

## Antibacterial activity of Sawa-wasabi (*Wasabia japonica*) on the Growth of Fish Pathogenic Bacteria

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### Introduction

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 antimicrobial effect against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and fish pathogenic bacteria such as *Edwardsiella ictaluri* and *Vibrio hollisae* (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, we investigated the antibacterial activity of the Sawa-wasabi on 6 strains of fish pathogenic bacteria for development of natural antibacterial 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°C. They were separated into root, stem and leaf, and washed cleanly with distilled water. The antibacterial components were extracted by diethyl ether.

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).

Culture of fish pathogenic bacteria: Six strains of fish pathogenic bacteria, *Vibrio hollisae* 1, 2, *V. anguillarum*, *Edwardsiella tarda* 1, 2, and *Staphylococcus captis* were

obtained from National Fisheries Research and Development Institute, Republic of Korea. They were cultured in Brain heart infusion broth (Difco Co.) at 25°C for 48 h. This medium was also used for minimum bactericidal concentration assay.

MBC assay of the Sawa-wasabi extracts: The Minimum bactericidal concentration (MBC) was assayed by method of Bamba et al. (1997). The MBC was defined as the lowest concentration that induced no colony of each bacterium.

## Results and Summary

1. AIT amount in Sawa-wasabi: 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.

2. MBCs of Sawa-wasabi against fish pathogenic bacteria: All parts of Sawa-wasabi showed bactericidal activity on the growth of 5 strains of gram-negative bacteria, but little inhibitory effect on *Staphy captis* (gram-positive bacteria). The Sawa-wasabi leaf showed the strongest bactericidal activity with MBC of 156.3 mg dry weight/ml against 5 strains of gram-negative bacteria, *V. hollisae*, *V. anguillarum* and *E. tarda*. The Sawa-wasabi root and stem showed a little weaker antibacterial activity with 312.5~625 mg dry weight/ml than Sawa-wasabi leaf against 5 strains of gram-negative bacteria.

## References

- Bamba, H., Kondo, Y., Wong, R.M., Sekine, S. and Matsuzki, F. 1997. Evaluation of an assay method of the susceptibility of to antimicrobial agents using a 96-well flat-bottom microplate and a microplate readrer. American J. Gastroenterology. 92 (4), 659-662.
- Forter, M.J. 1940. Bacterial properties of allyl isothiocyanate and related oils. Food Res. 5, 147-152.
- Foter, M.J. and Golick, A.M. 1938. Inhibition properties of horseradish vapors. Food Res. 3, 609-613.
- Inoue, S., Goi, H., Miyauchi, K., Muraki, S., Ogihara, M. and Iwatani, Y., 1983. Inhibitory effect of volatile constituents of plants on the proliferation of bacteria -Antibacterial activity of plant volatiles-. J. Antibact. Antifung. Agents. 11, 609-615.