

Basic study on the antibacterial effects of volatile fragrance of *Cercidiphyllaceae japonicum* and *Cedrus deodara*

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Abstract: This research examined the antibacterial effects of volatile fragrance of leaves of *Cercidiphyllaceae japonicum* and *Cedrus deodara* which have strong fragrance. The samples were collected 24 hours on 21st to 22nd of June and 10th to 11th of August in 2012. We used *Escherichia coli* and *Staphylococcus aureus* subsp. *aureus* which are ordinary bacterium in our daily life. The experiment results showed the volatile fragrance of *C. japonica* could resist the growing of both of bacteria in August, because of the fact that the hours of sunlight was 5.9 hour, however in the condition of 0.2 hour of sunlight in June, the resistant effects were not found on the both of bacteria. *C. deodara* did not show the antibacterial effects on the both of bacteria in June, but on the *S. aureus* subsp. *aureus* on the experiment in August, the antibacterial effects were found. These results suggested the antibacterial effects of volatile fragrance of trees were individually different and they were controlled by sunlight, seasonal, and individual condition.

Keywords: antibacterial effects, *Cercidiphyllaceae japonicum*, *Cedrus deodara*, *Escherichia coli*, *Staphylococcus aureus* subsp. *aureus*

要旨: 本研究では、葉の芳香の強い樹種であるカツラとヒマラヤスギを供試材料とし、その葉から放出される芳香揮発成分の抗菌作用について、日常の身近な細菌である大腸菌と黄色ブドウ球菌を用い、2012年6月21~22日と8月10~11日にそれぞれ24時間芳香をトラップして実験を行った。実験の結果、実施日に日照時間が0.2時間だった6月の実験条件下ではカツラ、ヒマラヤスギともに大腸菌、黄色ブドウ球菌の双方に対して葉の芳香による菌体の増殖を抑制する作用は認められなかった。また、日照時間が5.9時間だった8月の条件下においてはカツラには2つの菌の増殖を抑制する効果がある程度認められ、ヒマラヤスギでは大腸菌に対しては認められなかったものの、黄色ブドウ球菌に対してのみ認められた。これらの結果から、葉の抗菌作用は、樹種による特性のほか、日照条件や季節、個体によってその強さが異なることが示唆された。

キーワード: 抗菌作用, カツラ, ヒマラヤスギ, 大腸菌, 黄色ブドウ球菌

I Introduction

Recently, antibacterial effects of trees have been paid attention (1, 2, 3, 4, 5, 6). *Cercidiphyllaceae japonicum* is a special tree of one family and one genus. It contains maltol having special sweet fragrance. Maltol has been recently utilized for foods and cosmetics. *Cedrus deodara* is one of the popular planting trees for greening urban environment. It is also utilized the essence of leaves which contains mono terpene for aroma therapy, too. But the fragrance of these trees have not been researched their antibacterial effects yet. Therefore, this research examined the antibacterial effects of volatile fragrance of these trees.

II Materials and Methods

Experiment samples were leaves of *C. japonicum* and *C. deodara*. Extraction and culturing bacterium methods were as below.

1. Medium: Solution for culture medium was made from LB culture medium (NaCl 5g, Tryptone 5g, Yeast extract 2.5g, Agar 5g each stuff was g/500ml). The solution was sterilized by an autoclave and provided for a culture dish (8.5cm diameter) 20ml each and made solid medium.

2.Extraction method and an experiment tree: The volatile fragrance was trapped 24 hours (from 13:00 to next 13:00) by vinyl clear bags (25cm square) covering two branches (north and south at 2m height of *C. japonicum* tree (13.3m height and 24cm DBH) and *C.*

deodara tree (19m height and 36cm DBH) on the campus of Tokyo University of Agriculture. Trapping day was 21st to 22nd of June and 10th to 11th of August in 2012. The weather of each day was mostly cloudy, but the hour of sunlight was only 0.2 hour on 21st to 22nd of June. The climate conditions are shown on Table 1.

Table 1. Climate conditions on this experiment
表-1. 6月および8月の実験実施日の気象条件

	21 st to 22 nd of June	10 th to 11 th of August
Max.Temp.(°C)	26.5	31.2
Min. Temp.(°C)	18.2	23.8
Hour of Sun (h)	0.2	5.9

3.Bacterial strains and culture conditions: *Escherichia coli* and *Staphylococcus aureus* subsp. *aureus* were selected as examined bacterium, because they were ordinary existed in daily life. Examined bacterium were ones of culture collection center of Tokyo University Agriculture. Each 10μl bacterium drop (approximately 10⁶~10⁷ cfu / 10μL) was put on the center of the culture dish. 10ml of trapped volatile fragrance air was absorbed 10ml by a syringe and put into each culture dish through 0.2μm disk filter. Culture dishes were seal up and keep in an incubator and cultivated for a month (average temperature was 30°C). Growing bacterium area of 10 repeat culture dishes and 5

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control ones were periodically measured. Control dishes were not put anything and cultivated in the same conditions as other dishes.

(4) Method of measuring growing rate of bacterium: To measure the growing rate of the bacterium per a week, tracing the bacterium area on the paper, and scanning the area by measuring software. Expanding rate was calculated by the grown bacteria area divided by the first bacteria area.

III. Results and Discussion

1. Antibacterial effect on *E. coli*

The changes of expanding times of bacteria area of *E. coli* in June experiment is shown on Figs. 1 and 2. Expanding rate was calculated by the grown bacteria area divided by the first bacteria area. Both of expanding rates of two trees were higher than control, so the volatile fragrance of them could not show the antibacterial effect on *E. coli* and *S. aureus* subsp. *aureus* in June.

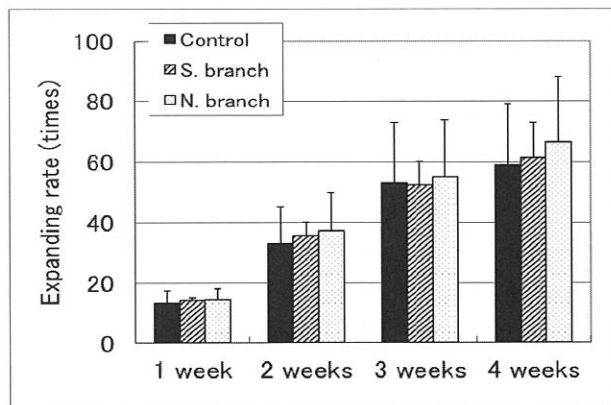


Fig.1. Expanding rate of *E. coli* by volatile fragrance treatment of *C. japonicum* on the June experiment (n=10 each)

図一 1. 6 月の実験におけるカツラ芳香成分処理下での大腸菌糸面積の拡大率

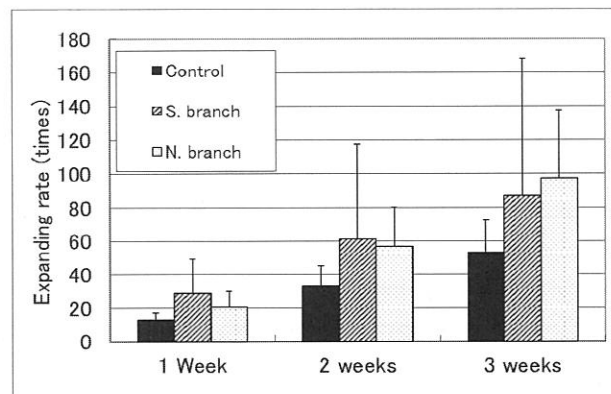


Fig.2. Expanding rate of *E. coli* by volatile fragrance treatment of *C. deodara* on the June experiment (n=10 each)

図一 2. 6 月の実験におけるヒマラヤスギ芳香成分処理下での大腸菌糸面積の拡大率

Next, the changes of expanding times of bacteria area of *E. coli* in August are shown on Figs. 3 and 4. Expanding rates of *C. japonicum* volatile fragrance treatment groups were statistically lower than the control ($p < 0.05$ each) at 3 weeks later. But *C. deodara* could not show the effect and also standard deviation of south branch's data was wide.

These results suggested the antibacterial effects are individually different and volatile fragrance was also controlled by the hours of sunlight, seasonal condition, and individual condition.

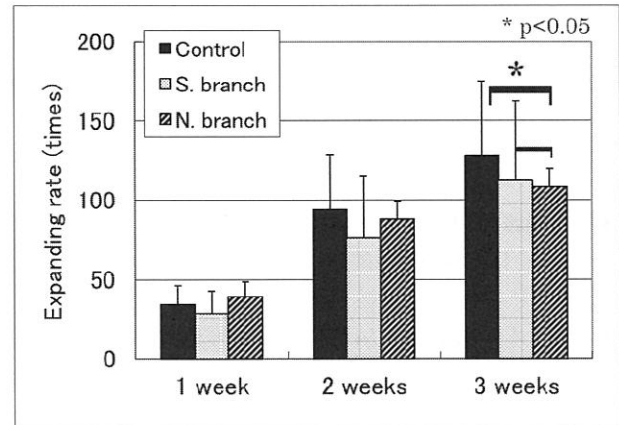


Fig.3. Expanding rate of *E. coli* by volatile fragrance treatment of *C. japonicum* on the August experiment (n=10 each)

図一 3. 8 月の実験におけるカツラ芳香成分処理下での大腸菌糸面積の拡大率

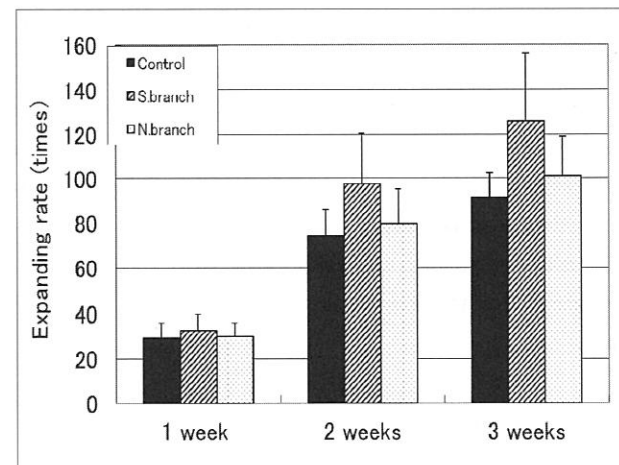


Fig.4. Expanding rate of *E. coli* by volatile fragrance treatment of *C. deodara* on the August experiment (n=10 each)

図一 4. 8 月の実験におけるヒマラヤスギ芳香成分処理下での大腸菌糸面積の拡大率

2. Antibacterial effect on *S. aureus* subsp. *aureus*

The changes of expanding times of bacteria area of *S. aureus* subsp. *aureus* in June experiment are shown on Figs. 5 and 6. Expanding rates of each experiment were double higher than control. These results suggested the volatile fragrance might rather stimulate the bacteria growing in some condition.

of volatile fragrance during daily or seasonal change. Also, the effects of other parts of the trees and species should be kept investigating.

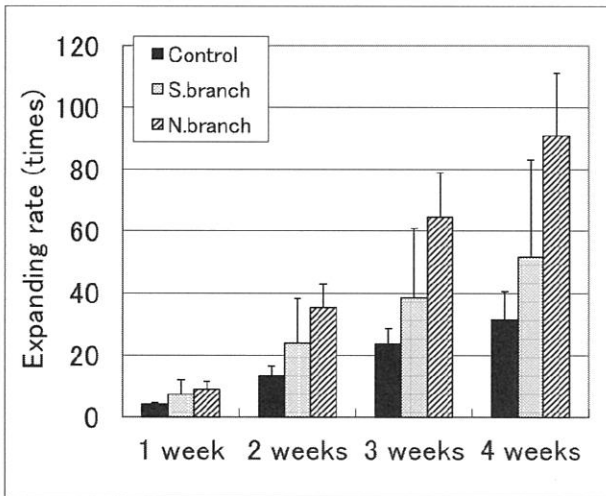


Fig.5. Expanding rate of *S. aureus* subsp. *aureus* by volatile fragrance treatment of *C. japonicum* on the June experiment (n=10 each)

図-5. 6月の実験におけるカツラ芳香成分処理下での黄色ブドウ球菌糸面積の拡大率

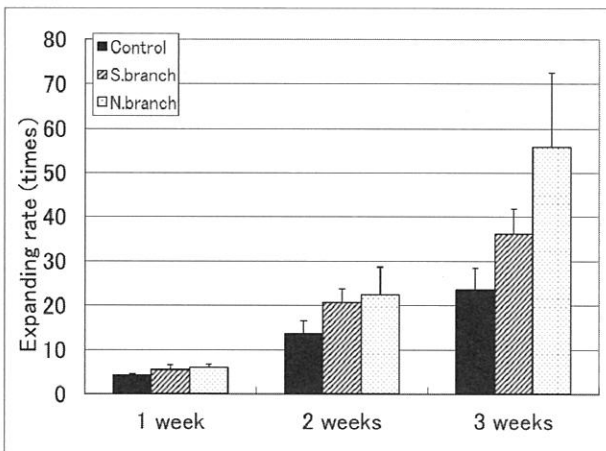


Fig.6. Expanding rate of *S. aureus* subsp. *aureus* by volatile fragrance treatment of *C. deodara* on the June experiment (n=10 each)

図-6. 6月の実験におけるヒマラヤスギ芳香成分処理下での黄色ブドウ球菌糸面積の拡大率

The changes of expanding times of bacteria area of *S. aureus* subsp. *aureus* in August experiment is shown on Figs. 7 and 8. Expanding rates of both branches of *C. japonicum* were statistically lower than the control ($p < 0.01$) at 3 weeks later and volatile fragrance of *C. deodara* also showed the antibacterial effects ($p < 0.05$). These results suggest the antibacterial effect of *C. japonicum* and *C. deodara* is controlled by hours of sunlight and seasonal condition.

This basic study showed antibacterial effects of the volatile fragrance of leaves of trees. The results suggest the effects were controlled by the hour and intensity of sunlight, seasonal conditions, and individual tree conditions. They also suggest volatile fragrance might be not only antibacterial effect but also sometimes stimulate bacteria by the case. However, some data of this study had wide standard deviation, so further researches are necessary to enhance the experiment accuracy. Especially, gas chromatography is necessary to make clear the contents

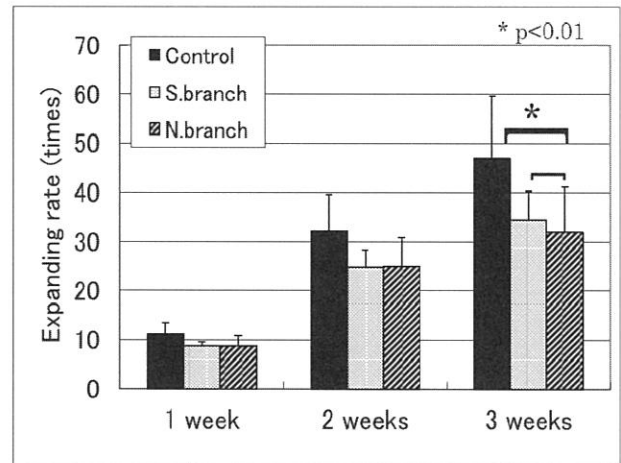


Fig.7. Expanding rate of *S. aureus* subsp. *aureus* by volatile fragrance treatment of *C. japonicum* on the August experiment (n=10 each)

図-7. 8月の実験におけるカツラ芳香成分処理下での黄色ブドウ球菌糸面積の拡大率

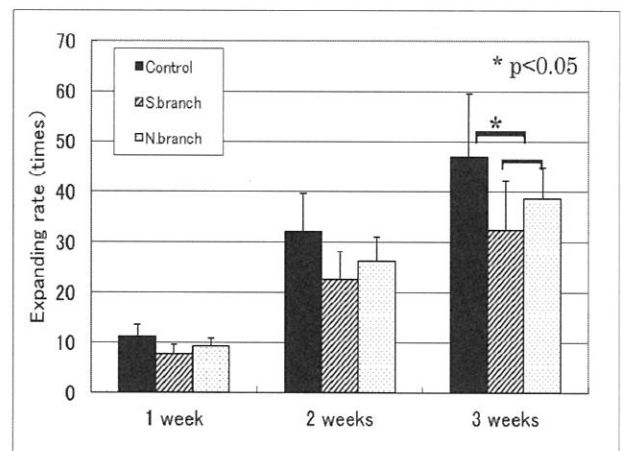


Fig.8. Expanding rate of *S. aureus* subsp. *aureus* by volatile fragrance treatment of *C. deodara* on the August experiment (n=10 each)

図-8. 8月の実験におけるヒマラヤスギ芳香成分処理下での黄色ブドウ球菌糸面積の拡大率

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