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Bactericidal Activity of Sawa-wasabi (*Wasabia japonica*) Against the Fish Pathogenic Bacteria

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In this study, the bactericidal activity of each extract from Sawa-wasabi (*Wasabia japonica*) root, stem and leaf against the fish pathogenic bacteria were examined. The main component related to bactericidal activity in Sawa-wasabi was well known to AIT. The Sawa-wasabi roots showed the highest AIT amount with 1.18 mg/g. Stems was 0.41 and leaves was 0.38 mg/g. All of them showed bactericidal activity against 2 strains of *Vibrio hollisae, V. anguillarum,* and 2 strains of *Edwardsiella tarda,* but weak effect against *Staphylococcus capitis.* The Sawa-wasabi leaves showed the strongest bactericidal activity with minimal bactericidal concentrations (MBCs) of 156.3 mg of dry weight/mL against 2 strains of *V. hollisae, V. anguillarum,* and 2 strains of *E. tarda.* The roots and stems showed a little weak bactericidal activities with 312~1,250 mg of dry weight/mL against them. These results suggest that certain components besides AIT in Sawa-wasabi are affective in killing fish pathogenic bacteria.

Key words: Sawa-wasabi (*Wasabia japonica*), Bactericidal activity, AIT, Fish pathogenic bacteria

Introduction

With the rapid progress in marine aquaculture, bacterial infectious diseases are emerged and causing problems for in this industry. Fish health is managed through the application of a wide variety of techniques. Among them, chemical (drug and pesticide) therapy is the most common techniques used to prevent, mitigate and treat disease in cultured fish. However, there are a few problems in chemical therapy to treat fish diseases by bacterial infection. Environmental issues that arise around fish health management practices most often involve the impacts of drugs and pesticides used by the fish farming industry. The primary environmental issues regarding their use in the aquaculture industry were: (1) the appearance of drug residues in seafood products (including farmed fish and non-target species), (2) the stimulation of antibacterial resistant strains of pathogens and their transfer to other species including man, and (3) effects on microbial and non-target communities. Therefore, a non-antibiotic agent that is both highly effective and safe might be of utmost importance for the eradication of fish pathogenic bacteria and to treatment of fish disease.

Many studies have been made on antimicrobial activity of Sawa-wasabi (*Wasabia japonica*). It has been reported that the essential oil of Sawa-wasabi has a particularly strong antibacterial effect against *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* (Nishida, 1958; Inoue et al., 1983). One of its components, allyl isothiocyanate (AIT), is mainly responsible for this bactericidal action (Foter and Golick, 1938; Foter, 1940; Kanemura and Miyamoto, 1990; Isshiki et al., 1992). Hasegawa et al. (1999) also reported that Sawa-wasabi or AIT inhibited the growth of *Vibrio parahaemolyticus.* Thus, it seems reasonable to explore the possibility of using the Sawa-wasabi for eradication of fish pathogenic bacteria.

In this study, the author investigated the bactericidal activity of the Sawa-wasabi on 6 strains of fish pathogenic bacteria for development of natural anti-

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bacterial agents.

Materials and Methods

Preparation of the Sawa-wasabi extracts

The Sawa-wasabi (*Wasabia japonica*) were obtained from a Sawa-wasabi farmer, who had cultivated it at a spring-fed limpid stream in a forest in Tawaramine, Shizuoka, Japan. The Sawa-wasabi used in this study were harvested in early spring after 2 years cultivation and stored at −80℃. They were separated into roots, stems and leaves, and washed cleanly with distilled water. The extraction procedures are described in Fig. 1. They were frozen with liquefied nitrogen for 5 min and then pulverized with blender, respectively. Two hundred grams of each part was mixed with 100 mL of milli Q and kept at 37℃ for an hour to maximize the producing of isothiocyanates. Three times volume of diethyl ether was added to the mixture and stirred for an hour. The mixture was centrifuged at 5,000×g for 30 min, and filtered through glass wool. The precipitates were extracted more twice with same volume of diethyl ether. The supernatants pooled were separated into aqueous and organic layers with separating funnel. The organic solvent layer was evaporated in vacuo, and the residues were dissolved in 20 mL of ethyl alcohol. The solution was applied to a Sep-Pak C_{18} cartridge (Waters Co.) and filtered by 0.22 μm filter of Millix-GS (Millipore). Each sample solution contained 10 g dry weight of root, stem or leaf per milliliter and was stored at −80℃ before use.

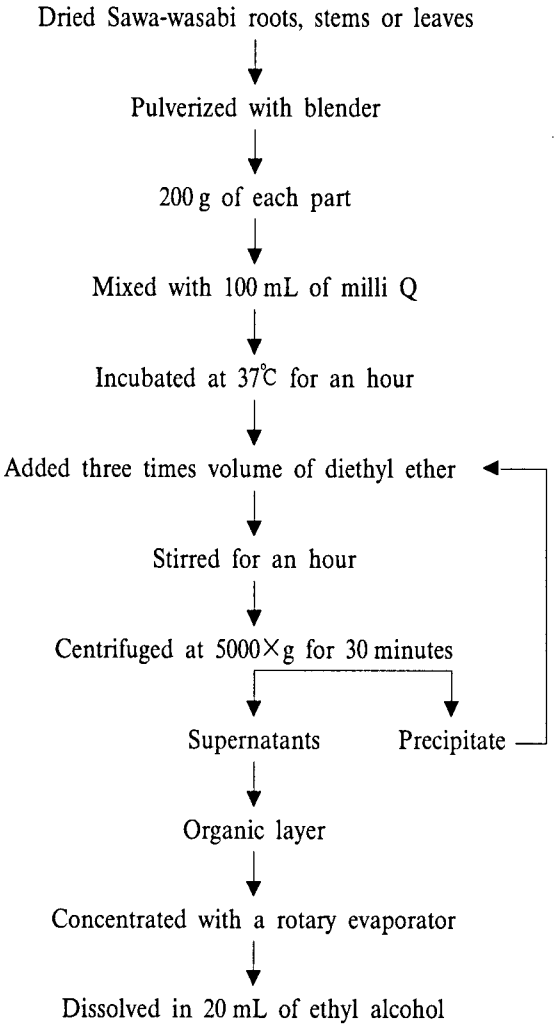

Fig. 1. Procedure for extraction of antibacterial components from Sawa-wasabi.

Quantitative analysis of AIT in Sawa-wasabi

The AIT concentrations in the Sawa-wasabi root, stem and leaf were estimated by using a gas chromatograph (GL Sciences GC-380) with a flame ionization detector (FID). A fused silica capillary column (0.25 mm i.d.×30 m) was used. The oven temperature programmed from 50℃ for 1 min to 200℃ for 3 min at 10℃/min. The temperature of injection port and detector was kept at 220℃. The carrier gas was helium, the split ratio being 1:50. Phenyl isothiocyanate (PIT, Wako Pure Chemical Industries LTD., Japan) was used as an internal standard substance because of the absence in the Sawa-wasabi.

Culture of fish pathogenic bacteria

Six strains of fish pathogenic bacteria, 2 strains of *Vibrio hollisae*, *V. anguillarum*, 2 strains of *Edwardsiella tarda* and *Staphylococcus capitis* were obtained from National Fisheries Research and Development Institute, Republic of Korea (Table 1). They were cultured in Brain heart infusion broth (Difco Co.) at 25℃ for 48 hours. This medium was also used for minimum bactericidal concentration (MBC) assay.

MBC assay of the Sawa-wasabi extracts

The MBC was assayed by method of Bamba et al. (1997). Various concentrations of Sawa-wasabi extract and authentic AIT (Wako Pure Chemical

Table 1. List of tested fish pathogenic bacteria

Strain	Source	Characteristics
Vibrio hollisae 1	Kidney of flounder	Gram neg. rod
V. hollisae 2	Kidney of rock fish	Septicemia to fish
V. anguillarum	Kidney of flounder	Gram neg. rod Typical fish pathogenic bacteria
Edwardsiella tarda 1	Cephalic tumor of flounder	Gram neg. short-rod,
E. tarda 2	Kidney of flounder	Nodule on kidney or liver of cultured eel
Staphylococcus capitis	Rock fish	Gram pos. coccus

Industries LTD., Japan) were initially dissolved in ethyl alcohol and then serially diluted in a two-fold series with culture medium. Each bacteria was adjusted to 10^7 CFU/mL with culture medium, and 5 μL of the adjusted culture was inoculated in each well of the 96-well flat-bottom microplate (Nunc), which had been filled with 100 μL of medium containing 50 μL of various concentrations of Sawa-wasabi extracts. The plates were incubated stationary at 25℃ for 2 days. The one loopful of each bacteria culture without growth in each microplate well was inoculated to the previously described culture medium containing 1.5% of agar plate, and incubated at 25℃ for 2 days. The MBC was defined as the lowest concentration that induced no colony of each bacterium.

Results

AIT amount in Sawa-wasabi

In general, the main component related to antimicrobial activity in Sawa-wasabi was well known to AIT (Nishida, 1958; Kanemura and Miyamoto, 1990; Isshiki et al., 1992). The author, therefore, measured the AIT amount in Sawa-wasabi root, stem and leaf. The retention time of AIT on a gas chromatogram was about 7.04~7.21 min (Fig. 1). The AIT amounts in Sawa-wasabi root, stem and leaf were shown in Table 2. Among them, Sawa-wasabi root showed the highest AIT amount with 1.18 mg/g. Stem was 0.41 mg/g and leaf was 0.38 mg/g.

Minimum bactericidal concentrations (MBCs) of Sawa-wasabi against fish pathogenic bacteria

The MBCs of Sawa-wasabi against 6 strains of fish pathogenic bacteria were shown in Table 3. All parts of Sawa-wasabi showed bactericidal activities on the growth of 5 strains of gram-negative bacteria,

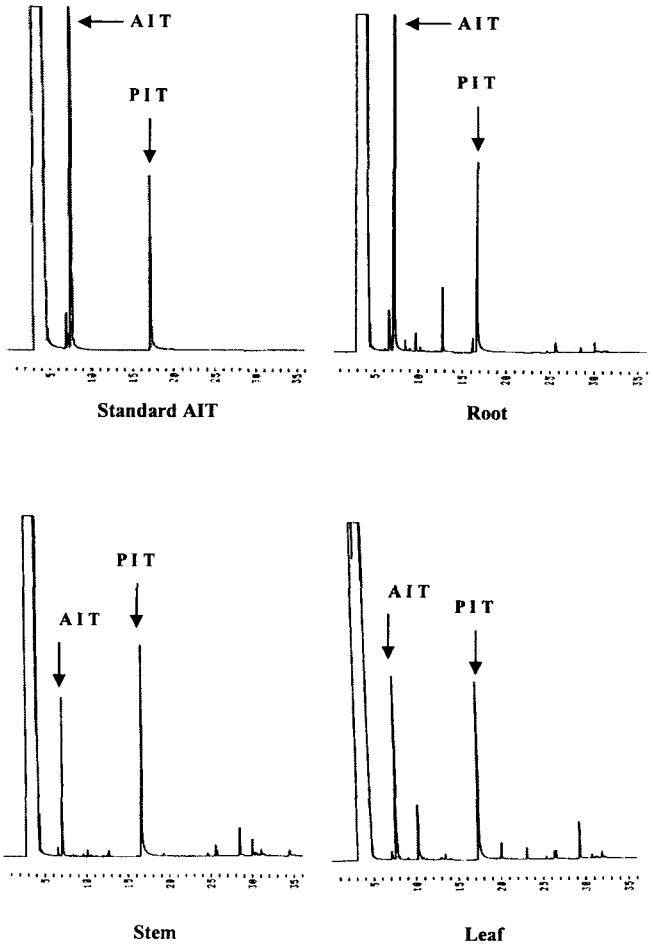

Fig. 2. Gas chromatogram of AIT in roots, stems and leaves of Sawa-wasabi.

Table 2. AIT amount of Sawa-wasabi root, stem and leaf

Sawa wasabi	AIT amount (mg/g)
Root	1.18
Stem	0.41
Leaf	0.38

Table 3. MBCs of Sawa-wasabi against fish pathogenic bacteria

Fish pathogenic bacteria	MBC of authentic AIT (mg/mL)	MBC of Sawa-wasabi (mg of dry weight/mL)		
		Roots	Stems	Leaves
Vibrio hollisae 1	1.00	312.5	312.5	56.3
V. hollisae 2	2.00	312.5	312.5	156.3
V. anguillarum	1.00	312.5	1,250	156.3
Edwardsiella tarda 1	4.00	625.0	312.5	156.3
E. tarda 2	4.00	625.0	1,250	156.3
Staphylococcus capitis	16.0	5,000	5,000	2,500

but weak bactericidal activity on *S. capitis* (gram-positive bacteria). The Sawa-wasabi leaves showed the strongest bactericidal activity with MBC of 156.3 mg dry weight/mL against 5 strains of gram-negative bacteria, 2 strains of *V. hollisae, V. anguillarum* and 2 strains of *E. tarda.* The Sawa-wasabi roots and stems showed a little weaker bactericidal activity with 312~1,250 mg of dry weight/mL against them.

Discussion

Over the years, extracts from plants believed to possess medicinal properties have indeed been shown to contain several components showing therapeutic values (Lewis and Elvin-Lewis, 1997; Valnet, 1994). On the other hand, despite (and perhaps because of) the high diversity of old and new synthesized antibiotics available for therapeutic purpose, bacterial pathogens have developed resistance mechanism, rendering their eradication highly difficult. Therefore, the search for antibacterial agents, such as plant extract compounds, has gained renewed impetus.

Japanese Sawa-wasabi (*Wasabia japonica*) is used as a spice to avoid both the food poisoning and odor (fish smelling) of Japanese traditional foods such as sashimi (sliced raw fish and shellfish) and sushi (vinegary rice ball covered with raw fish). AIT is a major pungent component of Sawa-wasabi, black mustard and horseradish and also well known to have strong antimicrobial activity (Foter and Golick, 1938; Foter, 1940; Kanemura and Miyamoto, 1990; Isshiki et al., 1992). Several mechanisms have been proposed for the antibacterial activity of AIT in Sawa-wasabi, including modulation of SH enzymes, inhibition of RNA synthesis, and partial inhibition of DNA and protein synthesis by action of thiocyanate moiety (−N=C=S).

In the present study, roots, stems and leaves of Sawa-wasabi were evaluated for their inhibitory potential *in vitro* on the growth of the fish pathogenic bacteria. All parts of Sawa-wasabi showed the bactericidal activity against 5 strains of the fish pathogenic bacteria. In common, the main component related to antimicrobial activity of Sawa-wasabi was well known to AIT (Nishida, 1958; Kanemura and Miyamoto, 1990; Isshiki et al., 1992). Almost isothiocyanates containing AIT and ω-methylthioalkyl isothiocyanate (5-methylthiopentyl, 6-methylthiohexyl, 7-methylthioheptyl and 8-methylthiooctyl isothiocyanate) were more contained in roots than stems and leaves (Ina et al., 1990; Kumagai et al., 1994). The Sawa-wasabi leaves, however, showed stronger bactericidal activity than roots against 5 strains of the fish pathogenic bacteria, even though AIT amount of leaves was lower than those of roots (Table 2 and 3). These results suggest that Sawa-wasabi contains certain components besides AIT related to bactericidal activity against the fish pathogenic bacteria.

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