Anti-bacterial action of onion (Allium cepa L.) extracts against oral pathogenic bacteria*

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Abstract: In this study, the focus was on the anti-bacterial activity of onions. This study researched the activities of onion extracts on Streptococcus mutans and Streptococcus sobrinus, the main causal bacteria for dental caries, and Porphyromonas gingivalis and Prevotella intermedia, considered to be the main causal bacteria of adult periodontitis.

The results showed that the onion extracts possess an effect on all test bacterial strains (S. mutans JC-2, S. sobrinus OMZ176, P. gingivalis ATCC 33277 and P. intermedia ATCC 25611), and the effects were bactericidal against cultured and resting bacterial cells. The activity of the onion extracts was stable even after 48 hours in the culture medium. This result suggests that no decomposition or volatility of onion extracts occurred in the culture medium. The antibacterial activity of onion extracts was not markedly influenced by cysteine (10 mM) treatment. However, activity significantly decreased with alkali treatment. Grated onion left to stand at 37°C for 48 hours did not show antibacterial activity. Also, activity of steam treated (100°C, 10 min.) onion was not observed. Using avicel plate by thin layer chromatography with the solvent of n-butanol: acetic acid:water (3 : 3 : 1), the main component of the substance (the substance which develops color with ninhydrin) was observed at an Rf value of about 0.9.

Key words: onion; antibacterial activity; oral pathogenic bacteria.

Introduction

As a result of having investigated natural substances which have the potential of effectively preventing dental caries and periodontitis, the author has focused on the onion extracts which have a peculiar odor and are a common food throughout the world. Though it has been suggested by the historical studies (1) that onion extracts contain many components such as a lacrimatory stimulating component, only a few studies on the antimicrobial activity of onion extracts have been reported. These reports describe antimicrobial activity exhibited by fungi such as Aspergillus flavus (2), and Penicillium rubrum (2,3), in addition to Gram-positive bacteria such as Bacillus cereus (3,4), Micrococcus luteus (3) and Gram-negative bacteria such as Klebsiella pneumoniae and Escherichia coli (3).

Bacteriological studies have identified the Streptococcus mutans group (5,6) as the main causative bacteria for dental caries, and the black pigmented Gram-negative obligative anaerobic rod (7,8) such as Porphyromonas gingivalis and Prevotella intermedia were considered to be the main causative bacteria of adult periodontitis. Although many potential preventive methods against these diseases have been investigated, no completely effective preventive method has ever been established. The purpose of the current study was to examine the possibility of the use of onion extracts as a preventive agent against dental caries and adult periodontitis.

Materials and Methods

1. Preparation of onion extracts

Extraction was made according to the method of Sharma (2) from 100 g of onion.

Treatment of onion. Onions were treated in one of the following two ways: Extraction was made after steam-processing of the onion in a steaming kettle for 10 minutes, or extraction was made after leaving the grated material stand at 37°C for 48 hours. The activities of these extracts were compared with that of non-treated materials.

2. Preparation of bacterial strains

The S. mutans JC-2 and S. sobrinus OMZ176 were cultured anaerobically by gas-substitution (95 % N₂, 5 % CO₂) at 37°C for 20 hours in Brain Heart Infusion (BHI, Difco). P. gingivalis ATCC33277 and P. intermedia ATCC25611 were cultured in GAM broth (Nissui) supplemented with hemin (5.0 mg/l) and menadione (1.0 mg/l) (H-M-GAM) at 37°C for 48 hours in an anaerobic glove box (Forma Scientific Anaerobic System Model 1024) under the conditions of 80 % N₂, 10 % CO₂ and 10 % H₂.

3. Measurement of antibacterial activity

1) Activity value. The activity was determined by using a modification of the method of Hayes et al. (9).

Briefly, the autoclaved Mitis Salivarius Agar (MS, Difco) was cooled to 50°C and poured into a plate which
contained 10 µl of bacterial suspension (approximately $1 \times 10^6$ colony forming unit, CFU). This plate was punched aseptically with a 3.5 mm in diameter steel pipe, and 10 µl of two times series-diluted sample was put into the wells. The diameter of the formed inhibition zone was measured after it was anaerobically cultured at 37°C for 24 hours. From the calibration curve, the activity showing an inhibition zone of 10 mm was defined as 10 units (Fig. 1).

2) The minimum inhibitory concentration (MIC) and bactericidal activity. The MIC of *S. mutans* and *S. sobrinus* in onion extracts was measured by the liquid culture medium dilution method using BHI. Briefly, 965 µl of BHI was put in a sterilized test tube, to which 25 µl of the onion extracts was added so that the final concentrations became 20, 40 and 80 µg/ml. Immediately, 10 µl of the cultured bacterial cell suspension of *S. mutans* JC-2 or *S. sobrinus* OMZ176 (final bacterial count in approximately $1 \times 10^6$ CFU/ml) was inoculated, and the mixture was anaerobically cultured at 37°C for 48 hours.

The minimum concentration of added onion extracts in which no growth was observed was defined as the MIC.

To estimate whether this inhibition of growth was bactericidal, the sample containing 40 µg/ml of the onion extracts was collected over an extended time period. This sample was diluted and inoculated on an MS plate. After

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**Fig. 1** Standard curve for antibacterial action of onion extracts against *S. mutans.*

**Fig. 2** Antibacterial effect of onion extracts against each growing bacterial cell

A: *S. mutans*, B: *S. sobrinus*, C: *P. gingivalis*, D: *P. intermedia*

○: None, ●: Onion extracts
anaerobically cultured for 48 hours, the number of colonies was counted. Further, to assess the bactericidal activity on resting bacterial cells, *S. mutans* or *S. sobrinus* growing cells were harvested and washed twice with 0.05 M tris-HCl buffer (pH 7.2, TB) and the inhibitory effects of onion extracts against resting cells was investigated in the same manner as above.

Antibacterial activities on *P. gingivalis* and *P. intermedia* were estimated by the same method as that for *S. mutans*, although the culture medium and condition of culture were different. The culture media used were H-M-GAM instead of BHI, Columbia Agar Base (Oxid) plate culture medium supplemented with hemin, menadione and 5 % horse-defibrillated blood instead of MS. These were cultured in an anaerobic glove box. Further, reduced transport medium (10) was used instead of TB.

4. Estimation of antibacterial substance extracted from onion by thin layer chromatography (TLC)

1) Developing solvents and plates.

(1) A developing solvent of n-butanol: acetic acid: water (3 : 3 : 1) (11) with an avicel plate (Funakoshi)

(2) A developing solvent of petroleum ether: diethyl ether: acetic acid (60 : 40 : 1) (12) and hexane: ethyl acetate (60 : 40) (13) with a silica gel plate (Wako).

2) Detecting reagents. Iodine steam, 50 % H₂SO₄, ninhydrin reagent (Wako) or glycine-formaldehyde reagent (14) were used as the detecting reagents. Subsequently, the developed substance in each site was scraped off of the TLC plate before the color developing manipulation and extraction, and before measurement of activity using *S. mutans* JC-2 strain. The obtained Rf values of the onion extracts were compared with the onion-constituting component reported previously (11-14).

5. Various properties of antibacterial substances extracted from onion

As no difference of the activity of the onion extracts among the tested bacterial strains was observed, the following experiments were performed using the *S. mutans* JC-2.

1) Influence by heat treatment. After heat treatment of the onion extracts (activity in 10 units) at 60°C for 30 minutes and 100°C for 10 and 30 minutes, its activity was

![Fig. 3 Antibacterial effect of onion extracts against each resting bacterial cell](image-url)
compared with that of non-treated onion extracts.

2) Influence by cysteine treatment. Cysteine was added to the sample (final activity concentration in 10 units) so that the concentrations became $5 \times 10^{-3}$ and $1 \times 10^{-2}$ moles. The mixture was adjusted to a pH of 7.0 with 10 % NaHCO$_3$, and the activity was estimated in the presence or absence of cysteine after incubation at 37°C for 1 hour.

3) Influence by alkali and acid treatments. After treating the onion extracts (final activity concentration in 10 units) with 0.1M tris-aminomethane solution or 0.1N HCl solution at 37°C for 5 hours, the pH was adjusted to 7.0, and the activity was estimated in comparison with that of 0.1M TB.

4) Volatility and decomposition of onion extracts. The culture medium solution containing onion extracts was pre-incubated at 37°C for 0, 24 and 48 hours using a screw-capped or a silicone-capped test tube. The activity of onion extracts was measured by the same experimental method as described in the Materials and Methods section [3,2].

Results

1. Influence of preparation of onion extracts

No antibacterial activity was observed in the sample extracted from steam-processed onions or from grated onions kept at 37°C for 48 hours.

2. Antibacterial effect of the onion extracts

1) MIC and bactericidal action. The MIC of onion extracts on all the test bacterial strains was about 40 µg/ml, and no difference was observed among the tested bacterial strains. Figs. 2-A, 2-B, 2-C and 2-D show the effects of onion extracts against growing tested bacterial cells. The growth of tested bacteria decreased gradually with the incubation time, and no colony on the medium plate was observed in S. mutans or S. sobrinus at 24 hours and few colonies were observed on the medium plate for P. gingivalis or P. intermedia at 48 hours. Figs. 3-A, 3-B, 3-C and 3-D show the effect of onion extracts against resting tested bacterial cells. The number of colonies on the medium plate decreased remarkably, rapidly and rectilinearly in all the bacterial strains and the survival rates became less than 1 percent in S. mutans and S. sobrinus after 3 hours and in P. gingivalis and P. intermedia after 1 hour.

Table 1: Effect of various treatments on antibacterial activity of onion extracts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td>Heat 60°C, 30min</td>
<td>10.0 ± 0.7</td>
</tr>
<tr>
<td>100°C, 10min</td>
<td>5.6 ± 0.1</td>
</tr>
<tr>
<td>100°C, 30min</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Cysteine 5mM</td>
<td>9.7 ± 0.2</td>
</tr>
<tr>
<td>10mM</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>Acid and Alkali</td>
<td></td>
</tr>
<tr>
<td>0.1N HCl</td>
<td>8.8 ± 0.2</td>
</tr>
<tr>
<td>0.1M Tris-aminomethane</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
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Fig. 4: TLC on an avicel plate of onion extracts
A: Extract without standing
B: Extract after leaving the grated material at 37°C for 48 hours
steam, though it was found slightly at the Rf values of 0.5, 0.3 and 0.1. Iodine steam-positive spots were also observed at the Rf values of 0.6, 0.3 and 0.15 by hexane:ethyl acetate-developing solvent. Although Rf values of these spots were seen slightly changed in the sample reacted with 10 mM cysteine, most of the activity remained at the starting point. Multiple ninhydrin-positive spots were confirmed to be present in a n-butanol:acetic acid:water solvent system using anavice plate (Fig. 4). As a result of measuring the activity, the author was able to find the activity only at the ninhydrin-positive spot with the Rf value of 0.9 (Lane A). No spots of the same Rf value were detected in the extracts from grated onion whose antibacterial activity disappeared after standing at 37°C for 48 hours, but clear ninhydrin-positive spots considered to be decomposed substances were detected (Lane B).

Discussion

It is widely known that the most common diseases causing tooth defects are dental caries and periodontitis, and that the important causal bacteria associated with these diseases are S. mutans and S. sobrinus for dental caries (5,6) and P. gingivalis and P. intermedia for adult periodontitis (7,8). Therefore, with the aim of preventing dental caries and adult periodontitis, an assessment was made of the antibacterial activity of onion extracts.

The components in onions have been comprehensively investigated, with the focus of this research has centering on sulfur compounds and aspects such as odor, pungency and lacrimatory property. It has been reported\(^1\) that onion extracts are antimicrobial to various bacteria\(^{[2,3]}\) and fungi (2,4). However, there is no report concerning the antibacterial activity to dental caries- or adult periodontitis-related bacteria.

Abdel et al. (3) reported that Gram-positive bacteria have a higher sensitivity than Gram-negative bacteria to onion oil, but almost the same activity was observed between two strains of Gram-positive bacteria and two strains of Gram-negative bacteria tested in this study. The difference in the results is ascribed to the difference in the bacterial strains used and obligative anaerobe used as Gram-negative bacteria. To conclude this point, it is considered that isolation of the antimicrobial components is required.

In the present experiment where concentrations of onion extracts and inoculated viable bacterial counts in which the effects were fully observed after keeping the culture medium with added onion extracts at 37°C for two days, no decrease was observed in the potency. From this fact, it is assumed that influence of the activity due to volatility or decomposition in a water solution of onion extracts is low.

Multiple alkyl-cysteine sulfoxides are present in intact onions, and it is reported that trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide, one of the main components, converts to alkylthiosulfinates rapidly by enzyme activity when crushed or cut and that antimicrobial activity as the product (1). Small et al. (15) reported that the thiosulfinate chain (-S(O)-S-) was very important in the antimicrobial activity from their study using synthesized alkylthiosulfinate. These authors described (16) that the effect of thiosulfonate (-S(O)=S-) was greater than that of thiosulfinate on Staphylococcus aureus and Klebsiella pneumoniae. Further, these authors demonstrated that low molecular thiosulfinates (2, 4 and 6 carbons) have a greater inhibitory effect on Gram-positive bacteria than on Gram-negative bacteria, and conversely, results are reversed if the carbon chain becomes longer but that it also becomes more liposoluble and less water-soluble (15). It was also reported that this activity of thiosulfinates is inactivated further by adding a complex with cysteine\(^{[17]}\). The data obtained from the present study suggests that the thiosulfinates were not markedly present in the sample based on the results of TLC analysis and treatment with cysteine.

The substances were unstable with alkali treatment, and the activity decreased to about 5%. As Whitaker\(^{[1]}\) described that trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide, which is the main component of onion, converts to 5-methyl-1,4-thiazan-3-carboxylic acid 1-oxide in alkali, the possibility is considered that the decrease of the activity was due to alkali treatment.

The component taking charge of lacrimatory activity, one of the features of onions, is produced as an intermediate substance which converts to thiosulfinates from trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide, which is one of the main components of intact onions by S-alkyl-L-cysteine sulfoxide lyase enzyme activity. The component was identified as thiopropanal-oxide (or 1-propenyl sulfenic acid) (18), which has been reported to have antimicrobial activity (3,4). Color reactions were used to detect the lacrimatory component in this extracts by TLC. Spots were observed near the Rf values of 0.1, 0.3 and 0.5, but the lacrimatory component was considered to be minor since most of the active substances were collected from the starting point.

It was found during this study that the antibacterial activity disappeared from the onion extracts kept at 37°C for more than two days after grating. This finding suggests that the thiosulfinate probably changed by enzyme reaction into di-, mono-, tri-sulfides or thiosulfonate, etc. which are relatively unstable substances (1). Actually, no antibacterial activity was observed in the commercially available disulfides on the test bacterial strains (data not shown). Sharma et al. (2) also reported that no antimicrobial activity was observed in synthesized dipropylsulfide.

In this summary, the main antibacterial components in these onion extracts may be trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide which exists in intact onions. The results of this study suggest that there is a possibility of preventing dental caries and adult periodontitis with onion extracts as these were found to have bactericidal activity against dental caries and adult periodontitis related bacteria. In the discussion of the usefulness of onion extracts in clinical practice, the safety factor should also be considered.
References