only difference between the two sugars was the structural formula.

Fructose could change the color until red. Normally, it took longer to shift to RY, but when mixing was stopped, it changed immediately to RY. It appeared that Ketone group had reducibility, and that speed was faster than glucose. Fructose contains ketone groups which results in reducibility, whereas glucose contains aldehyde groups, leading to this difference between glucose and fructose.

The color of sucrose could not be changed to yellow when mixing the solution. It only changed from green to red in the first change. Sucrose does not normally demonstrate reducibility, but it did in this experiment. Sucrose normally does not demonstrate reducibility because it is a disaccharide.

Future plan

In the future I would like to do an experiment using other disaccharides to measure the reaction of the color and use a stirrer which can change the temperature in order to observe how changing the temperature of the solution affects the time between color changes.

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研究番号	20SS047	研究について	□個人　■グループ
研究テーマ	Making Antibacterial Sheets by Using Psoralen　～ソラレンを使用した抗菌シートの作成～		

A：研究目的（Purpose）

ソラレンを用いて人体への影響の少ない抗菌シートの作成。

B：研究方法（Materials & Methods）

実験Iではソラレンと、Mock control で用いる DMSO、ブロッコリーから採取した DNA を用いスペクトル解析を行った。実験IIでは蛍光励起スペクトルによりソラレンのピークトップを調査した。実験IIIでは、サンプルとソラレン、または DMSO を大腸菌または酵母と共培養し、希釈して培地に塗布し、UV を照射した。実験IVではソラレンと寒天を用い、乾燥させて抗菌シートを作成した。

C：研究結果（Result）

実験Iからソラレンは DNA と結合してスペクトルの波形がシフトした。実験IIかソラレンを 250 nm 以上の UV の波長で励起させると蛍光を発した。実験IIIから、ソラレン濃度が原液 2.0 Mの 1/100 と UV 10 分の組み合わせで最も抗菌性を示した。実験IVからソラレンを使用したシートの作成は可能であった。

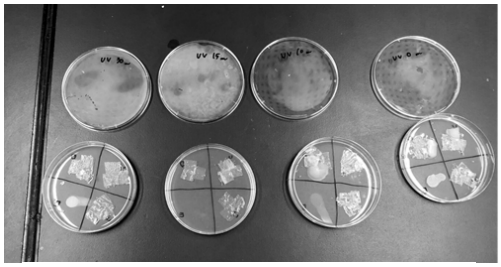

図１　作成したシートの抗菌実験

D：考察（Discussion）

ソラレンは波長が約 300 nm で蛍光を示し、DNA と結合した。ソラレンと UV の組み合わせは抗菌作用を持つ。

E：今後の展望（Future Direction of Research）

真核生物と原核生物への影響の違いを調べるために他のサンプルを使用する。金属イオンとの併用も検討している。野菜、果物から採取したソラレンから抗菌シートを作成する。

F：参考文献（References）

(Ref. 1) Ghasemi, F., Rostami, S., Nabavinia, M. S., & Meshkat, Z. (2016). Developing Michigan Cancer Foundation 7 Cells with Stable Expression of E7 Gene of Human Papillomavirus Type 16. *Iranian journal of pathology*, *11*(1), 41–46. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4749194/

(Ref. 2) Panno, M. L., & Giordano, F. (2014). Effects of psoralens as anti-tumoral agents in breast cancer cells. *World journal of clinical oncology*, *5*(3), 348–358. https://doi.org/10.5306/wjco.v5.i3.348　https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4127606/

1. 序論

ほとんどの抗菌シートにはアルコールが含まれているが、アルコールは皮膚の炎症の原因となる。ソラレン(図 1)は分子量 186.16 の物質で、酢酸エチルやアセトンに溶け、光毒性や抗酸化性、抗菌性を持つ。ソラレンはオランダビユの種子や柑橘類などに含まれており、UVA 波との組み合わせで乾癬の治療法である PUVA 療法として使われている。果物や野菜から抽出したソラレンを利用して抗菌シートの作成ができれば、人体への害の少ない抗菌シートができるだけでなく、廃棄食品の有効活用にもつながる。

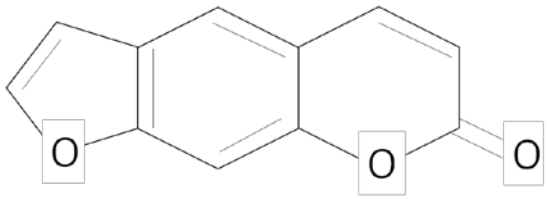

図 1　ソラレンの構造式

2. 方法

実験器具

・Psoralen　（原液 2 M）
・Dimethyl Sulfoxide　（以下 DMSO）
・ブロッコリー
・遠心分離機
・Tris-HCl
・EDTA
・NaCl
・SDS
・乳鉢、乳棒
・漏斗
・試験管
・EtOH
・パスツールピペット
・駒込ピペット
・メスシリンダー
・キムワイプ
・吸光光度計
・石英キュベット
・マイクロピペット
・15 mL チューブ
・*E.coli*
・*S.cerevisiae*
・Trypton
・NaCl
・Yeast extract
・Agar
・Polypeptone
・Glucose
・三角フラスコ
・ビーカー
・メジューム瓶
・インキュベーター
・クリーンベンチ
・シャーレ
・キムタオル

I—１　ブロッコリーから DNA 抽出

1 M の Tris-HCl を 1.0 mL、0.5 M の EDTA を 2.0 mL、1 M の NaCl を 15.0 mL、1％の SDS を 10.0 mL を混ぜて 100 mL にメスアップし Buffer を作成した。ブロッコリーを乳鉢と乳棒ですりつぶし､Buffer を適量かけキムワイプで試験管に濾した｡試験管に EtOH を適量入れ、浮遊してきた DNA をパスツールピペットでチューブに取り出した。チューブを 10,000 rpm の遠心分離機に 2 分かけ、上澄みをパスツールピペットで取り除き、70％ EtOH で洗浄した。その後、10,000 rpm の遠心分離機に 2 分かけチューブをひっくり返して乾燥させた。

I—２　H_2O ベースでスペクトル解析

実験I—1 で抽出した DNA をイオン交換水 200 μL に溶かし、13000 rpm の遠心分離機に５分かけ上澄みを集めた。H_2O 2.5 mL と DNA の上澄み液 500μL を混ぜた、以下 DNA 抽出溶液と、H_2O 3 mL にそれぞれソラレンまたは DMSO を入れ、ソラレン濃度が 0, 1/500, 1/1000 の溶液を作成し、200 nm から 400 nm の範囲でスペクトル解析を行った。

I—３　EtOH ベースでスペクトル解析

EtOH を溶媒としソラレンが 1.97×10^{-4} mol/L の溶液のスペクトル解析を 300 nm から 1000 nm の範囲で行った。

II　EtOH ベースで蛍光励起スペクトル測定

溶媒が EtOH でソラレンが 1.97×10^{-4} mol/L の溶液を用い蛍光励起スペクトルを測定した。

III—１　培地の作成

LB 培地は、Tryptone 10.0 g、Yeast extract 5.0 g、NaCl 10.0 g、Agar 15.0 g を H_2O1000 mL に加え作成した。YPD 培地は Polypeptone 10.0 g、Yeast extract 5.0 g、Glucose 10.0 g、Agar 5.0 g を H_2O1000 mL に加えて作成した。液体培地の場合、Agar を入れずに同じ手順で作成した。

III—２　共培養

ソラレン、または DMSO と各サンプルを 10 mL の液体培地に加えた。このときサンプルが *E.coli* の場合には LB 培地、*S.cerevisiase* の場合では YPD 培地を用いた。ソラレン、DMSO の濃度は 0, 1/100, 1/500, 1/1000 に設定した (表 1)。その後 144 rpm、のシェイカーで攪拌しながら一晩培養した。温度は *E.coli* の場合には 37℃、*S.cerevisiase* の場合では 30℃で行った。攪拌した培地を原液とし、100 μL チューブにとりその溶液を No.0 とし、No.0 から 10 μL とり H_2O を 90 μL 加えて No.1 の溶液を作成するという手順で次の溶液が前の溶液の 1/10 の濃度になるように希釈を繰り返した。希釈した溶液を 10μL ずつ各々のサンプルに適した

培地に塗布を行い(図 2)、UV を照射した。照射時間は 0 分、10 分、30 分に設定した。最後に *E.coli* の場合には 37℃、*S.cerevisiase* の場合では 30℃で一晩培養した。

表 1 実験IIIの実験条件

	Culture medium	Solvent	Concentrations	Organism used
A	LB medium			*E.coli*
B				
C		Psoralen	1/100	
D		DMSO		
E		Psoralen	1/500	
F		DMSO		
G		Psoralen	1/1000	
H		DMSO		

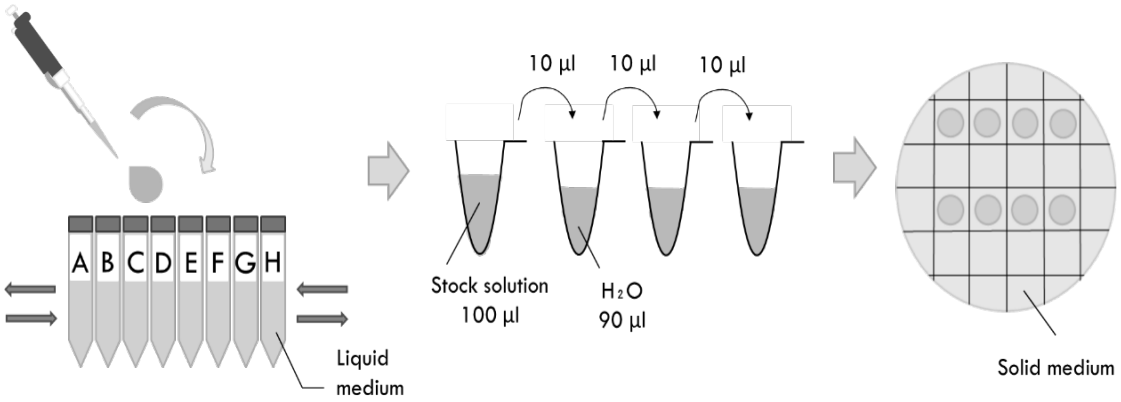

図 2　攪拌から塗布までの流れ

IV　シートの作成

H_2O 20 mL ベースで Agar 加え 2%の溶液を作成し、シャーレに流し込み、ソラレンまたは DMSO を 100 µL 加えた。硬化してゲル状になったものをキムワイプの上で一晩乾燥させ、剥がしとった。比較実験のため Agarose と H_2O 以外に何も加えないものも作成した。1 cm 平方ずつに切り、一か所に 3 枚ずつ繋げて LB 培地にのせ、その上に *E.coli* を 10 µL 塗布した(図 3)。その後 UV 照射を 0 分、1 分、10 分、15 分、30 分で設定して行った。最後に 37℃で培養した。

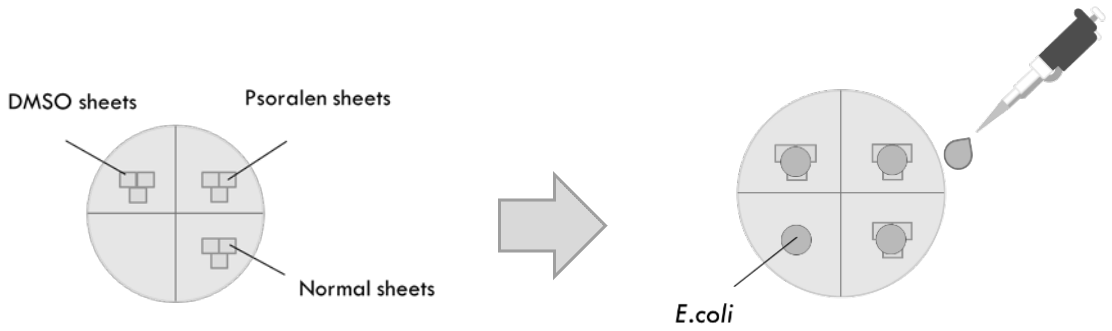

図 3　シートの貼り付けから大腸菌塗布の流れ

3.　結果

3-1. ソラレンを DNA 抽出液と掛け合わせた時、結合し、スペクトルの波形がシフトした。また、溶液が水、エタノールに関わらず光を吸収した（図 4、5、6、7）。

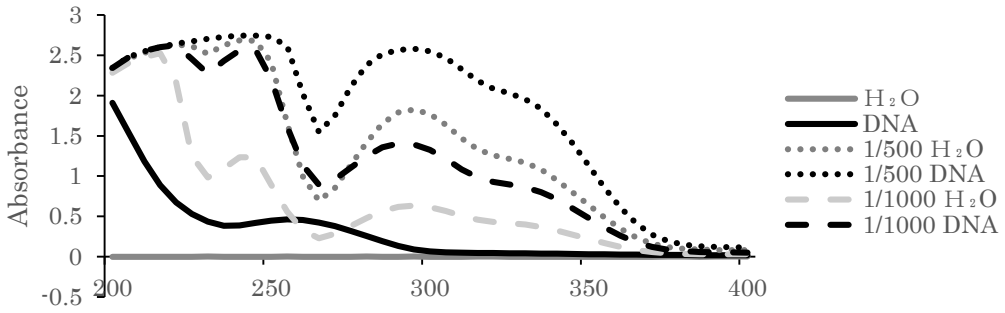

図 4　ソラレンのスペクトル解析

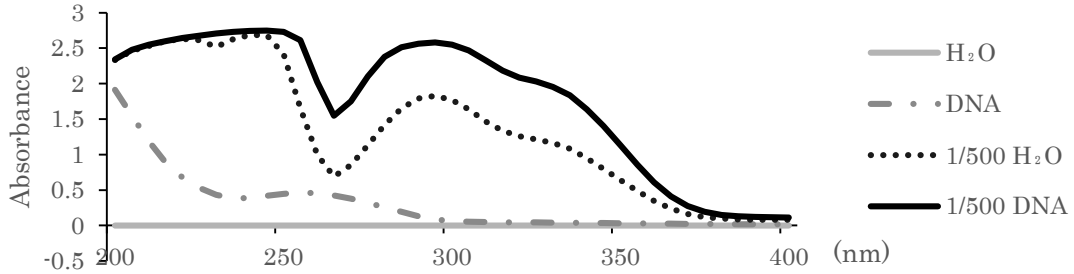

図 5　1/500 のソラレンのスペクトル解析

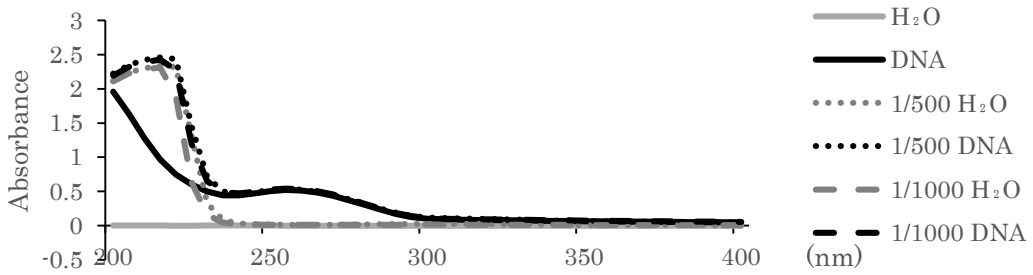

図 6　DMSO のスペクトル解析

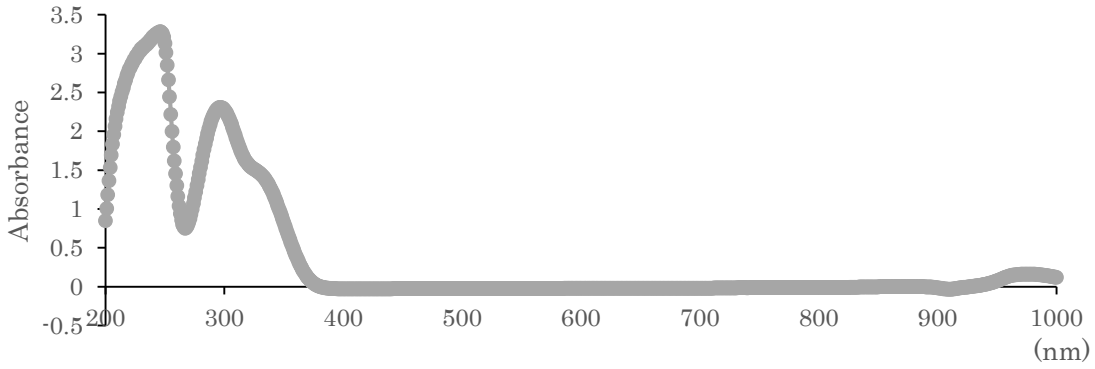

図 7　ソラレンの吸光度

3-2. ソラレンを 250 nm 以上の UV の波長で励起させると蛍光を出した。
蛍光励起 450 nm の時に現れた（図 8、9）。

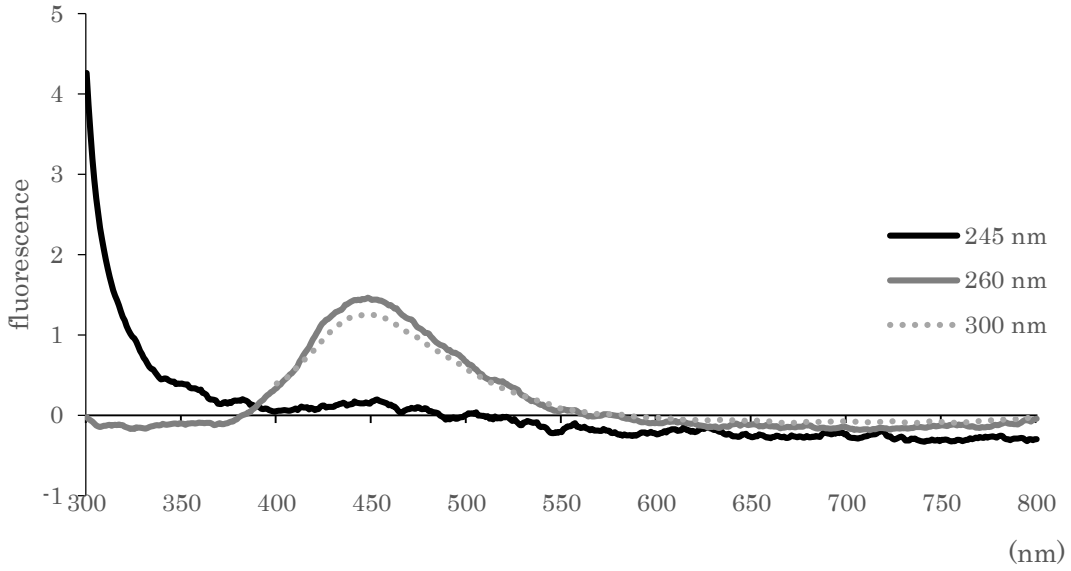

図 8　ソラレンの蛍光励起

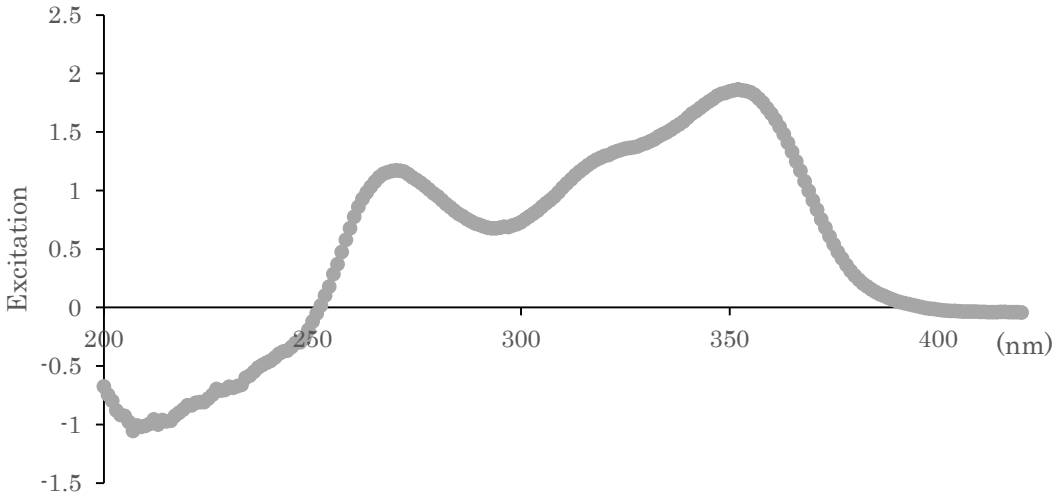

図 9　ソラレンの励起

3-3. ソラレン濃度が原液 2.0 M の 1/100 と UV 照射時間が 10 分の組み合わせの時、最も抗菌性を示した。

大腸菌、酵母に関わらず死滅した（表 2、3)。

表 2　LB 培地における結果

	A	B	C	D	E	F	G	H
UV0	○○○○	●●●●						
UV10	○○○○	●○○○	○○○○	●●●○	●○○○	●●●○	●○○○	●●●○
UV30	○○○○	●○○○	○○○○		●○○○		○○●○	●●○●

表 3　YDP 培地における結果

	A	B	C	D	E	F	G	H
UV0	○○○○	●●●●						
UV10	○○○○			●●○○	○○○○	●○○○	●●●○	●●○○
UV30	○○○○							●○○○

○ indicates samples did not breed

● indicates samples bred

3-4. ソラレンを使用した抗菌シートの作成は可能。

今回は汚染されてしまい、よい結果が取れていないが作成自体は可能である(図 10、11)。

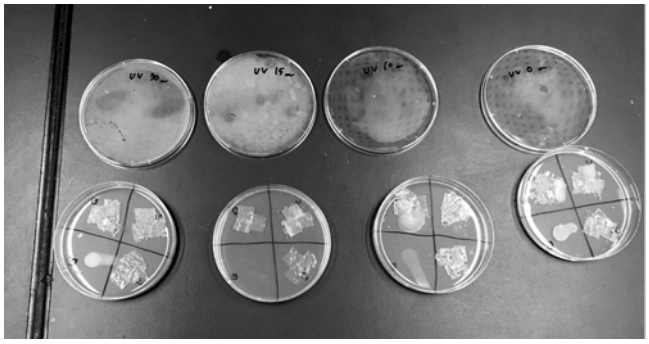

図 10　実験 4 の結果

図 11　シート

4. 考察

実験Iのグラフから、ソラレンは溶媒が H_2O、EtOH のどちらかにかかわらず、光吸収の作用を示したといえる。ソラレンは 300 nm で蛍光を示し、DNA と結合したと考えられる。また、ソラレンと UV との組み合わせによって抗菌作用を示す。加えて、共培養を行った際のみ抗菌作用が見られたことから、サンプルは分裂時にソラレンを取り込んでいると考えられる。

5. 今後の展望

すでに使用した大腸菌や酵母とは異なるサンプルを用いることで真核生物や原核生物の違いが関与しているかを見出せる可能性が高い。また、抗菌作用を持つ亜鉛イオンなどの金

属イオンを用い、抗菌作用の相乗効果を確かめる必要がある。加えて現在は粉末のソラレンを用いて実験を行っているので実際の野菜、果物から抽出して効果を確かめてみる必要もある。

6. 参考文献

(Ref. 1) Ghasemi, F., Rostami, S., Nabavinia, M. S., & Meshkat, Z. (2016). Developing Michigan Cancer Foundation 7 Cells with Stable Expression of E7 Gene of Human Papillomavirus Type 16. *Iranian journal of pathology*, *11*(1), 41–46.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4749194/

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https://www.livescience.com/38039-what-causes-sunburns.html

(Ref. 3) Panno, M. L., & Giordano, F. (2014). Effects of psoralens as anti-tumoral agents in breast cancer cells. *World journal of clinical oncology*, *5*(3), 348–358.
https://doi.org/10.5306/wjco.v5.i3.348
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4127606/

(Ref. 4) Panno, M. L., Giordano, F., Mastroianni, F., Palma, M. G., Bartella, V., Carpino, A., Aquila, S. & Andò, S. (2010). Breast cancer cell survival signal is affected by bergapten combined with an ultraviolet I irradiation, *FEBS Letters*, 584.
https://doi.org/10.1016/j.febslet.2010.04.001

7. 謝辞

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Making Antibacterial Sheets by Using Psoralen

G12 SSG
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JSSF 2020
January 08, 2021

Abstract

This paper describes and analyses the antibacterial effect of Psoralen while focusing on using it to create antibacterial sheets. Most antibacterial sheets contain alcohol, which can cause inflammation to the skin. We hypothesized that making antibacterial sheets utilizing the properties of Psoralen, which are not harmful to the human body, would help alleviate these problems. Psoralen, DMSO as mock controls and samples of *E. coli and S. cerevisiae* were used to confirm if Psoralen really has an antibacterial effect. Spectrum analysis, fluorescence activation, excitation activation and coculture were carried out. An antibacterial effect was most clearly demonstrated when the concentration of Psoralen was 1/100 and irradiation time was 10 minutes. Also, Psoralen absorbed UV light and combined with DNA extract. It was found that sheets can be made by using Psoralen. However, it was not found yet if it has significant antibacterial effects.

Keywords: psoralen, antibacterial, *E.* coli, S. *cerevisiae*, UV light

Introduction

Most antibacterial sheets contain alcohol. However, the central issue in using alcohol is allergies. Some people have an allergic reaction to alcohol. Also, alcohol can cause inflammation to the skin. Sometimes it also causes the skin to become dry. Making antibacterial sheets, which are not harmful to the human body, will help alleviate these problems. Psoralen's molecular weight is 186.16, and it dissolves in ethyl acetate and acetone. In addition, it is one of the components contained in phototoxic substances (Ref. 3). Phototoxicity is a substance that increases UV absorption and causes skin irritation and pigmentation. Also, Psoralen is used as a therapeutic agent in a psoriasis treatment called PUVA therapy (Ref. 1). In addition, according to report, Psoralen is known as the substance which has anticancer, antibacterial, antioxidant and other beneficial properties (Ref. 3). If Psoralen has antibacterial effects, we can make the harmless antibacterial sheets by using Psoralen extracted from vegetables and fruits. In this study, we focused on using the properties of Psoralen to create antibacterial sheets.

Materials and Method

Experiment I

The spectrum analysis of Psoralen and Dimethyl sulfoxide (DMSO) was investigated. Solvents were only H_2O or DNA extracted from broccoli. Also, the concentrations of Psoralen and DMSO were changed to 0, 1/5000, 1/1000, 1/500 and 1/100. The wavelength was from 200 to 400 nm. In addition, light absorption of Psoralen whose solvent is ethanol was researched by using a 1.97×10^{-4} mol/L solution of EtOH and solute Psoralen as solvent. The conditions were medium sensitivity, 2 nm broad of band, 400 nm/s scanning speed and 300-1000 nm wavelength.

Experiment II

Fluorescence and excitation were investigated by using a 1.97×10^{-4} mol/L solution of Psoralen and EtOH. The conditions were 4 second response, medium sensitivity, 200 nm/s scanning speed and 1 nm data acquisition.

Experiment III

Coculture was carried out. First, samples (*E. coli* or *S. cerevisiae*) and Psoralen or DMSO were added to a liquid medium. Second, they were cultured overnight. After that, they were diluted and applied. Finally, the mediums were irradiated with UV radiation. They were kept at 37℃ and observed overnight. In all experiments and conditions, DMSO was used as a mock control. The effect of Psoralen on each sample was compared.

Experiment IV

The sheets were made from 100 μl Psoralen, 2％ agar and 20 ml H_2O, and their antibacterial effect was observed. Sheets were dried and cut into 1 cm squares and attached to the LB medium. *E. coli* was diluted on the sheets, and they were irradiated with UV light (0, 10, 15, 30 min). After that, the sheets were turned over, and *E.coli* were applied to the mediums. The mediums were kept at 37℃ and observed overnight.

Data Analysis

In Experiment I, the solvents used were H_2O and DNA extract. It was found that Psoralen had an effect on light absorption. When Psoralen was combined with DNA extract, the pattern of wavelengths shifted (Fig.1, 2 and 3). Psoralen absorbed the light regardless of whether the solvent was H_2O or ethanol (Fig. 1, 2, 3, and 4).

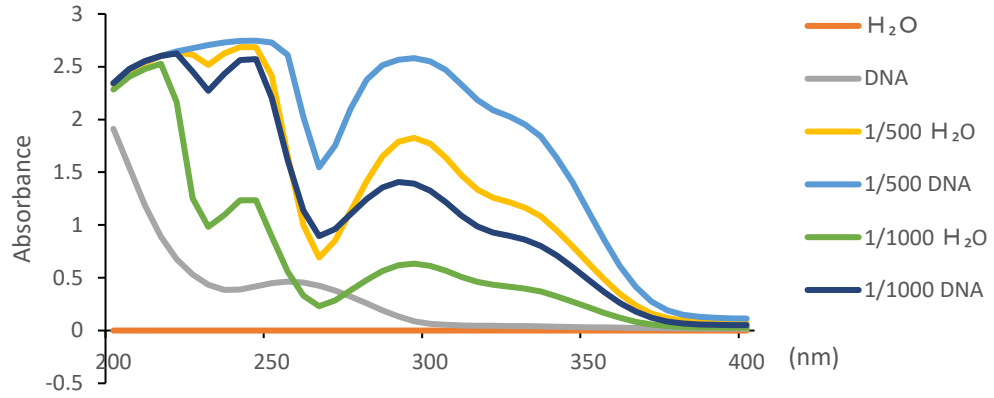

Fig. 1 Spectrum analysis of Psoralen

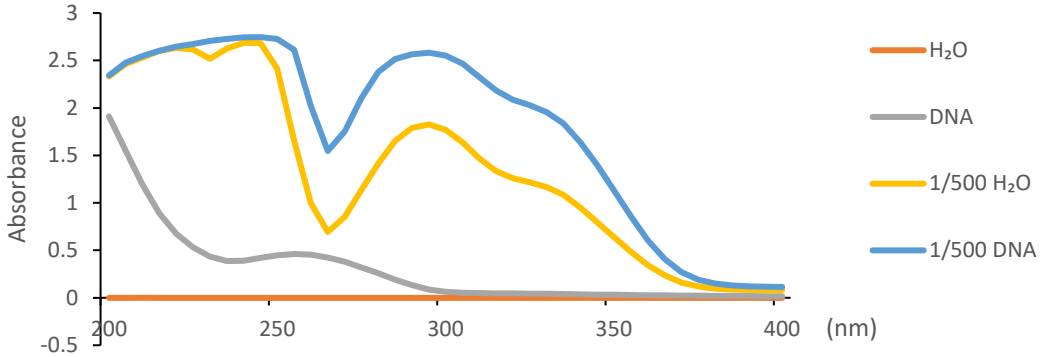

Fig. 2 Spectrum analysis of 1/500 Psoralen

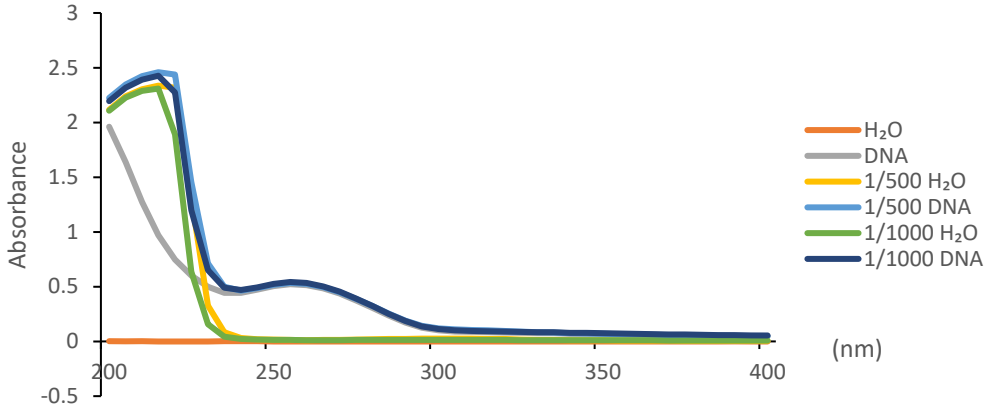

Fig. 3 Spectrum analysis of DMSO

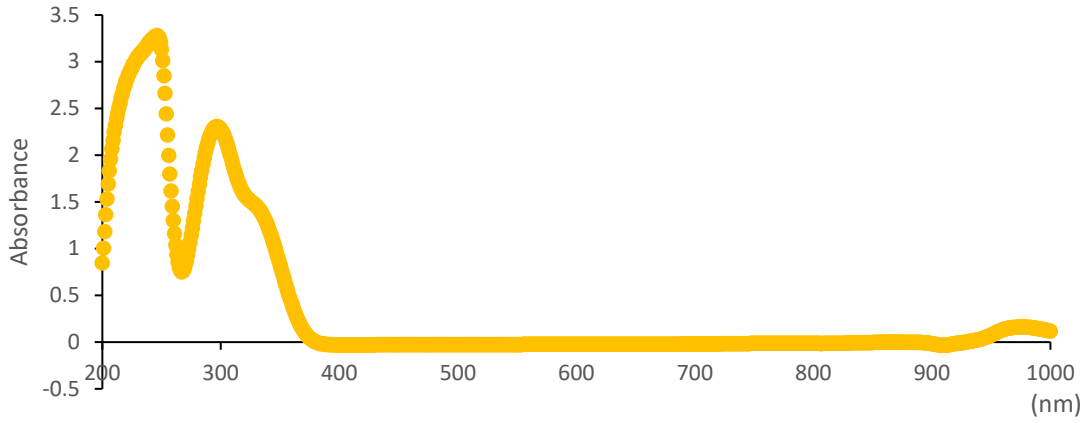

Fig. 4 Absorbance of Psoralen EtOH

In Experiment II, when the wavelength, 260 nm and 300 nm was applied to the solution, fluorescence appeared at 450 nm (Fig.5). From the result of excitation, it was found that Psoralen absorbs UV light less than 250 nm, but it was not used for fluorescence (Fig. 6).

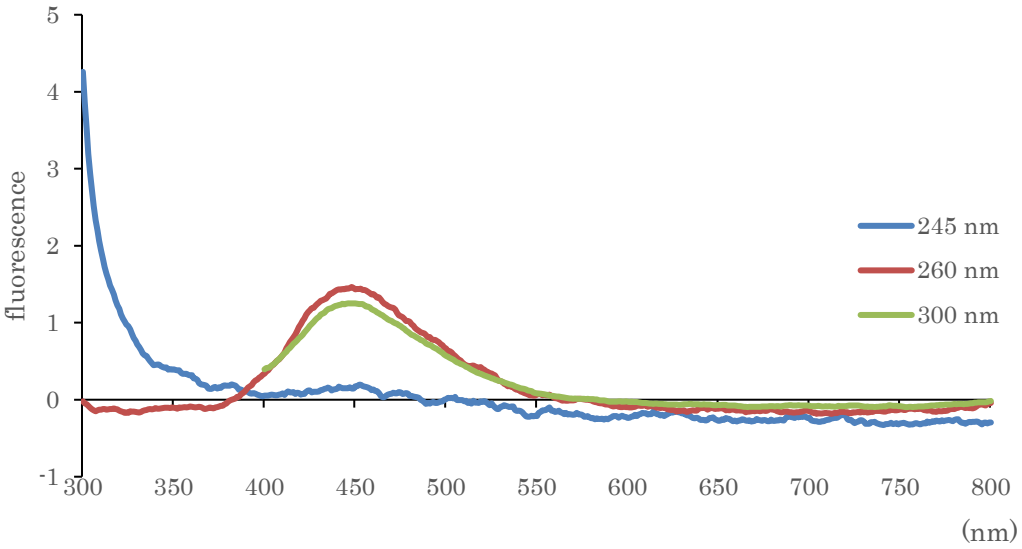

Fig.5 Fluorescence of Psoralen

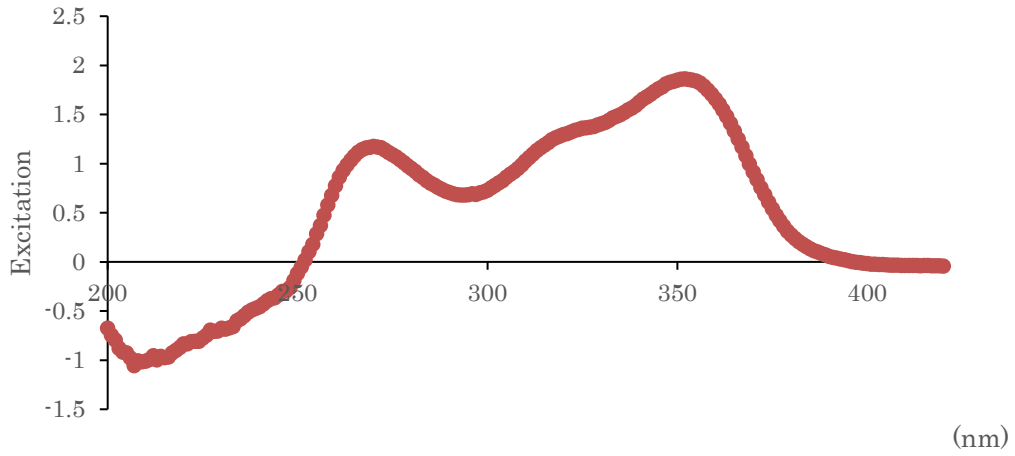

Fig.6 Excitation of Psoralen

In Experiment III, all the samples died when irradiated for 30 minutes in every condition. When irradiated for only 10 minutes, the 1/1000 concentration of either Psoralen or DMSO was found to decrease the reproductive rate of the samples. This means that the reduced reproductive rate is not related to the presence of Psoralen or DMSO. When the Psoralen concentration was 1/100 and the UV radiation was for 10 minutes, then the sample was killed in the case of both *E. coli* and *S. cerevisiae* (Tables 1 and 2).

Table 1 Results of Experiment III, LB medium

	A	B	C	D	E	F	G	H
UV0	○○○○	●●●●						
UV10	○○○○	●○○○	○○○○	●●●○	●○○○	●●●○	●○○○	●●●○
UV30	○○○○	●○○○	○○○○		●○○○		○○●○	●●○●

Table 2 Results of Experiment III, YDP medium

	A	B	C	D	E	F	G	H
UV0	○○○○	●●●●						
UV10	○○○○			●●○○	○○○○	●○○○	●●●○	●●○○
UV30	○○○○							●○○○

○ indicates samples did not breed ● indicates samples bred

In Experiment IV, sheets were successfully made, but a sufficient result was not found because they were contaminated (Fig. 7 and 8).

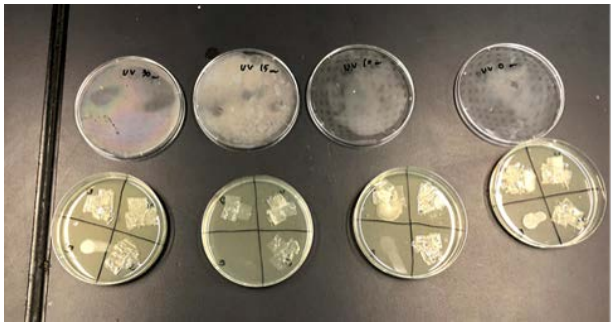

Fig. 7 Result of Experiment IV

Fig. 8 Sheets

Discussion

It was found that Psoralen influenced light absorption whether the solvent was H_2O and EtOH. It was determined that H_2O can be used as a solvent instead of EtOH and still result in an antibacterial effect. Psoralen absorbs UV light, and passes the energy of UV light to the samples in some way. In the spectrum analyses of Psoralen and DMSO we could see an expected peak at the 260nm wavelength with broccoli DNA. In addition, the spectrum analysis of Psoralen contained a third peak around the 300nm range that was not present in DMSO (Fig. 2). There was also a notable difference between the controls without Psoralen and the solvents containing it. This peak was more pronounced in the broccoli DNA solvent than the water solvent. This could indicate that the DNA is interacting with Psoralen. Irradiating UV radiation

for more than 30 minutes had an antibacterial effect regardless of the Psoralen effect. The UV irradiation time of 10 minutes was found to have the most antibacterial effect when the concentration of Psoralen was 1/100. Psoralen was not proven to influence the growth of *E. coli and S. cerevisiae.* However, making sheets using the prescribed method was successful.

Conclusion

Our purpose was to make antibacterial sheets by using the properties of Psoralen. Our results proved that making sheets by using Psoralen was possible, and Psoralen has an antibacterial effect. Psoralen absorbed the light in 300 nm, and passed the energy of UV light to the samples in some way. This property of Psoralen indicated an antibacterial effect. The antibacterial effect was the most pronounced under the conditions that UV irradiation time was 10 minutes and the concentration of Psoralen was 1/100. As a future plan, our suggestion is to attempt to make antibacterial sheets by using Psoralen extracted from vegetables and fruits.

Practical Application

By using Psoralen at a concentration of 1/100 and applying 10 minutes UV radiation, effective antibacterial sheets can be made which are not harmful to the human body.

Also, if abandoned vegetables and fruits are used to extract Psoralen, it will enable a reduction of food waste. In this study, Psoralen in powder form was used, which is not practical. If it can be extracted from the peels of fruits such as citrus, it will be possible to make antibacterial sheets that are good not only for the environment but also the human body.

Ideas for Future Research

First, we will use other samples to investigate whether there is a difference in the effects in eukaryotes and prokaryotes or not, because a difference in breeding between eukaryotes such as mold and yeast and prokaryotes such as *E.coli* was detected. For example, we will use *B.*

subtilis and *Euglena*. Second, we will use metal ions together with Psoralen, to find a multiplier effect, because metal ions have an antibacterial effect. For instance, we will use zinc ion, iron ion, manganese ion and aluminum ion. Third, we will make antibacterial sheets by using Psoralen again. Finally, we will make sheets from Psoralen extracted from vegetables and fruits.

Acknowledgements

We would like to thank relators from the Leave a Nest corporation for giving us funding to purchase Psoralen. We would also like to thank Ms. Koyama from the University of Shiga Prefecture, Dr. Ichikawa and Mr. Greenleaf from Ritsumeikan High School for giving us scientific advice and suggestions.

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Making Antibacterial Sheets by Using Psoralen

Ritsumeikan High School

Introduction

Most antibacterial sheets contain alcohol. However there are people who are allergic to alcohol. Also, alcohol can cause inflammation to the skin. Sometimes it also causes the skin to become dry. Making antibacterial sheets which are not harmful to the human body will help alleviate these problems. Psoralen's molecular weight is 186.16, and it dissolves in ethyl acetate and acetone. In addition, it is one of the components contained in phototoxic substances (See Ref. 3). Phototoxicity is a substance that increases UV absorption and causes skin irritation and pigmentation. Also, Psoralen is used as a therapeutic agent in a psoriasis treatment called PUVA therapy (See Ref. 1). In this study, we focused on using the properties of Psoralen to create antibacterial sheets.

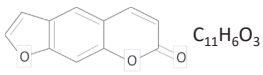

Fig. 1 Structural formula of Psoralen

Materials and Methods

Experiment Ⅰ

The spectrum analysis of Psoralen and Dimethyl sulfoxide (DMSO) was investigated. Solvents were only H_2O or DNA extracted from broccoli. Also, the concentrations of Psoralen and DMSO were changed to 0, 1/5000, 1/1000, 1/500 and 1/100. The wavelength was from 200 to 400 nm. In addition, light absorption of Psoralen whose solvent is ethanol was researched by using the 1.97×10^{-4} mol/L solution of EtOH and solute Psoralen as solvent. The conditions were medium sensitivity, 2 nm broad of band, 400 nm/s scanning speed and 300-1000 nm wavelength.

Experiment Ⅱ

Fluorescence and excitation were investigated by using the 1.97×10^{-4} mol/L solution of Psoralen and EtOH. The conditions were 4 second response, medium sensitivity, 200 nm/s scanning speed and 1 nm data acquisition.

Experiment Ⅲ

The coculture was carried out. First, sample (*E.coli* or *S.cerevisiae*) and Psoralen or DMSO were added to a liquid medium. Second, it was cultured overnight. After that, it was diluted and applied. Finally, UV radiation was irradiated. It was kept at 37℃ and observed overnight. In all experiments and conditions, DMSO was used as mock controls. The effect of Psoralen on each sample was compared.

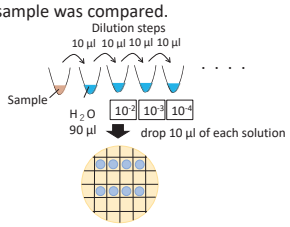

Fig. 2 Scheme of method

Table 1 Conditions for each trial of Experiment Ⅲ

	Culture medium	Solvent	Concentrations	Organism used
A	LB medium or YPD medium			*E.coli* or *S.cerevisiae*
B				
C		Psoralen	1/100	
D		DMSO		
E		Psoralen	1/500	
F		DMSO		
G		Psoralen	1/1000	
H		DMSO		

Experiment Ⅳ

The sheets were made from 100 µl Psoralen, 2% agar and 20 ml H_2O, and their antibacterial effect was observed. Sheets were dried and cut into 1 cm square, and attached to the LB medium. *E.coli* was diluted on the sheets , and UV light (0, 10, 15, 30 min) was irradiated to them. After that, the sheets were turned over, attached *E.coli* to the medium. It was kept at 37℃ and observed overnight.

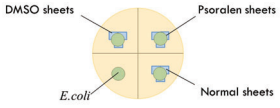

Fig.3 Scheme of experiment Ⅳ

Discussion

It was found that Psoralen had an effect on light absorption nevertheless solvent was H_2O and EtOH. It was determined that H_2O can be used as a solvent instead of EtOH and still has an antibacterial effect. Psoralen absorbs the UV light, and passes the energy of UV light to the samples in some way.

In the spectrum analyses of Psoralen and DMSO we can see an expected peak at the 260nm wavelength with broccoli DNA. In addition, the spectrum analysis of Psoralen contained a third peak around the 300nm range that was not present in DMSO (Fig. 5). There was also a notable difference between the controls without Psoralen and the solvents containing it. This peak was more pronounced in the broccoli DNA solvent than the water solvent. This could indicate that the DNA is interacting with Psoralen.

Irradiating UV radiation for more than 30 minutes had an antibacterial effect regardless of the Psoralen effect. Psoralen was incorporated while samples were divided. The UV irradiation time of 10 minutes was found to have the most antibacterial effect under the condition where the concentration of Psoralen was 1/100. Psoralen does not have an effect on the growth of *E.coli and S.cerevisiae.* Psoralen has an inhibition effect on breeding.

Making sheets using Psoralen in the prescribed method was successful.

Future Plan

First, we will use other samples to investigate whether there is a difference of effect in eukaryote and prokaryote or not, because the difference of breeding between eukaryote such as mold and yeast and prokaryote such as *E.coli* was found. For example, we will use *B.subtilis* and *Euglena*.

Second, we will use metal ions together with Psoralen, to find a multiplier effect , because metal ions have an antibacterial effect. For instance, we will use zinc ion, iron ion, manganese ion and aluminum ion.

Third, we will make antibacterial sheets by using Psoralen again.

Finally, we will make sheets from Psoralen extracted from vegetables and fruits.

Results

In Experiment Ⅰ, the solvents used were H_2O and DNA extract. It was found that Psoralen had an effect on light absorption. When Psoralen was combined with DNA extract, the pattern of wavelengths were shifted (Fig.4, 5 and 6). Psoralen absorbed the light regardless of whether the solvent was H_2O or ethanol (Fig. 4, 5, 6 and 7).

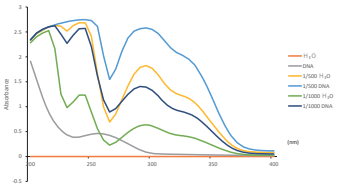

Fig. 4 Spectrum analysis of Psoralen

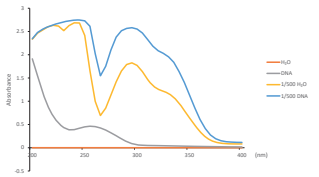

Fig. 5 Spectrum analysis of 1/500 Psoralen

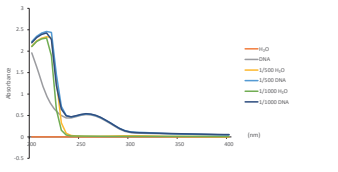

Fig. 6 Spectrum analysis of DMSO

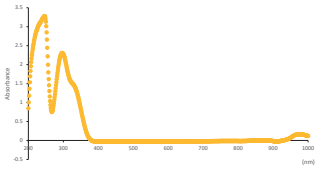

Fig. 7 Absorbance of Psoralen EtOH

In Experiment Ⅱ, when the wavelength, 260 nm and 300 nm was applied to the solution, fluorescence appeared at 450 nm (Fig.8). From the result of excitation, it was found that Psoralen absorbs the UV light less than 250 nm, but it was not used for fluorescence (Fig. 9).

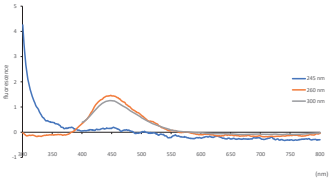

Fig.8 Fluorescence of Psoralen

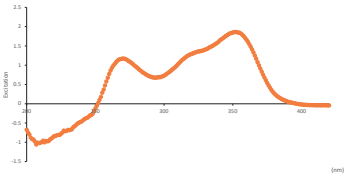

Fig.9 Excitation of Psoralen

In Experiment Ⅲ, all of the samples died when irradiated for 30 minutes in every condition. When irradiated for only 10 minutes, the 1/1000 concentration of either Psoralen or DMSO was found to decrease the reproductive rate of the samples. This means that the reduced reproductive rate is not related to the presence of Psoralen or DMSO. When the Psoralen concentration was 1/100 and the UV radiation was for 10 minutes, then the sample was killed regardless of *E.coli* or *S.cerevisiae* (Table 2 and 3).

Table 2 Results of Experiment Ⅲ, LB medium

	A	B	C	D	E	F	G	H
UV0	○○○○	●●●●						
UV10	○○○○	●○○○	○○○○	●●●○	●○○○	●●●○	●○○○	●●●○
UV30	○○○○	●○○○	○○○○		●○○○		○○●○	●●●●

Table 3 Results of Experiment Ⅲ, YDP medium

	A	B	C	D	E	F	G	H
UV0	○○○○	●●●●						
UV10		○○○○		●●○○	○○○○	●○○○	●●●○	●●○○
UV30		○○○○						●○○○

○ indicates samples did not breed
● indicates samples bred

In Experiment Ⅳ, Making sheets was successful, but a sufficient result was not found because it was contaminated (Fig. 10 and 11).

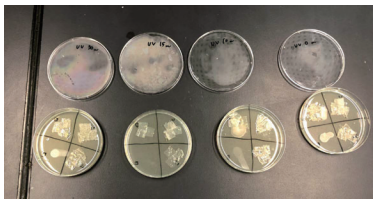

Fig. 10 Result of Experiment Ⅳ

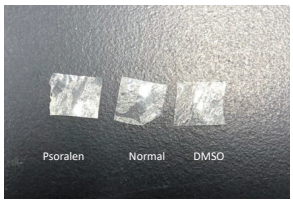

Fig. 11 Sheets

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(Ref. 3) Panno, M. L., & Giordano, F. (2014). Effects of psoralens as anti-tumoral agents in breast cancer cells. *World journal of clinical oncology*, *5*(3), 348–358. https://doi.org/10.5306/wjco.v5.i3.348 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4127606/

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