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Effects of Storage Temperature and pH on the Stability of Antibacterial Effectiveness of Garlic Extract against *Escherichia coli* B34

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Abstract The effect of long-term storage on garlic antibacterial activity was investigated. A concentration of 5% or more garlic was found to be necessary to completely inhibit *Escherichia coli* growth in tryptic soy broth. This value is substantially higher than the minimum inhibitory concentration of 1% for *E. coli* reported previously. pH-modified garlic extract was stored at different temperatures to investigate the impact of storage conditions (i.e., temperature, pH, period of storage) on the stability of the antibacterial activity of the garlic extract used against *E. coli* B34. The antibacterial effectiveness of the garlic extract against *E. coli* remained stable when both the storage temperature and the pH of the extract were kept low. When the garlic extract was stored at 40°C and above, most or all of the garlic antibacterial activity disappeared after a 24-h storage period, regardless of the storage pH. The antibacterial activity was weakened when the pH of the garlic extract was adjusted to 8, and at low temperatures.

Key words: Garlic, *Escherichia coli*, pH, temperature, antibacterial activity, combined effects of temperature and pH

The antimicrobial activity of garlic extract has been recognized for many years in all parts of the world. The antibacterial [8], antifungal [15, 21], antiviral [16], and enzyme inhibitory [9, 10, 22] activities of garlic have been widely investigated. One to two percent concentrations of garlic extract have been reported to inhibit microbial growth, and higher concentrations are noted to be germicidal. Dababneh and Al-Delaimy [8] reported that 1% garlic extract inhibited *Staphylococcus aureus*. Mantis *et al.* [14] reported that 2% garlic extract had growth inhibitory effects against *S. aureus*, while extracts of <1% were noninhibitory, and those of >5% were bactericidal. Karaioannoglou *et al.* [12] reported that a garlic extract of >1% concentration in culture media inhibited *Lactobacillus plantarum*, while 2% was bactericidal. Fungal growth is more strongly inhibited by garlic extract than bacterial growth is [7, 15].

Cavallito and Bailey [5] discovered the principal antimicrobial compound of garlic during the rush to discover penicillin, and named it allicin (diallyl thiosulfinate). Garlic also contains methyl methanethiosulfinate [3], an analog of diallyl thiosulfinate. These antimicrobial compounds are absent in intact garlic, yet generated enzymatically when garlic is damaged. Garlic is known to contain up to 1.4% alliin on a wet weight basis [25]. The antimicrobial activity of thiosulfinates is due to the S(O)-O-group, which reacts with SH groups of essential cellular proteins [6, 19, 20]. The antimicrobial activity of garlic is unstable at high temperatures and high pH. Brodnitz *et al.* [4] observed that allicin breaks down almost completely after 20 h at 20°C.

This study investigated the long-term effect of garlic extract on the growth of *Escherichia coli*, and the combined effects of pH, temperature, and storage period on the stability of garlic antibacterial activity.

Pre-peeled garlic was purchased from a local market in the Seoul area. A water extract of garlic was prepared by grinding the garlic with the same volume of water and filtering it through several layers of cheesecloth. The pH of this garlic extract was then adjusted to 2, 4, 6, and 8 using 1 N HCl or 1 N NaOH, dispensed into screw-capped test tubes in 15 ml aliquots, and stored at different temperatures (4, 10, 20, 30, 40, and 50°C) for 24 h before each antibacterial test was performed against *E. coli*. A second portion of garlic extract was prepared by adjusting the pH to 4 and storing it for 15 days at 10°C before the antibacterial activity was measured. Finally, the pH level of the various extracts was readjusted to a pH between 6.0 and 6.5 after storage for the defined time periods, a level near to that of natural garlic.

Filter-sterilized fresh garlic extract was added to tryptic soy broth (TSB; Difco Lab., Detroit, MI, U.S.A.) at concentrations of 0.5, 1.0, 2, 5, 7.5, and 10%. This was

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then dispensed into sterile culture tubes, and each tube was inoculated with a culture of *E. coli* grown overnight to provide an initial cell assay of 1.0×10^5 cfu/ml. The tubes were incubated at 30°C for 10 days, and viable cells were counted everyday. The pH-adjusted garlic extract was filtered (Whatman #44, Whatman International LTD, Kent, England), centrifuged to remove any fine particles, and filter-sterilized (membrane filter 0.45mm pore size, Whatman). The filter-sterilized garlic extract was added to TSB at 1.0% level (as garlic) and dispensed into sterile culture tubes, which were then inoculated with the *E. coli* culture to provide an initial cell concentration of 1.0×10^5 cfu/ml. The culture tubes were incubated at 30°C, and counts of viable cells were made after 24, 48, and 72 h of incubation using a spiral plater (Autoplate 4000, Spiral Biotech, U.S.A.). The result was expressed as colony-forming units (cfu)/ml.

Most previous studies examining garlic antibacterial activity have been performed for relatively short periods of time, usually less than 2 days [1, 8, 11, 13, 16, 17, 18]. Accordingly, a 10-day experiment was performed to examine the long-term effect of garlic antibacterial activity. The results showed that 5% or higher concentrations of garlic extract destroyed all bacterial cells, and no recovery of bacterial activity was noted during the remaining10-day test period (Fig. 1). In contrast, with 1 and 2% garlic extract in TSB, the bacterium recovered from the garlic antibacterial activity after between 4 and 7 days. Before the observed recovery, viable cell numbers were decreased, and then, sudden increases in viable bacterial cells were observed.

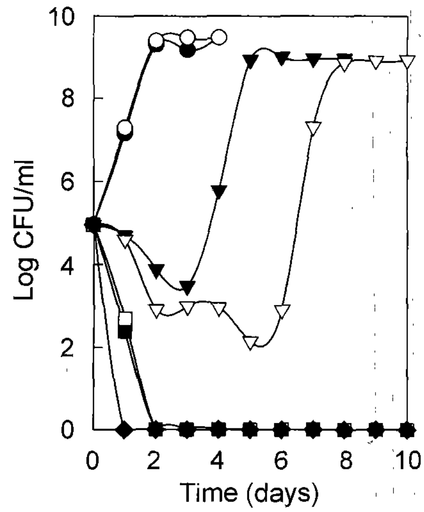

Fig. 1. Effect of concentration of fresh garlic extract on growth of *E. coli* B34 during extended period of cultivation at 30°C.
●: 0%; ○: 0.5%; ▼: 1.0%; ▽: 2.0%; ■: 5.0%; □: 7.5%; ◆: 10%.

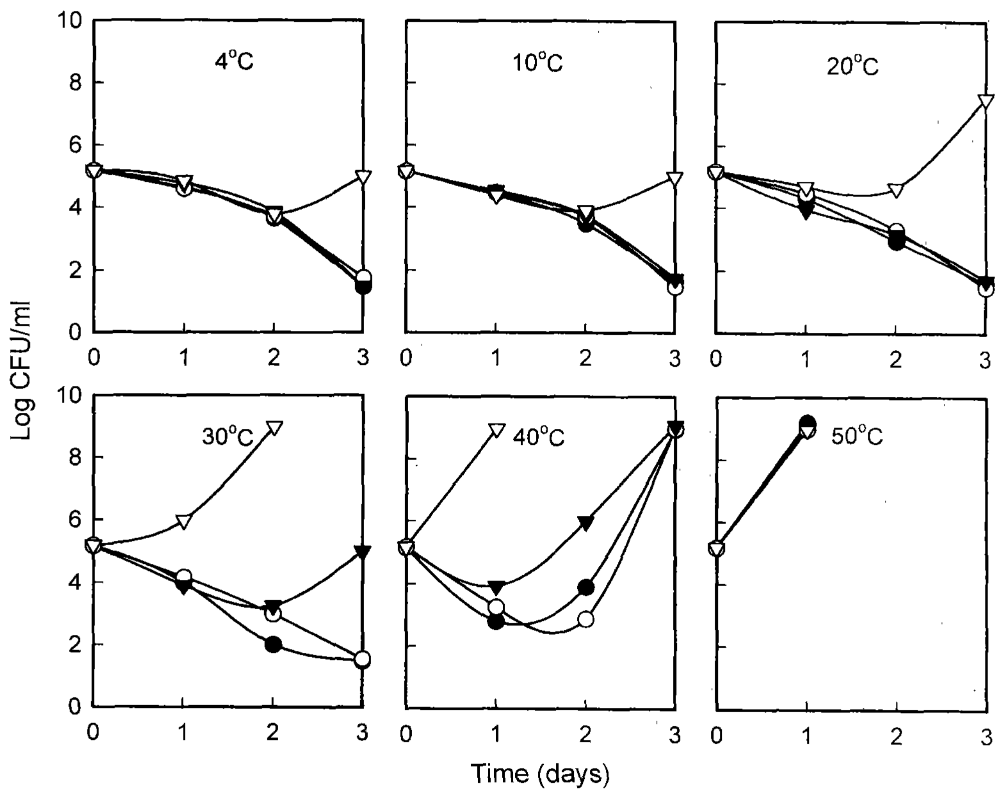

Fig. 2. Combined effects of pH and temperature on the stability of the antibacterial activity of garlic extract during storage.
●: pH 2.0; ○: pH 4.0; ▼: pH 6.0; ▽: pH 8.0

These results indicated that the expression of the MIC as a function of time (i.e., MIC at 24 h=1% garlic for *E. coli*) is more useful than describing it simply in terms of a simple percentage of garlic extract [16]. This study demonstrated that a MIC of 1% garlic applied to *E. coli* was effective until the third day, yet became increasingly less effective from the fourth day onward (Fig. 1).

There was no significant difference in the final number of cells once the bacteria recovered from the effect of the garlic antibacterial activity. It is unknown whether the rise in the number of viable cells was attributable to adaptation, the instability of the inhibitory compounds of garlic during incubation at 30°C, or due to interplay between both processes.

The stability of the antibacterial effectiveness of the garlic extract was found to be strongly dependent on both pH and temperature during storage (Fig. 2). During the 24-h storage period at temperatures of 20°C and below, and pH levels of 6 and below, the antibacterial activity of the stored garlic extract was stable. However, when the pH was adjusted to 8, the antibacterial activity weakened and an increase in the number of viable bacterial cells was observed at low storage temperatures of 4 and 20°C. After 24-h storage at 40°C and above, the garlic extract lost most or all antibacterial activity, regardless of the pH level.

Exposing garlic extracts to temperatures of 80 to 90°C for 5 min completely destroyed the antibacterial activity [8, 17]. Normally, the loss of antibacterial activity through heating is due to volatilization, and to the chemical and physical changes that take place during heating [17]. However, in the current study, it is unlikely that the garlic extract antibacterial compounds kept in glass tubes were evaporated between 40 and 50°C. Instead, the primary reason for loss of antimicrobial activity appeared to be due to compound instability at moderately high temperatures.

Low pH levels have been found to assist in the maintenance of garlic antibacterial activity. Block [2] reported that allicin, the principal antibacterial compound of garlic, is more stable at pH levels between 2 and 4. In the current study, garlic extract with a pH of 4 stored at 10°C for up to 15 days maintained stable levels of antibacterial activity (data not shown). Allicin has been reported to be more stable under acidic conditions than at other pH levels [24].

Brodnitz *et al.* [4] reported that after 20 h at 20°C, allicin undergoes nearly complete decomposition. In contrast, Yu and Wu [23] reported the complete decomposition of allicin in garlic extract kept at 40°C after 144 h, and postulated that allicin is more stable in garlic juice than in a pure state. They found that approximately 50% of the allicin content disappears at 40°C after 24 h. In the current experiment, the antibacterial activity of garlic completely disappeared at 40°C after 24 h, and a 0.5% garlic extract was noninhibitory against *E. coli* (Fig. 1). As described by Yu and Wu, 1% garlic extract, in which approximately 50% of the allicin in the solution had broken down after 24 h at 40°C, was not expected to inhibit bacterial growth [23].

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