Research Note

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Effects of Garlic Extract on Acid Production and Growth of *Streptococcus mutans*

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ABSTRACT

Garlic extracts have been used for medicinal purposes for about one thousand years. The antibacterial properties of garlic have been known for more than 60 years. This study was aimed to examine the effects of filtered garlic extracts and diallyl sulfide on the acid production and the growth of *Streptococcus mutans* 10449 and BHT. Results showed that both garlic extracts and diallyl sulfide could enhance the rate of acid production and inhibit the growth of *S. mutans*. Strain BHT was highly susceptible, and strain 10449 less sensitive. These data indicate that garlic extracts stimulates the acid production and may thus be harmful to the teeth if eaten with glucose-containing foods, but may inhibit the growth of *S. mutans* and subsequently reduce the caries incidence.

Key words: garlic extracts, diallyl sulfide, acid production, *Streptococcus mutans*

INTRODUCTION

Dental caries is a common disease in the oral cavity[(1,2)]. A series of animal experiments and clinical trials indicate that multiple factors contribute to the occurrence of dental caries[(1-3)]. The contributing factors include sugar and other dietary components. Research has focused on various aspects of sugars in foods which may have etiologic roles in the caries processes[(4)]. The diet (food and drink) may exert an effect on caries locally in the mouth by reacting with the enamel surface or by serving as a substrate for cariogenic microorganisms. S*treptococcus mutans* has been suggested to play an important role in the initiation of dental caries in humans[(2,5)]. Its cariogenicity is due to the bacterium's capacity to produce various acids from dietary sugars with a resultant demineralization of tooth enamel[(1-5)].

Garlic has been used for some 3,000 years as a flavor-enhancing food and folk medicine by Chinese and Egyptians[(6,7)]. Garlic has strong odors, sharp tastes and marked physiological effects that induce tears, sweat and salivary secretion. A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be sensitive to garlic extract[(8-10)]. Animal and *in vitro* experiments showed that garlic juice inhibits the growth of bacteria of the genera *Streptococcus*, *Staphylococcus*, and *Bacillus*[(9,11,12)]. Other studies have reported that garlic extract has antimicrobial activities against oral bacteria[(13)] and oral streptococci[(14)], but there are few data on its effects against dental caries pathogen, particularly *S. mutans*.

Diallyl sulfide is representative of thioethers that occur naturally in garlic; such sulfur-containing chemical has been of pharmacologic interest[(8,12,15)]. Chemical analyses of garlic cloves have revealed an unusual concentration of sulfur-containing compounds (1-3%). Therefore, diallyl sulfide was used in this study to explore how such compounds might be responsible for the cariogenesis.

Recognition of the anti-microbial role of garlic has stimulated research on its whether and how it might act as a protectant against dental caries. Despite numerous reports of the antibacterial properties of garlic extracts, the effects of garlic on glucose fermentation is barely known. Therefore, this study was conducted to investigate the effect of garlic extract on acid production in two strains of *S. mutans*. In addition, growth inhibition of tested microorganisms was also determined.

MATERIALS AND METHODS

I. *Growth and Harvesting of Bacteria*

S. mutans 10449 (serotype c) and BHT (serotype b) were examined in this study. Cultures were grown on blood agar plates and were renewed from lyophylized stocks after four successive subcultures. Several colonies from blood-agar plates were inoculated into TSYG broth (3%

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Trypticase soy, 0.5% yeast extract, 0.25% dextrose) and were grown overnight (18 h) in an anaerobic jar at 37°C. Cultures were harvested by centrifugation (8,000 × g for 10 min at 4°C), washed twice with one volume of 2 mM potassium phosphate buffer (pH 7.0) and resuspended in the same buffer. Cell suspensions were adjusted to ca. 10 mg dry weight/mL (OD=10) and were held on ice for no more than four hours before use.

II. *Preparation of Garlic Extract*

Fresh garlic was purchased from a local market, washed and prepared for the crude juice extraction. One hundred grams of cleaned garlic bulbs with 125 mL of distilled water were crushed in a juicer. The mixture was then filtrated and further centrifuged at 10,000 × g for 20 min. The supernatant was filtered through a 0.45 μm Millipore filter (Corning, USA) to remove microorganisms. By subtracting the weight of the insoluble material from the original garlic cloves, the final concentration of the garlic extract in solution was determined to be 249 mg/mL (24.9%, w/v). Aliquots were stored at -20°C until required.

III. *Rate of Acid Production*

The rate of acid production by the non-growing cells was estimated with a Metrohm pH stat (Brinkmann Co., Westbury, NY) by measuring of the titration volume of 20 mM KOH at a pH of 7.0[16]. The pH stat system consists of a model 632 pH meter, an automatically controlled titrant delivery unit (model 614 Impulsomat), an electronic strip chart recorder (Brinkmann BR-100), and a titration assembly with a 5 ml autoburette (model 665 Dosimat). The reaction mixture contained bacterial cells (323 μg dry weight/mL, OD=1.0), 2 mM potassium phosphate buffer, 18 mM KCl and 0.4 mM $MgCl_2$. Fermentations were started by the addition of 20 mM glucose and proceeded at 37°C in air. The reaction mixture was agitated by a magnetic stirrer and the amount of KOH added was recorded continuously. The rate of acid production was calculated as described before[16].

In order to examine the effects of garlic extract and diallyl sulfide (Sigma Company, St. Louis, MO. U.S.A.) on glucose fermentation, the garlic extract or diallyl sulfide was added 6 minutes after the addition of glucose. Tested agents were omitted from the controls. The amounts of KOH added from 4 to 6, 12 to 14, and 18 to 20 min. were recorded. The difference between control and experimental fermentations without inhibiting agents (4-6 min.) was no more than 5%. The effect of tested agents on fermentation rate (percentage of control) was calculated from the equation: % of control = (the amount of KOH added from 12 to 14 min or 18 to 20 min) in the experimental group/the amount of KOH added from 12 to 14 min or 18 to 20 min in the control group)/(the amount of KOH added from 4 to 6 min in the experimental group/the amount of KOH added from 4 to 6 min in the control group) × 100. Thus, the rate of acid production calculated from 12 to 14 min represents the rate of acid production at 7 min, and the rate of acid production calculated between 18 to 20 min represents the rate of acid production at 13 min.

IV. *Bacterial Growth Experiments*

Several colonies from blood-agar plates were inoculated into TSYG broth and were grown overnight in an anaerobic jar at 37°C. Overnight cultures were diluted 10 fold in 10 ml TSYG broth in a glass tube. Bacterial cultures which contained bacterial cells with an optical density of 0.11 to 0.13, were incubated and shaken in a water bath at 37°C in air. The optical density of growth was measured with a spectrophotometer at 600 nm wavelength. The tested agents (garlic extracts or diallyl sulfide) were added in bacterial cultures at the beginning of each experiment. Each assay was performed twice on different days.

RESULTS

I. *Effect of Garlic Extract on Rate of Acid Production*

The dose responses for the effect garlic extract on acid production of *S. mutans* cells are shown in Figure 1. Garlic extract stimulated the acid production of *S. mutans* cells. Strain BHT had a greater response to the garlic extract in the rate of acid production than did strain 10449.

For strain BHT, when the garlic extract's concentrations were between 0.75 mg/mL and 2.5 mg/mL, the rates of acid production were faster at 7 min than at 13 min. With garlic extract at other concentrations, the rates of acid production were not different between 7 min and 13 min (Figure 1).

For strain 10449, when the garlic extract's concentrations were between 0.25 mg/mL and 0.75 mg/mL, the rates of acid production were greater at 13 min than at 7 min. When the garlic extract's concentration was at or above 0.75 mg/mL, the rates of acid production showed no difference between 7 and 13 min (Figure 1).

II. *Effect of Diallyl Sulfide on Rate of Acid Production*

Figure 2 shows that diallyl sulfide increased the rate of acid production of *S. mutans*. The increase in rate of acid production of strain BHT was more obvious than that for strain 10449. The rate of acid production of strain BHT at 7 min was faster than at 13 min. When strain 10449 was tested in the same range for concentration of diallyl sulfide, there was no difference between 7 min and 13 min in the rate of acid production.

III. *Effect of Garlic Extract on Growth of S. mutans*

The growth inhibition activity of garlic extract

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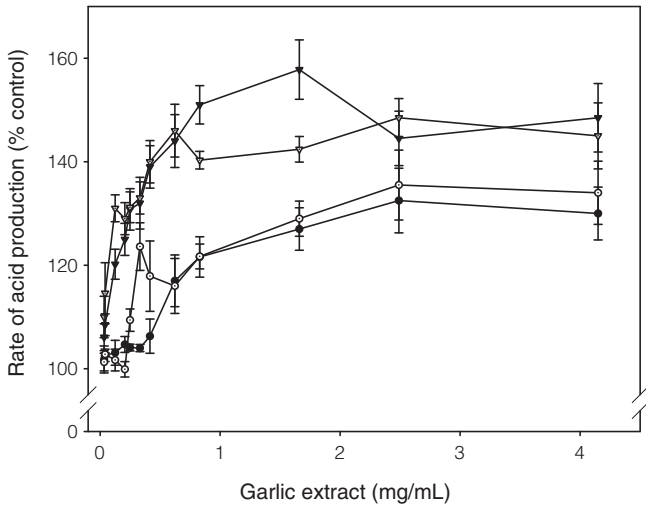

Figure 1. Effect of garlic extract on the rate of acid production by *S. mutans* BHT and 10449. Rate of acid production was determined at 7 min and 13 min after incubation with garlic extract. Results represent the mean ± SD of four experiments. Symbols: (▼) strain BHT at 7 min; (▽) strain BHT at 13 min; (●) strain 10449 at 7 min; (○) strain 10449 at 13 min.

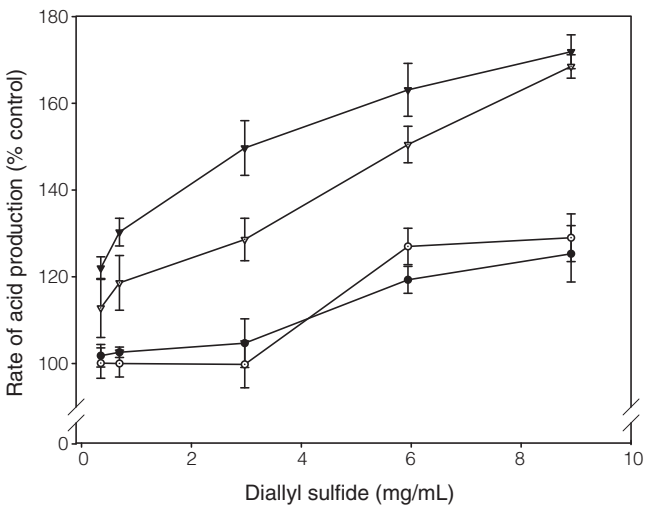

Figure 2. Effect of dially sulfide on the rate of acid production by *S. mutans* BHT and 10449. Experimental condition as for Figure 1. Results represent the mean ± SD of four experiments. Symbols: (▼) strain BHT at 7 min; (▽) strain BHT at 13 min; (●) strain 10449 at 7 min; (○) strain 10449 at 13 min.

was investigated (Figure 3). When garlic concentration increased, the suppression of growth of *S. mutans* was more obvious. The suppressive effect of garlic extract on strain 10449 was greater (Figure 3A). However the effect of garlic extract on the suppression of the growth of strain BHT exhibited a threshold effect. When the garlic extract concentration was less than 2.08 mg/mL, the suppression of growth was not obvious, but when garlic extract concentration was at or above 4.15 mg/mL, the suppression of growth was more obvious (Figure 3B).

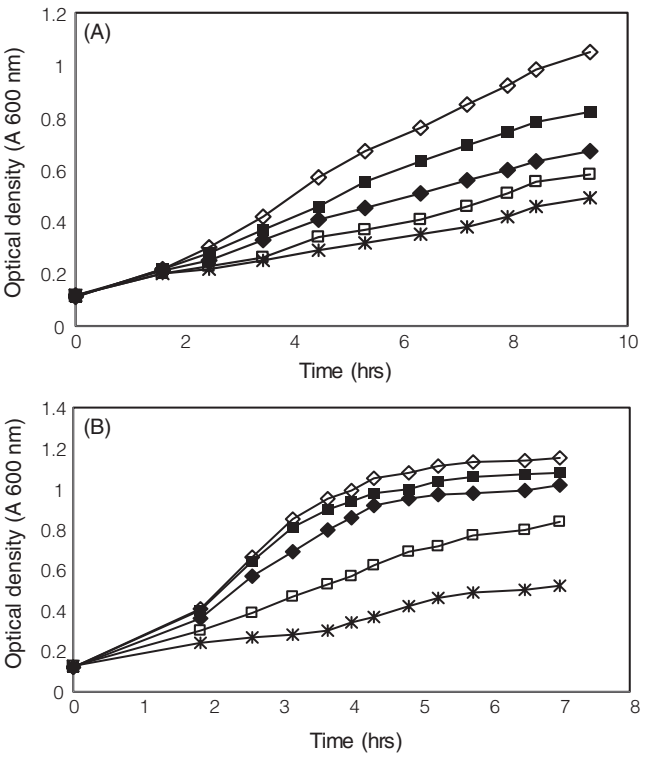

Figure 3. Effect of garlic extract on growth curve of *S. mutans* 10449 (panel A) and BHT (panel B). See text for details. Data represent the mean of two experiments. Symbols: Control (◇); 0.83 mg/mL (■); 2.08 mg/mL (◆); 4.15 mg/mL (□); 8.30 mg/mL (∗).

IV. *Effect of Diallyl Sulfide on Growth of S. mutans*

The growth of *S. mutans* cells was also affected by diallyl sulfide (Figure 4). As the concentration of diallyl sulfide increased, the growth inhibition of *S. mutans* was more obvious. The result was the same as that for garlic extract, i.e., diallyl sulfide's inhibition of growth of strain 10449 was greater (Figure 4A). The effects of diallyl sulfide on strain BHT also exhibited a threshold. For concentrations less than 1.49 mg/mL, suppression of growth was not obvious, but when diallyl sulfide concentration was at or above 2.97 mg/mL, the growth of strain BHT was almost completely suppressed (Figure 4B).

DISCUSSION

This investigation clearly demonstrated that garlic extracts and diallyl sulfide enhanced the acid production (glucose fermentation) of *S. mutans* BHT and 10449. The stimulation of acid production by garlic extract was concentration- and incubation time-dependent. Surprisingly, no inhibitory effect on acid production of *S. mutans* was observed, although a bactericidal effect on *S. mutans* was reported previously[13,14]. This *in vitro* study demonstrated the stimulatory effect of garlic extracts on certain strains of *S. mutans*. It should, however, be stressed that

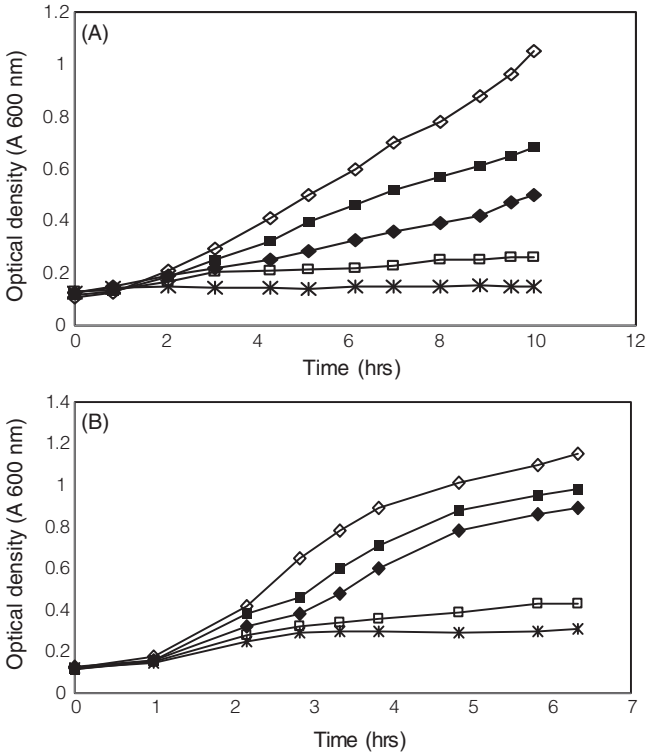

Figure 4. Effect of diallyl sulfide on growth curve of *S. mutans* 10449 (panel A) and BHT (panel B). Experimental condition as for Figure 3. Symbols: Control (◇); 0.74 mg/mL (■); 1.49 mg/mL (◆); 2.97 mg/mL (□); 5.94 mg/mL (∗).

little is known about the conditions *in vivo*. *In vivo* plaque acidogenicity tests are needed to provide more relevant information on the relative cariogenicity or anti-cariogenicity of garlic.

Different susceptibilities to garlic extract were demonstrated with different bacterial strains by measuring the acid production. We found that strains of serotype b (BHT) were highly susceptible, and serotype c (10449) less sensitive. Bacterial strain-dependent differences in sensitivity to garlic extract were also demonstrated by means of a bacterial growth experiment. Diallyl sulfide had similar outcomes to garlic extract. The findings of serotype c susceptibility are of particular interest since it is the most frequently related serotype of *S. mutans* from human plaque[17].

The basis for inter-strain variation in garlic extracts and diallyl sulfide sensitivity is unknown for *S. mutans*. It is likely that strain-dependent changes in the proportion and composition of surface molecules, such as peptidoglycans, lipoteichoic acid, fibrils and capsular material, occured[17-19]. Such changes could modulate garlic extract binding at its active site on the cell surface. Since cell surface composition is changed in different strains, it is possible that the strain variation is due in part to cell surface composition[17-19].

There should be normal flora in the oral cavity, including the species of *S. mutans*; thus the antibacterial agents used in the oral cavity need not totally eliminate the caries pathogens. In the present study, we demonstrated that garlic extract inhibited *S. mutans* cell growth. This is an important cascade in caries prevention. Garlic extract could block this cascade by acting to inhibit the *S. mutans* growth. As shown in a recent study, mouthwash containing garlic extract (2.5%) reduces the salivary levels of streptococci after 2 weeks of mouthwash use[14]. Therefore, low concentration of garlic could have benefits for oral health.

There is no doubt that acid production is involved in the formation of caries. In dental plaque, the pH decreases following a rinse with a sugar containing diet for bacterial fermentation[20]. In the oral cavity, garlic may have two protective properties: antibacterial activity and stimulation of saliva secretion by the garlic spice taste. Although this study showed that acid production of *S. mutans* was enhanced by garlic extract, the protective properties of garlic could be potentiated by salivary stimulation. Saliva plays an important role in caries prevention[21,22]. Saliva can neutralize the acid from bacteria. Salivation stimulated by garlic consumption would reduce the fall in plaque pH that could lead to demineralization and increase the potential for remineralization.

CONCLUSIONS

The present study indicates that garlic extract stimulates acid production and so could be harmful to the teeth if eaten with glucose-containing foods. We also presented evidence for the growth inhibition activity of garlic extract and diallyl sulfide against *S mutans*. Despite the stimulation of acid production, garlic may prevent dental caries by the stimulation of salivary secretion and inhibition of bacterial growth in the oral cavity. Thus, garlic may have potential to prevent dental caries.

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