Bacteriocidal Activity of Garlic Powder against Bacillus anthracis

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Summary The antibacterial activity of garlic powder was examined against Bacillus anthracis using agar plate cultivation and test tube methods. On the agar plate test, 1–5% garlic powder inhibited the growth of B. anthracis and Escherichia coli O157 used as references. A 1% water solution of garlic powder in the test tube method killed B. anthracis at 10^7 cfu/mL within 3 h of treatment at room temperature. A number of intestinal bacteria in a BALB/c mouse decreased after the oral administration of 1 mL of 1% garlic powder solution once a day for 3 d. These results suggest that the oral administration of garlic powder is effective against pathogenic bacteria invasion into the intestine as an infection.

Key Words Garlic powder, Bacillus anthracis, killing potency, oral administration

The outbreak of anthrax that occurred last year in America was considered to be started by terrorists, and four persons became victims of pulmonary anthrax. Anxious citizens were asking doctors for antibiotics to prevent serious infections.

Anthrax is primarily a disease of cattle and sheep; horses and swine are also susceptible, but are less commonly affected. Three routes of infection to human beings are known: (a) through the skin, (b) through the respiratory tract, and (c) through the alimentary tract. The bacillus is almost always transmitted to humans from lower animals rather than from other humans. The pulmonary form of anthrax, due to inhalation of the microorganisms (spores) set floating in the air, is the most dangerous (1).

In 1996, a huge outbreak of food poisoning due to Escherichia coli O157 occurred in Japan without being able to identify the causative material, and the total number of patients was estimated to be over 12,000, including three cases of death. The nation panicked with infection scares similar to that in America. This incidence led us to research anti-O157 foodstuffs, and eventually garlic powder was discovered to be effective against O157 (2).

Based on this finding, we tested the anti-Bacillus anthracis potency of garlic powder, and were able to successfully confirm the bacteria killing activity of garlic powder.

Materials and Methods
The garlic powder used was prepared from 1-y-old dried garlic bulbs, which were further dried at 60°C for 2 h and then milled.

The organism B. anthracis was an isolate from the skin focus of a patient infected by cow anthrax in 1970 in Japan.

For testing the bacteria killing potency of garlic powder, agar plate and test tube methods were used. For the agar plate method, a nutrient agar plate (Oxoid, England) was prepared, and then half of it was replaced by garlic powder containing nutrient agar (0.1–5%). B. anthracis cultivated on the agar plate the previous day was streaked onto both parts (with and without garlic powder) and incubated at 37°C overnight to examine activity.

A quantitative analysis of the bacteria killing potency of garlic powder was done by the test tube method to find out the time course of activity. The 0.1 mL of B. anthracis cultivated in a nutrient broth the previous day was added to test tube with 10 mL of distilled water (with and without 1% garlic powder). After keeping them at room temperature, both of the liquid were used for counting the living bacteria by the agar plate method. Briefly, 0.1 mL of serially diluted liquid from both test tubes was placed on an agar plate surface, spread, and cultivated overnight at 37°C. The number of living bacteria was calculated from the number of colonies grown on the agar plate.

One-milliliter of 1% garlic powder solution per mouse was orally administered into BALB/c mice (n= 5) once a day for 3 d to examine the number of floral intestinal bacteria. On the fourth day after administration, the mice were sacrificed to aseptically remove five feces from the intestines, which were then dissolved in 5 mL of distilled saline, followed by cultivation on agar plates to count the number of living bacteria after serial dilution in saline, in the same manner as described above. Animal experiments were performed under the approval of the Guidelines for Animal Experimentation of the Animal Committee, Hirosaki University.

The statistical significance between the experimental and control groups was examined by Student’s t-test.
Fig. 1 Growth inhibition of B. anthracis and E. coli O157 on garlic powder-supplemented nutrient agar plate. Left line (E. coli O157) and right line (B. anthracis) in plates A or B indicate the growth of each bacterium on the upper control parts without garlic powder. No growth of bacteria was observed on the lower parts of plates A or B, in which the plates contained 5% garlic powder (A) and 1% garlic powder (B) respectively.

Results

B. anthracis and E. coli O157 did not grow on 1 or 5% garlic powder on the agar plates (Fig. 1), suggesting the presence of growth inhibition activity in the garlic powder against both bacteria under these concentrations. However, a 0.1% concentration of garlic powder allowed the growth of B. anthracis in the agar plate test (not shown).

The results of the test tube method were similar to that of the agar plate method, and the number of B. anthracis at 2.0±1.8×10⁷ cfu/mL was reduced to 4.1±2.0×10⁴ cfu/mL (p<0.001) after 1 h treatment in a 1% garlic powder solution, and no more living bacteria was detected after 3 h of incubation at room temperature (Table 1). In contrast, the number of B. anthracis in distilled water (i.e., control) was 1.0±0.5×10⁷ cfu/mL after 3 h of incubation, and no change was observed in the number of living bacteria (Table 1).

Regarding the oral administration test for garlic powder, the number of living bacteria was 2.3±1.9×10⁵ (p<0.01) from the feces mice fed 1% garlic powder, which was a statistically significant reduction in comparison to that of the control, 5.4±2.3×10⁶/mL/feces (Table 2).

Discussion

Ten years ago, the Institute of Medicine published a report entitled "Emerging Infections: Microbial Threats to Health in the United States." The report mentioned newly defined and emerging infectious diseases as those that have: (i) newly appeared in humans, (ii) rapidly increased in incidence, (iii) expanded in geographic range, (iv) and developed increasing or novel mechanisms of antimicrobial resistance (3).

Actually, in 1996, the food poisoning caused by the emerging infectious agent E. coli O157, was virtually an outbreak in Japan, and the nation was exposed to a threat that gained prominent attention. We immediately initiated research to find foodstuffs with anti-O157 potency, and were able to demonstrate this activity in garlic powder (2).

Generally, the bacteria killing potency of garlic powder has long been known, and recently, persuasive experimental data of the bacteriocidal effect of garlic have been accumulated against Staphylococcus aureus (MRSA), Salmonella enteritidis, Candida albicans, O157, Vibrio parahaemolyticus, and other bacteria (2, 4, 5).

Based on these findings, we tested the antibacterial potency of garlic powder against aerobic spore-bearing B. anthracis. This bacillus is non-motile, forms capsules in the animal body and grows on agar in characteristically long, segmented, parallel or interwoven chains. Anthrax bacillus is naturally pathogenic, mainly to the herbivora and humans. The most certain route is intramuscular, and B. anthracis produces a fatal infection after inhalation (6).

Through the agar plate test, it was found that both 1% and 5% of garlic powder is capable of completely inhibiting the growth of B. anthracis as shown in Fig. 1; however, a concentration of 0.1% garlic powder was less effective and allowed growth of the bacterium (not shown). Next, a test tube method was carried out to obtain more detailed data. It showed that a 1% solution of garlic powder was effective for eradicating B. anthracis at 10⁷ cfu/mL with a 3-h treatment (Table 1). Since spores are never found in the infected animal body and appears more slowly in cultures than the bacterium of the other members of the group, B. anthracis killed by garlic powder is probably a vegetative bacilli, and not of the spore form. When spores germinate in favorable conditions such as in vivo, the garlic powder works effectively to kill the vegetative bacilli germinated from the spores.

| Incubation time (h) | No. living bacteria (cfu/mL) |
|--------------------|-------------------------------|
|                    | in 1% garlic powder | in distilled water |
| 0                  | 2.0±1.8×10⁷ | 2.0±1.8×10⁷ |
| 1                  | 4.1±2.0×10⁴* | ND |
| 3                  | 0              | 1.0±0.5×10⁷ |
| 6                  | 0              | 4.0±2.1×10⁷ |

ND: not determined. * p<0.001.

B. anthracis was added into 1% garlic powder-water and kept at room temperature for analysis.

Table 2. Decrease in number of living bacteria in feces of BALB/c mouse fed the 1% garlic powder solution.

| Group                  | No. living bacteria (cfu/feces) |
|------------------------|---------------------------------|
| 1% garlic powder fed   | 5.4±2.3×10⁶                   |
| Water fed              | 2.3±1.9×10⁶*                  |

* p<0.01.

1 mL of 1% garlic powder in water per day was orally administered to 5 BALB/c mice for 3 d, and five feces samples were collected from individual mouse intestines to count the number of living bacteria.
No direct evidence of the bacteria killing potency of garlic powder *in vivo* has been reported; therefore, we tested its activity using a mouse model. The oral administration of a 1% garlic powder solution reduced the number of intestinal bacteria (Table 2). Although these bacteria are mostly beneficial bacteria found as normal flora in the intestine, a similar result may be expected against pathogenic bacteria such as O157 (gram-negative) and *B. anthracis* (gram-positive) in intestine too. The active antibacterial compounds in garlic powder are unclear at present, but a sulfides complex derived from heat labile allicin is probably associated with the activity. We previously reported that the properties of the bacteria killing compounds in garlic powder were heat stable, and its activity remains following heat treatment at 100°C for 20 min (2). This suggests that the antibacterial activity of the garlic powder used in the present experiment originates from heat-stable compound(s) such as the sulfides complex (7) derived from allicin, but not heat labile allicin itself.

Since the garlic (powder) is a beneficial foodstuff with antibacterial potency against pathogenic bacteria such as *B. anthracis*, *E. coli* O157 (2), MRSA (2), and other bacteria, a broad range of uses of this foodstuff is expected to protect and prevent the emergence or reemergence of infections by being ingested in daily life.

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