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Emission of Odor in Plants: A Strategy to Fight Infection?

Jin-ichi Sasaki[1*] • Chihiro Sutoh[1] • Ayako Norigami[1] • Tomomi Satoh[1] • Nana Kumaki[1] • Hidemitsu Uchisawa[2] • Shinya Yamaguchi[2] • Vachik Lazarian[3]

[1] Department of Medical Technology, Hirosaki University, School of Health Sciences, Honcho, Hirosaki 036-8564, Japan
[2] Aomori Industrial Research Center, Daini-Tonyacyo, Aomori 030-0113, Japan
[3] University of Tennessee at Martin, Martin, Tennessee 38238, USA

***Corresponding author*: * jnsasaki@tk2.so-net.ne.jp**

ABSTRACT

The bacteriocidal potency of odor was studied by the Petri dish method using eight types of plant odors from Hiba tree/Japanese cypress (*Thujopsis dolabrata* var. *hondae*), garlic (*Allium sativum*), onion (*Allium cepa*), horseradish (*Armoracia rusticana*), cinnamon (*Cinnamomum zeylanicum*), dokudami (*Houttuynia cordata*), clove (*Syzygium aromaticum*) and sage (*Salvia officinalis*). Target bacteria in the test were *Pseudomonas aeruginosa*, *Bacillus natto*, enterohemorragic *Escherichia coli* O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Candida albicans*. Seven out of eight plant odors showed antibacterial activity with a different spectrum of activity against the above described bacteria. Odor released from Hiba tree, garlic, onion, horseradish and dried whole clove demonstrated the strongest antibacterial activity, followed by dried cinnamon and dokudami, but the odor of dried sage did not show any activity against these bacteria. Gas chromatography analysis clarified the presence of 15 composites in fresh garlic odor, but two of them were unidentified in an analysis 24 hours later. When viewed by scanning electron microscopy, the bacteria surface became fluffy due to exposure to Hiba odor accompanied with bleb formation on the surface, which may have been caused by protein degeneration, while formaldehyde (a representative disinfectant)-exposed bacteria maintained a smooth surface without any formation of blebs. These results suggest that anti-bacteria mechanisms of odor vary depending on the odor (i.e. chemical(s) included) emitted from plants. One of the significances of plant odor might be the protection from attacks by harmful agents such as bacteria and insects in environmental surroundings.

Keywords: bacteria killing potency, defense system, role of plant odor, strategy to survive

INTRODUCTION

There is an increasing interest in finding trustworthy sources of information about functional foods that can maintain good health (Mazza 1998; Bratman 2000; Sasaki 2006). This theme has been covered in detail in a Special Issue of FOOD dedicated to natural biological compounds and food preservation (Benkeblia 2007). Under these social demands, we initiated 15 years ago bio-functional research of plants (vegetables) to provide individuals beneficial information.

In 1996, in Japan, a historically huge outbreak of food poisoning caused by enterohemorrhagic *Escherichia coli* O157:H7 resulted in the total number of patients to exceed 15,000 with 12 deaths. Our group immediately initiated a search to find functional food with potential antibacterial activity aimed at preventing food poisoning by the application of vegetables with antibacterial potency. During our study we observed an interesting phenomenon: did not grow on culture medium when exposed to odor from the Hiba tree/Japanese cypress (*Thujopsis dolabrata* var. *hondae*), even when the test was repeated.

We speculated from these results that this strange phenomenon was probably due to the effect of odor released from the Hiba tree. In this paper, we extend these initial findings to describe an antibacterial act of plant odors which we generally observed in ordinary plants and which were not limited to a specific event.

MATERIALS AND METHODS

Plant materials

Plants (vegetables) employed in the tests were Hiba tree/Japanese cypress (oil and crystal), fresh raw materials of garlic (*Allium sativum*), onion (*Allium cepa*), horseradish (*Armoracia rusticana*), and dokudami (*Houttuynia cordata*), and dried samples of cinnamon (*Cinnamomum zeylanicum*), sage (*Salvia officinalis*) and whole clove (*Syzygium aromaticum*). Garlic, onion, horseradish and dokudami were purchased from a foods market in Japan, and dried cinnamon, sage and clove spices (Associated Wholesale Grocers, INC, Kansas City, USA) were obtained from a market in the USA.

Target bacteria

Target pathogens used were *Pseudomonas aeruginosa*, *Bacillus natto*, enterohemorrhagic *Escherichia coli* O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Candida albicans*, which were clinically isolated from patients in Hirosaki University Hospital. All bacteria except for *Bacillus natto* are critically important in the medical field as causative agents of human diseases such as a food poisoning and hospital-acquired infection in compromised hosts.

Anti-bacteria test by plant odor

A test was performed by the nutrient agar plate method as described previously (Sasaki *et al*. 1999). Briefly 5.0 g of fresh test sample was ground to emit an odor from the test plant and the resulting extract was placed in a lid of a Petri dish. Dried material was ground together with 3.0 mL of sterilized distilled water. An inner plate dish on which bacteria had been streaked on the surface of nutrient agar was placed upside-down on the lid of the Petri dish, then sealed immediately with an adherent tape as shown in **Fig. 1**. The antibacterial effect of plant odor was evaluated by growth inhibition of bacteria on the nutrient agar plate as seen in **Figs. 1** and **2** after overnight cultivation at 37°C. Growth was not statistically assessed.

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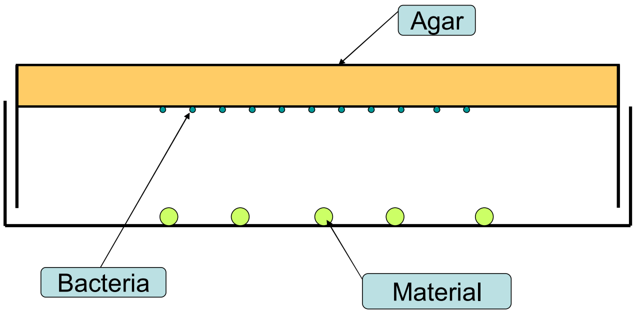

Fig. 1 Experimental design of anti-bacteria act by plant emitted odor.

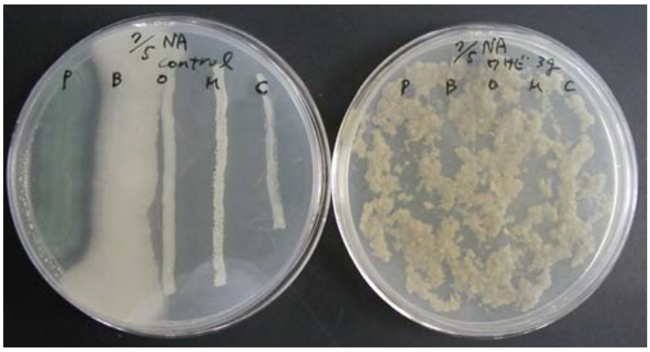

Fig. 2 Bactericidal effect of horseradish-emitted odor. The growth of five species of bacteria (streaked lines) were totally inhibited by exposure to volatiles released from 5.0 g ground horseradish (right) while all bacteria streaked on the culture medium without horseradish odor grew as visible lines on the control plate (left). Bacteria streaked (from left to right): *Pseudomonas aeruginosa*, *Bacillus natto*, *Escherichia coli* O157:H7, MRSA (methicillin-resistant *Staphylococcus aureus*), and *Candida albicans*.

Gas-chromatograph analysis of garlic odor

Garlic clove was smashed in a plastic bag and odor emitted from the ground materials was analyzed by HITACHI 17A gas chromatogram under the following conditions: detector = Hydro Flame Ion Detector (FID), column = J & W Scientific DB-1, split rate = 13; temperature conditions: vaporization room: 250.0°C; interface: 275.0°C; column: 320.0°C.

Scanning electron microscope observation

Affection of Hiba tree odor on bacteria enterohemorrhagic *Escherichia coli* O157:H7 was studied using a scanning electron microscope (Nihon-Denshi, JSM 25S-11). Bacteria that were grown on nutrient agar plates and exposed to any of the odors for 24 h at 37°C were fixed in 3.0% glutaraldehyde for 3 hours. After washing in distilled water they were serially dehydrated in ethyl-alcohol followed by a standard method (Ahsan and Sasaki 1989). Then, alcohol was substituted by isoamyl-alcohol for more than a day. Bacteria were shadowed with vaporized platinum-palladium in a vacuum chamber for observation by scanning electron microscope.

RESULTS

Antibacterial nature of plant odor

The bactericidal potency of plant odors tested is summarized in **Table 1**. Garlic, onion, horseradish, whole clove and Hiba tree odors showed the strongest bactericidal activity among all bacteria tested (**Fig. 2**). Dokudami and cinnamon odors were placed in the second strongest group, which killed several bacteria but not all of them. By contrast dried sage odor did not show any bactericidal effect.

The odor of certain plants was bactericidal and not simply bacteriostatic: this result was judged through a simple assay in which bacteria that were not growing on medium under odor failed to grow when re-cultured. The only exception was the odor of dried sage, which was not effectively bactericidal, unlike fresh sage.

Table 1 Bactericidal potency of plant emitted odor.

	P. aeruginosa	*B. natto*	O157	MRSA	*C. albicans*
Hiba tree	+	+	+	+	+
Garlic	+	+	+	+	ND
Onion	+	+	+	+	ND
Dokudami	—	+	—	+	ND
Horseradish	+	+	+	+	+
Sage	—	—	—	—	—
Whole clove	+	+	+	+	+
Cinnamon	—	+	+	+	+
Control	—	—	—	—	—

+: bactericidal; -: not bactericidal; ND: not determined.
Hiba oil used was 500 μL per Petri dish while other samples were 5.0 g per Petri dish.

The bactericidal concentration of odor was calculated by using a crystal sample prepared from Hiba tree extract and compared to that of representative disinfectant formalin volatile. Odor released from 5.0 mg of Hiba crystal was the minimum inhibitory concentration (MIC) in the Petri dish (10.0 cm (diameter) × 1.0 cm (depth). Since bacteria growth inhibitory dosage of Hiba crystal was equivalent to gas released from 50 μL of formalin liquid. Therefore, the MIC of Hiba tree odor was calculated at 64 $\mu g/cm^3$ and that of formalin at 0.65 $\mu g/cm^3$, respectively.

Gas chromatography analysis of garlic odor

Plant odor was a complex of volatiles, and fifteen elements were detectable in fresh garlic odor. Two of them detected at 12 and 19 retention time were unstable and abolished in 24 hour later analysis (**Fig. 3**). These results suggested that these unstable composites might be an extremely unstable compound such as allicin or allicin-derivative compounds.

Scanning electron micrograph of odor exposed bacteria

There existed morphological differences between plant odor-and formalin gas-treated bacteria as seen in **Fig 4**. Hiba tree odor-treated bacteria showed a chapped surface with bleb formation. By contrast formalin gas-treated bacteria col-

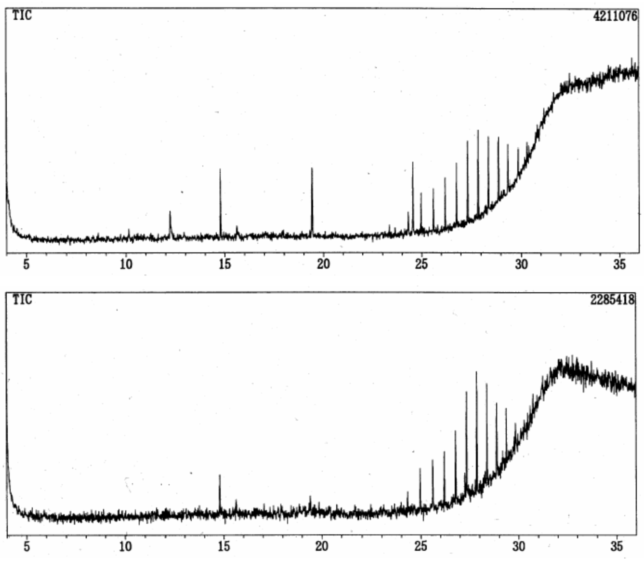

Fig. 3 Profile of gas chromatography analysis of garlic-emitted odor. Fifteen compounds were detected in fresh garlic odor (upper). Two major peaks at retention times of 12 and 19 disappeared twenty four hours later (lower).

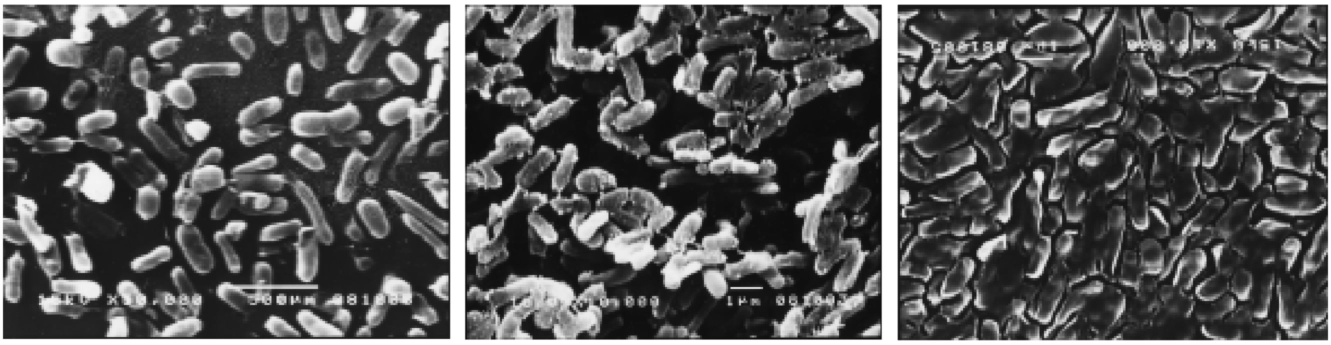

Fig. 4 Scanning electron micrograph of enterohemorrhagic *Escherichia coli* O157:H7 exposed to Hiba tree-emitted odor and formalin gas respectively. Hiba tree odor-exposed O157 produced rough surfaces and showed transformed shape (**center**), different from that of formaldehyde-treated bacteria (**right**), indicating that the mechanisms by Hiba odor and formalin gas were different. Control on the **left**.

lapsed to become flat in shape without bleb formation. It suggested that working manner of plant odor probably differed from each other emitted odor to kill bacteria.

DISCUSSION

Plants ordinarily generate a variety of odors (volatiles) from sweet to sour, bitter, irritating depending on the plant species, whose use in herbal medicine is particularly attractive due to the scent. The number of volatiles is estimated to be more than 1,000 compounds emitted from plants (Natalia *et al* 2004).

Plant odors or volatiles are associated with their protection from attacks by herbivores and pathogens and provide a reproductive advantage (Natalia *et al*. 2004, 2006). Plant volatiles are also involved in wound healing by triggering the immune response to aid the plant in stressful environmental conditions (Lesney 2002), and act as a way to communicate and interact with their surrounding environment (Natalia *et al*. 2006). The bactericidal activity of many plant oils are widely reported (reviewed in Benkeblia 2007) but all such studies are based on the use of extracted plant oils and not on natural plant odors (volatile).

To broaden the base of knowledge on traditional roles of plant extracts, we conducted initial studies that hinted at the potential of Hiba tree odor to inhibit bacterial growth. To some degree this was confirmed by the fact that insects and weeds do not gather and grow around Hiba trees within a forest (pers. obs.), suggesting their release of inhibitory volatiles or substances. Branching from these initial findings we set up these experiments and conclusively supported our initial hypothesis that several plant volatiles have bactericidal activity. More extensive experiments should be conducted to further strengthen this claim.

Most plant flavors and volatiles are a complexity mix of components. The analysis of fresh garlic odor by GC, for example, revealed at least 15 compounds (**Fig. 3**), two of which, at 12 and 19 retention times disappeared within 24 hours later, and these are likely to be allicin or allicin-related compounds such as allyl sulfides compounds because of instability of these compounds (Garlic and Health Group 2007).

In additional, we wanted to examine the mechanism by which plant odors kill bacteria. In the experiment using Hiba tree odor we believe, through SEM observations, that odor first works on the bacterial surface protein, since the surface became rough after exposure to the odor (**Fig. 4**). Thereafter, it is most likely that odor chemicals penetrate cell walls to deactivate life-related enzymes, which eventually leads to bacterial death (Smith-Palmer *et al.* 1998; Inoue *et al* 2001; Natalia *et al* 2004), a mode of functioning that is different from formalin gas (formaldehyde), a representative disinfectant used in the medical field.

Living organisms exposed to stress immediately react against it by generating heat shock proteins (HSPs) to restore denatured proteins (Young and Elliot 1992). In cancer cell, HSP is constantly expressed on cell membrane likely to be a cell constituent, suggesting that cancer cell is preparing against inappropriate situation to survive by the aid of HSP. We, then, can use this specific phenomenon for identification of tumor cell by detecting HSP by using such as immune-fluorescence technique (Ahsan and Sasaki 1993; Ahsan *et al*. 1993). Plant odor may have a similar mechanism as HSP by protecting the plant from undesirable conditions in nature.

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REFERENCES

Ahsan CR, Sasaki J (1989) Isolation of line 10 hepatoma-cell membrane by the water extraction method and immunochemical analysis. *Microbiology Immunology* **33**, 219-227

Ahsan CR, Sasaki J (1993) The *Mycobacterium bovis* BCG 64-kDa surface protein is antigenically shared with different mouse tumor cells and has anti-tumor activity in immunized mice. *Immunology Letters* **36**, 235-238

Ahsan CR, Sasaki J, Nomaguchi H (1993) The 65-kDa stress protein: is it related to the tumor specific antigen? *Immunology Letters* **35**, 291-292

Benkeblia N (Ed) (2007) Natural biological compounds and food preservation. *Food* **1**, pp 111-245

Bratman S (2000) *Your Complete Guide to Herbs*, Prima Publishing, California, pp 1-210

Garlic and Health Group (2007) Collection of six reviews. *Medicinal and Aromatic Plant Science and Biotechnology* **1**, 1-36

Inouye S, Takizawa T, Yamaguchi H (2001) Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. Journal of Antimicrobial Chemotherapy **47**, 565-573

Lesney MS (2002) A fragrant feast. *Today's Chemist at Work* **11**, 26-31

Mazza G (1998) *Functional Foods: Biochemical and Processing Aspect*. Technomic Publishing Co. Inc., Canada, pp 1-460

Natalia D, Eran P, Jonathan G (2004) Biochemistry of plant volatiles. *Plant Physiology* **135**, 1893-1902

Natalia D, Florence N, Dinesh N, Irina O (2006) Plant volatiles. Recent advances and future perspectives. *Critical Reviews in Plant Sciences* **25**, 417-440

Sasaki J (2006) Bioactive phytocompounds and products traditionally used in Japan. In: Iqubal A, Farrukh A, Mohammad O (Eds) *Modern Phytomedicine – Turning Medicinal Plants into Drugs*, Wiley VCH, Weinheim, pp 79-96

Sasaki J, Kita T, Uchisawa H, Matsue H (1999) Antibacterial activity of garlic powder against *Escherichia coli* O157. *Journal of Nutrition Science Vitaminology* **45**, 785-90

Smith-Palmer A, Stewart J, Fyfe L (1998) Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology* **26**, 118-122

Young RA, Elliot TJ (1992) Heat shock protein: immunity and autoimmunity. *Current Opinion in Immunology* **4**, 396-400