Antibacterial Activity of Garlic Powder against
Escherichia coli O-157

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Summary The antibacterial activity of garlic powder against O-157
was tested by using garlic bulbs post-harvested 1 y. O-157 at 10⁶-⁷ cfu/mL
perished after incubation for 24 h with a 1% solution of garlic powder.
The use of powder from fresh garlic was more effective for antibacterial
activity than that from old garlic; the 1% solution of fresh garlic powder
eradicating the O-157 in 6 h. The antibacterial activity was resistant to
heat treatment of 100°C for 20 min. The water-soluble components of
garlic powder were fractionated into three fractions (Fr. 1–3) by Sephadex
G-100 column chromatography, among which Fr. 3 showed antibacterial
activity against O-157 but the other fractions were scarce in activity. The
antibacterial activity was also shown against other types of pathogenic
bacteria such as methicillin-resistant Staphylococcus aureus (MRSA),
Salmonella enteritidis, and Candida albicans. Thus, the practical use of
garlic powder is expected to prevent bacteria-caused food poisoning.

Key Words garlic powder, anti-O-157 activity, food poisoning

The food poisoning caused by enterohaemorrhagic Escherichia coli O-157:H7
was first reported by Riley et al, who carried epidemiologic and laboratory in-
vestigations forward (1). Then the symptoms of an unusual gastrointestinal illness
were characterized by the sudden onset of severe abdominal cramps and bloody
diarrhea with no fever or low-grade fever (1–4). The illness sometimes develops a
haemolytic uremic syndrome (HUS), which is a critical different property from
other types of food poisoning, and can often be fatal to the patient especially in
infant infections.

We experienced a huge outbreak of O-157-caused food poisoning in 1996 in
Japan, and the number of patients was estimated at over 12,000 including 12 deaths.

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However, the causative materials of food poisoning were little identified in spite of extensive research, and remained unsolved in many cases. The nation fell into a panic in fear of O-157 infection because of the ambiguity of the causative foodstuffs. Sporadic outbreaks of O-157-caused infection continue nation-wide even now. This implies the settlement of O-157 bacteria in our country.

Under such situations, we have begun to search for a potential anti-O-157 food and found that anti-O-157 activity exists in garlic powder. This is the first report for the O-157 killing potency of garlic powder, and we can expect a broad range of usage of garlic not only to prevent bacterial infection but also to preserve health in daily life.

MATERIALS AND METHODS

The garlic powder (old garlic) used for this experiment was prepared from garlic harvested 1 y previously in Aomori, Japan. Briefly, garlic bulbs were air-dried in the shade for 1 y, and cut into small pieces followed by drying at 60°C for 6 h to prepare powder by milling. Fresh garlic powder was similarly prepared exclusive of air-drying from fresh garlic after harvesting, and used for antibacterial tests against O-157.

O-157 producing verotoxins 1 and 2, which was isolated from an infant patient, was courteously provided by the Aomori Environmental Health Center. Other types of pathogenic bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), Salmonella enteritidis, Escherichia coli and Candida albicans, which have been maintained in our laboratory, were also used for testing the antibacterial activity of garlic powder.

The antibacterial activity of garlic powder was mainly tested by the test tube method occasionally combined with nutrient agar plate (Oxoid, England) analysis. O-157 cultivated at 37°C overnight in nutrient broth was added to a solution of garlic powder in distilled water. After incubation at 37°C for 6 or 24 h, the number of living O-157 in the garlic powder solution was counted by the nutrient agar plate method after serial dilution of the O-157 cultured liquid.

In addition, other types of pathogenic bacteria such as MRSA, Salmonella enteritidis, Candida albicans and E. coli were used for antibacterial activity test by the agar plate method. Nutrient agar in Petri dishes was divided into two parts, and half was replaced by nutrient agar containing 1–2% garlic powder. Then, bacteria were streaked on both parts of the plate surface and the growth inhibition (antibacterial activity) was evaluated after 24 h incubation.

The thermostability of antibacterial activity was examined by boiling the garlic powder solution for 10 or 20 min, adding O-157 and incubating in the same way as described above.

To separate the anti-O-157 constituent, garlic powder solubilized in water (150 mg/mL) was fractionated using a Sephadex G-100 column (1.8 × 60.0 cm) equilibrated with PBS (pH 7.2) at a flow rate of one drop/s under UV monitoring.
Table 1. Antibacterial activity against O-157 of garlic powder.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of O-157 (cfu*/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Garlic powder</td>
<td>0</td>
</tr>
<tr>
<td>1% Horseradish</td>
<td>$3.5 \times 10^6$</td>
</tr>
<tr>
<td>1% Aloe</td>
<td>$4.4 \times 10^9$</td>
</tr>
<tr>
<td>Control (water)</td>
<td>$3.4 \times 10^8$</td>
</tr>
</tbody>
</table>

Garlic powder used was prepared from 1-y-old garlic. The initial number of O-157 tested was $5.5 \times 10^7$ cfu/mL.

*cfu, colony-forming units.

Table 2. Comparative study of antibacterial activity against O-157 activity of garlic powder prepared from old and fresh garlic.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of O-157 (cfu/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
</tr>
<tr>
<td>1% Old garlic</td>
<td>$8.0 \times 10^6$</td>
</tr>
<tr>
<td>1% Fresh garlic</td>
<td>0</td>
</tr>
<tr>
<td>Control (water)</td>
<td>$8.0 \times 10^7$</td>
</tr>
</tbody>
</table>

Old garlic powder: prepared from 1-y-old garlic. Fresh garlic powder: prepared from fresh garlic. The initial number of O-157 tested was $4.0 \times 10^7$ cfu/mL.

at 280 nm. After lyophilization, each fraction was subjected to testing for antibacterial activity against O-157.

RESULTS

A 1% solution of garlic powder in water showed strong anti-O-157 activity capable of killing all of the cells of O-157 at $5.5 \times 10^7$ colony-forming units (cfu)/mL in the 24 h treatment, whereas the control O-157 incubated in distilled water grew from $5.5 \times 10^7$ cfu/mL to $3.4 \times 10^8$ cfu/mL 24 h later (Table 1). A 1% horseradish powder in water, which was used as a reference, showed weak anti-O-157 activity, while a 1% aloe plant powder in water allowed O-157 growth to increase from $5.5 \times 10^7$ cfu/mL to $4.4 \times 10^9$ cfu/mL 24 h later (Table 1).

Old garlic powder was compared with fresh garlic powder for anti-O-157 activity. Total cell death was not attained with the 1% old garlic powder after 6 h treatment, but fresh garlic powder could eradicate O-157 after 6 h treatment as shown in Table 2.

The anti-O-157 activity of old garlic powder resisted heat treatment at 100°C
Table 3. Thermostability of anti-O-157 activity of garlic powder (old garlic).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of O-157 (cfu/mL) 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Garlic powder</td>
<td>0</td>
</tr>
<tr>
<td>1% Garlic powder (100°C, 10min)</td>
<td>0</td>
</tr>
<tr>
<td>1% Garlic powder (100°C, 20min)</td>
<td>0</td>
</tr>
<tr>
<td>Control (water)</td>
<td>$6.2 \times 10^7$</td>
</tr>
</tbody>
</table>

Old garlic powder: prepared from 1-y-old garlic. The initial number of O-157 tested was $4.3 \times 10^6$ cfu/mL.

Table 4. Anti-O-157 activity of garlic powder (old garlic) fractions separated using a Sephadex G-100 column.

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Number of O-157 (cfu/mL) 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Fr. 1</td>
<td>$2.4 \times 10^3$</td>
</tr>
<tr>
<td>1% Fr. 2</td>
<td>$1.0 \times 10^2$</td>
</tr>
<tr>
<td>1% Fr. 3</td>
<td>0</td>
</tr>
<tr>
<td>Control (water)</td>
<td>$2.8 \times 10^4$</td>
</tr>
</tbody>
</table>

Old garlic powder prepared from 1-y-old garlic was separated using a Sephadex G-100 column. The initial number of O-157 tested was $3.6 \times 10^4$ cfu/mL.

for 20 min, and the heat-treated powder could kill O-157 at $4.3 \times 10^6$ cfu/mL after 24 h incubation (Table 3).

Old garlic powder was fractionated by Sephadex G-100 column chromatography, and three major fractions (Fr.) were obtained. Fractions 1 and 2 were whitish in color, but Fr. 3 was yellowish concomitantly with garlic smell. Fractions 1 and 2 at a concentration of 1% in water solution were weak in anti-O-157 activity, but the Fr. 3 killed O-157 at $3.6 \times 10^4$ cfu/mL after 24 h treatment (Table 4).

As a result of antibacterial testing, the growth inhibition of pathogenic bacteria such as MRSA, Salmonella enteritidis, E. coli, and Candida albicans was observed on the nutrient agar supplemented with 2% garlic powder, suggesting the presence of bactericidal factors in the garlic powder (Fig. 1).

DISCUSSION

In the past, it had been known that garlic had not only beneficial effects on health improvement such as reduction of blood cholesterol and lipid in serum or decrease in blood pressure (5), but also antibacterial effects against several types of bacteria such as gram-negative and gram-positive bacteria and fungi (6–11).
Antibacterial Activity of Garlic against O-157

Fig. 1. Bacteria growth inhibition of garlic powder (old garlic). A garlic powder-supplemented agar plate was prepared and pathogenic bacteria (from left to right, MRSA, E. coli, O-157, Salmonella enteritidis, and Candida albicans) were streaked on control and garlic-supplemented parts for cultivation. Bacteria failed to grow on the 2% garlic powder-supplemented section (bottom). This suggests the presence of antibacterial activity in garlic powder.

However, there was no report on whether or not garlic has anti-O-157 activity, because O-157 itself has only recently been designated as one of the bacteria causing emerging infection. We are now accumulating diverse data about this newly rising bacteria such as therapeutic manner, vaccination efficacy, how widely spread in environment and so on.

Our experiments showed that a 1% garlic powder solution, which was prepared from 1-y old garlic, had a strong anti-O-157 activity in which O-157 at $10^6-7$ cfu/mL was totally killed after 24 h treatment. The bacteria killing potency of garlic powder was also demonstrated against other types of pathogenic bacteria such as MRSA, Salmonella enteritidis, E. coli, and Candida albicans in the 1–2% concentration range of garlic powder (Fig. 1). It is generally accepted that O-157-caused food poisoning develops with $10^2$ or a lesser number of bacteria in human patient cases. We speculate from our results that garlic powder at various concentrations might work effectively against O-157 of lesser numbers ($10^{1-2}$) in a short period of time.

Concerning the antibacterial components of garlic, it is easy to consider that the principal constituent is very allicin or its derivative, and that heat treatment destroys the antibacterial activity. As shown in our experiment, however, the anti-O-157 activity in garlic resisted heat treatment at 100°C for 20 min. This suggests the presence of heat stable anti-O-157 substances even in old garlic. Since the garlic powder prepared from fresh garlic was stronger in antibacterial activity than the old garlic powder (Table 2), it is probable that the antibacterial composites in garlic are composed of both heat-labile and -stable compounds such as thiosulfinates, sulfides, dithiins and so on (5). In any case, we speculate from this finding that
either fresh or old garlic powders include antibacterial components and are positively recommendable foodstuffs to prevent bacteria-caused food poisoning, in addition to the other various food-functional values of garlic in eating habits.

REFERENCES


