Antibacterial Actions of Seasonings and Spices on the Viability of *Vibrio parahaemolyticus*

I. Co-operation of Soy Sauce and Sawa-wasabi, Ginger or Garlic

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(Received August 24, 1994)

(Accepted September 26, 1994)

The antibacterial actions of soy sauce, sawa-wasabi, ginger and garlic on the viability of *Vibrio parahaemolyticus* were examined mainly at 5°C. Sawa-wasabi acted most strongly and rapidly of these spices, only in soy sauce, and not alone. Allylisoithiocyanate, the main pungent component of sawa-wasabi had the same action as sawa-wasabi. Ginger had a strong inhibitory action by itself, but showed no synergy with soy sauce. Garlic did not exert its action even in soy sauce. The antibacterial mechanism of soy sauce is suggested to be due at least to the co-operation of its pH (4.5-4.7), Aw (below 0.9) and the content of sodium chloride (10-18%).

Key words: Antibacterial action, Soy sauce, Wasabi, Ginger, *Vibrio parahaemolyticus*.

Introduction

There are many kinds of spices for giving special pungency, flavor and color to foods, and some of them are known to have antibacterial activities. From olden times we have used many spices for the preservation of fishes and meats around the world. In our country, there is also the food custom of dipping fresh and cold slices of fishes and shell meats into soy sauce with wasabi and so on, and then taking them on our tongues. So it is possible that, besides giving better taste, the combination of soy sauce and spices exerts a strong antibacterial action and prevents some food-born diseases. However, there are almost no reports concerning the antibacterial actions of these combinations.

Here, we would like to show our data obtained at mainly 5°C on the effect of sawa-wasabi, ginger and garlic in combination with soy sauce as well as for the inhibitory actions of each of them against *Vibrio parahaemolyticus*, which is still one of the major bacteria responsible for the foodborn diseases in our country.

Materials and Methods

Test organisms

Vibrio parahaemolyticus used is a Kanagawa phenomenon-positive strain (T-81-383) isolated from the feces of a patient and identified at Tokyo Metropolitan Research Laboratory of Public Health.

Culture media and inocula

The strain was maintained on 2% polypeptone (Nihon Pharmaceutical Co., Ltd.) containing 2% NaCl and 1.5% agar at room temperature. The preincubation was done aerobically at 37°C

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Table 1. Effect of temperature on viability of *Vibrio parahaemolyticus* in soy sauce

Temperature	Viable cells/ml						
	0	5 min	10 min	1 hr	2 hr	4 hr	23 hr
5°C	1.5×10^7	9.3×10^6	9.3×10^6	2.4×10^6	2.4×10^6	2.4×10^6	2.3×10^3
15°C	1.1×10^7	9.3×10^6	1.1×10^7	3.9×10^5	4.6×10^3	$< 3 \times 10^1$	
35°C	2.4×10^7	4.3×10^6	2.4×10^5				

Cells were inoculated after preincubation at 5°C, 15°C or 35°C for 1 hr and incubated in soy sauce (pH 4.7). Viability was examined by the MPN method, a triplet of test tubes with BHI broth (pH 8.0) with 0.1 ml from each serial ten fold dilutions of the samples were observed for the growth by turbidity after overnight culture at 37°C.

overnight in a medium consisting of 2% polypeptone with 2% NaCl.

After being kept at the experimental temperature for 1 hr, the preincubated cells were inoculated in soy sauce or 0.05 M citrate buffer containing NaCl with or without the spices to be approximately 10^7 /ml.

Determination of the antibacterial action

Antimicrobial actions were determined as their abilities to decrease the viability of the bacterium. Sampling at 0 time was done immediately after a 5-sec mixing of the cells added in soy sauce or in the citrate buffer with or without the spices. One ml of all samples were successively diluted ten fold by brain heart infusion bouillon (Nissui Pharmaceutical Co., Ltd.) at pH 8.0. The number of viable cells was obtained by the MPN method in triplets for which growth was judged by turbidity after overnight culture at 37°C and ascertained to be *V. parahaemolyticus* on BTB-teepol agar (Nissui Pharmaceutical Co., Ltd.).

Aw (water activity) and pH

The values of Aw were obtained by an Ageless Aw Meter TR-9100 (Mitsubishi Gas Chemical Co., Inc.) and pH was measured by a pH Meter F-13 (Horiba, Ltd.).

Spices and Soy sauces

Fresh sawa-wasabi (*Wasabia japonica* Matsumura), ginger and garlic were crushed into pieces and juice, just before the experiments were started, and used at approximately 10 to 20% concentrations (w/v). Two kinds of soy sauces were tested: one is normal type (Koikuchi) with 18% NaCl and pH 4.7, and the other is reduced-salt type (Gen-en) with 10% NaCl and pH 4.5. They were both commercial products of Kikkoman Co., Ltd. A 0.05 M citrate buffer with the same % of NaCl and the same pH as those of soy sauces was used as the control.

Allylisothiocyanate

Sawa-wasabi is known to contain allylisothiocyanate as the main pungent component. Allyl-isothiocyanate (Wako Pure Chemical Industries, Ltd., special grade) was dissolved in ethylalcohol to be 200 mg/ml and added in soy sauce or 0.05 M citrate buffer with NaCl.

Results

Antibacterial activity of soy sauce

Table 1 shows the effect of temperature on the viability of *V. parahaemolyticus* in soy sauce. At 5°C, the viability was not reduced within 10 min, but decreased to be 17% after 60 min and 0.015% after 23 hr of incubation. At 15°C it decreased to be 3.5% after 1 hr and 0.042% after 2 hr of incubation. At 35°C the number of viable cells decreased markedly to be 18% after 5 min and 1% after 10 min of incubation.

Table 2 shows the antibacterial actions of diluted soy sauces. Even at 35°C, the viability did not decrease at all in the 75% dilution of Koikuchi, but it decreased to be about 0.01% after 10 min of incubation in the 75% dilution of Gen-en. In 0.05 M citrate buffer with 3% NaCl at pH 4.7, the number of viable cells was the same as the inoculum size after 10 min of incubation, but it decreased

Table 2. The viability of *Vibrio parahaemolyticus* in diluted soy sauce at 35°C

	Aw	Viable cells/ml		
		0	5	10 min
Normal type (Koikuchi)				
Soy sauce (pH 4.7) 100%	0.85	4.3×10^7	2.4×10^6	9.3×10^4
75%	0.91	4.3×10^7	4.6×10^7	4.6×10^7
50%	0.93	4.3×10^7	4.3×10^7	4.3×10^7
0.05 M citrate buffer (pH 4.7)				
+ 0.5% NaCl		4.6×10^7	4.6×10^7	2.4×10^7
+ 3.0% NaCl		4.6×10^7	4.6×10^7	4.6×10^7
+ 18% NaCl		1.1×10^8	4.6×10^7	2.4×10^7
Reduced type (Gen-en)				
Soy sauce (pH 4.5) 100%		4.3×10^7	9.3×10^4	4.3×10^2
75%		9.3×10^7	9.3×10^6	9.3×10^3
50%		4.3×10^7	2.1×10^7	4.6×10^5
0.05 M citrate buffer (pH 4.5)				
+ 2.0% NaCl		4.3×10^7	9.3×10^7	1.5×10^7

Cells were incubated at 35°C. The experimental conditions are the same as in Table 1.

Table 3. Effect of sawa-wasabi on viability of *Vibrio parahaemolyticus* at 5°C in the presence of soy sauce

	Viable cells/ml		
	0	7.5	15 min
Soy sauce (pH 4.7)	9.3×10^6	4.3×10^6	4.3×10^6
+ sawa-wasabi (9.2%)	9.3×10^4	7.0×10^2	9.0×10^1
+ sawa-wasabi (25.8%)	2.4×10^5	9.3×10^2	9.0×10^1
18% NaCl·0.05 M citrate buffer (pH 4.7)	2.4×10^7	2.4×10^7	4.6×10^7
+ sawa-wasabi (20%)	2.4×10^7	1.5×10^7	$\geq 2.4 \times 10^7$

Cells were inoculated after preincubation at 5°C for 1 hr. Other experimental conditions are the same as in Table 1.

Table 4. Effect of allylisothiocyanate (AIC) on viability of *Vibrio parahaemolyticus* in soy sauce at 5°C

	Viable cells/ml		
	0	5	10 min
Soy sauce (pH 4.7)	2.4×10^7	2.4×10^7	4.6×10^7
+ AIC 100 ppm	3.9×10^6	2.4×10^6	4.6×10^6
+ AIC 200 ppm	9.3×10^4	4.3×10^3	2.4×10^3
+ AIC 400 ppm	$< 3 \times 10^2$	$< 3 \times 10^1$	$< 3 \times 10^1$
50% soy sauce (pH 4.7)			
+ AIC 400 ppm	2.4×10^7	1.1×10^7	1.1×10^7
Soy sauce (pH 4.5)	3.9×10^7	9.3×10^6	
+ AIC 400 ppm	9×10^1	$< 3 \times 10^1$	
18% NaCl·0.05 M citrate buffer (pH 4.5)	3.9×10^7		2.4×10^7
+ AIC 400 ppm	4.3×10^7	4.3×10^7	2.4×10^7

The experimental conditions are the same as in Table 3.

slowly in the same buffer with 0.5% and 18% NaCl.

Antibacterial activity of sawa-wasabi

Table 3 shows the antibacterial actions of sawa-wasabi in the combination with soy sauce. At 5°C, the number of viable cells in soy sauce with 9.2% sawa-wasabi was immediately decreased to be 1% just after inoculation, although sawa-wasabi could not exert its antibacterial action at all in the citrate buffer. Therefore, we concluded that sawa-wasabi exerts its strong antibacterial action

Table 5. Effect of ginger on viability of *Vibrio parahaemolyticus* at 5°C

	Viable cells/ml				
	0	5	7.5	10	15 min
Soy sauce (pH 4.7)	9.3×10^6		2.3×10^6		2.3×10^6
+ ginger (21.7%)	4.3×10^6		9.3×10^3		4.3×10^2
18% NaCl·0.05 M citrate buffer (pH 4.7)	4.6×10^7	4.6×10^7		4.3×10^7	
+ ginger (16.7%)	2.4×10^7	4.3×10^5		9.3×10^2	

The experimental conditions are the same as in Table 3.

Table 6. Effect of garlic on viability of *Vibrio parahaemolyticus* at 5°C

	Viable cells/ml		
	0	7.5	15 min
Soy sauce (pH 4.5)	4.6×10^6	4.6×10^6	4.3×10^5
+ garlic (21.3%)	2.4×10^7	1.1×10^7	2.4×10^6

The experimental conditions are the same as in Table 3.

with soy sauce.

Table 4 shows that, in the soy sauce with allylisothiocyanate at 200 ppm the viability decreased to be 0.39% just after inoculation, and the higher the concentration of allylisothiocyanate, the stronger the antibacterial effect became. Allylisothiocyanate could not act on the viable cells in the citrate buffer, that is, without soy sauce.

Antibacterial activity of ginger

Ginger exerted its antibacterial action strongly without soy sauce. As Table 5 shows, in the citrate buffer with 16.7% ginger the number of viable cells decreased to be 1.8% after 5 min and 0.0039% after 10 min of incubation. The viability in soy sauce with ginger was decreased to almost the same rate as that in the citrate buffer with ginger. This means ginger did not act synergistically with soy sauce to inhibit *V. parahaemolyticus*.

Antibacterial activity of garlic

The number of viable cells did not decrease below 10% even in soy sauce with 21.3% garlic after 15 min of incubation as shown in Table 6. At 5°C garlic did not effect the viability of the cells of *V. parahaemolyticus*, either by itself, in the presence of soy sauce.

Discussion

Before the appearance of chemical food additives, some spices had been used very frequently as preservatives for fishes and meats. Therefore, many edible plants were examined for the presence of antibacterial substances. However, *Vibrio parahaemolyticus* was not known yet in those days, we have almost no report on the antibacterial activity of soy sauce and spices against this bacterium except for the recent papers^{1, 5)}.

Soy sauce has been used as an effective preservative for many kinds of foods to date. Therefore its antibacterial effects on this bacterium are not surprising. However, the mechanism of the antibacterial activity of soy sauce has not been clearly elucidated yet. Our present data seem to add suggestions for its mechanism to be due at least to the combined effects of pH, Aw and NaCl. First of all, soy sauces generally have a pH around 4.5 to 4.7, and diluted soy sauce has almost the same pH because of its strong buffer action. The pH might play the main role on the antibacterial action, as we examined previously the effect of pH on the viability of *V. parahaemolyticus* by some organic acids and found it critical^{8, 9)}. Next, many soy sauces have Aw of below 0.9. Therefore, Aw should be considered as an important factor. It seems that Aw for the viability is lower than 0.95 for the

growth, because as shown in Table 2 the viable cells were kept at the same level at least for 10 min at 35°C in the 75% soy sauce dilution with an Aw of 0.91. Third, soy sauces have a high concentration (10–18%) of NaCl, with which this bacterium is not able to grow in any medium. As seen in Table 2, the viability decreased in 0.05 M citrate buffer with 18% NaCl, but it did not decrease in the same buffer with 3% NaCl. As one more factor, we should consider the organic acid contents. Besides contributing to the pH, they seem to be one reason why Gen-en could effect the viability in the 50% and 75% dilutions and Koikuchi could not at the same percent dilutions, although we have no data on any organic acids in soy sauces.

Concerning spices, wasabi has been reported to act effectively against bacteria^{6, 10)} and such parasites as *anisakis*⁷⁾ in some sea fishes. In the present paper, even at a low temperature, sawa-wasabi in soy sauce reduced the viability of *V. parahaemolyticus* rapidly, although it could not decrease it alone. Accordingly, the traditional and combined usage of soy sauce and sawa-wasabi for raw and small pieces of fresh fishes and shells without skin or bone, would be concluded to be reasonable in order to prevent some seafish-born diseases. Allylisothiocyanate, a main pungent component of wasabi and mustard, has been reported to be effective for the growth inhibition of the microorganisms in foods^{3, 4)} recently. Then, their combined usage may be useful to protect some foods from spoilage and pathogenic bacteria.

Shogaol and zingerone from ginger, and allycin²⁾ from garlic are well known as antibacterial substances. We could not find their synergistic action with soy sauce against *V. parahaemolyticus*. Finally we have to mention that a concentration higher than 10% used in this report was sufficient for the three fresh spices to exert their actions fully. It is interesting that sawa-wasabi, ginger and garlic exert their actions in different ways on this bacterium in addition to giving their characteristic flavors to foods.

References

- 1) Beuchat, L. R.: Sensitivity of *Vibrio parahaemolyticus* to Spices and Organic Acids. J. Food Sci., **41**, 899–902 (1976).
- 2) Chester, J. C. and Bailey, J. H.: Allicin, the Antibacterial Principle of *Allium sativum*. I. Isolation, Physical Properties and Antibacterial Action. J. Am. Chem. Soc., **66**, 1950–1951 (1944).
- 3) Goi, H., Inoue, S. and Iwatani, Y.: Antifungal Activity of Powdery Black Mustard, Powdery Wasabi (Japanese Horseradish) and Allylisothiocyanate by Gaseous Contact—Antifungal Activity of Plant Volatiles—. J. Antibact. Antifung. Agents, **13**, 199–204 (1985).
- 4) Inoue, S., Goi, H., Miyauchi, K., Muraki, S., Ogihara, M. and Iwatani, Y.: Inhibitory Effect of Volatile Constituents of Plants on the Proliferation of Bacteria—Antibacterial Activity of Plant Volatiles—. J. Antibact. Antifung. Agents, **11**, 609–615 (1983).
- 5) Isshiki, K., Tokuoka, K., Mori, R. and Chiba, S.: Preliminary Examination of Allyl Isothiocyanate Vapor for Food Preservation. Biosci. Biotech. Biochem., **56**, 1476–1477 (1992).
- 6) Nishida, M.: Studies on the Pungent Component. IV. Antibacterial Properties of Essential Oil of *Eutrema Wasabi* Maxim. Yakugaku Zasshi (In Japanese), **78**, 435–436 (1958).
- 7) Murata, I., Miyazawa, S., Kuni, M., Nakajima, Y., Shibuya, T. and Nakanishi, H.: Inhibitory Effects of Partial Freezing, Several Kinds of Condiments, Spices, and Seasonings on the Activity of *Anisakis* Type 1 Larvae Collected from the Codfishes and Mackerels. Ann. Rep. Tokyo Metr. Res. Lab. P. H., **38**, 13–21 (1987).
- 8) Nose, M., Kazato, M. and Sakai, S.: Effect of Several Organic Acids and Sodium Chloride on the Viability of *Vibrio parahaemolyticus*. J. Food Hyg. Soc. Japan, **26**, 579–584 (1985).
- 9) Nose, M., Kazato, M. and Sakai, S.: Acid Injury of *Vibrio parahaemolyticus*. Jpn. J. Food Microbiol., **3**, 135–140 (1986).
- 10) Tetsumoto, S.: Antibacterial Activities of Some Spices and Seasonings against *Salmonella typhi* and *Vibrio Cholerae*. Nippon Nogei Kagaku Kaishi (In Japanese), **10**, 123–127 (1934).

調味料と香辛料が腸炎ビブリオの生存に及ぼす抗菌作用

I. 醤油と沢わさび, しょうが, にんにくの共働抗菌作用

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腸炎ビブリオの生存に及ぼす醤油と沢わさび, しょうが, にんにくの共働抗菌作用を検討した。その結果, 沢わさび添加醤油の抗菌作用が最も大で, 5℃においても急激な生菌数の減少が認められた。なお, 沢わさびの主要香気成分アリルイソチオシアネートも同様な共働抗菌作用を示した。しょうがは単独で強い抗菌作用を有するが, 醤油との相乗効果は観察されなかった。にんにくは, 5℃では醤油との共存下でも抗菌作用を示さなかった。醤油の希釈液, 醤油と同一 pH・食塩含有クエン酸緩衝液中での腸炎ビブリオの生存性を検討した結果, 醤油の抗菌メカニズムは pH, Aw, 食塩などの共働作用によるものと推論された。