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# Antifungal Activity of Ajoene Derived from Garlic

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**The antifungal activity of six fractions derived from garlic was investigated in an in vitro system. Ajoene had the strongest activity in these fractions. The growth of both *Aspergillus niger* and *Candida albicans* was inhibited by ajoene at <20 μg/ml.**

Garlic has been shown to inhibit the growth of a variety of microorganisms, not only bacteria (7, 8, 12) but also fungi (4, 14, 16–18) and viruses (9, 10, 15). The antimicrobial activity of garlic is believed to be due to the effect of allicin, the main ingredient in garlic, generated by the phosphopyridoxal enzyme allinase (1, 5, 7). It is necessary to investigate whether or not the other components of garlic have antimicrobial activity. We examined the antifungal activity of ajoene, which was recently reported by Block and Ahmad to be an anticoagulant agent isolated from garlic (2, 3, 6), and the activity was compared with that of five fractions (A to D and allicin; Fig. 1).

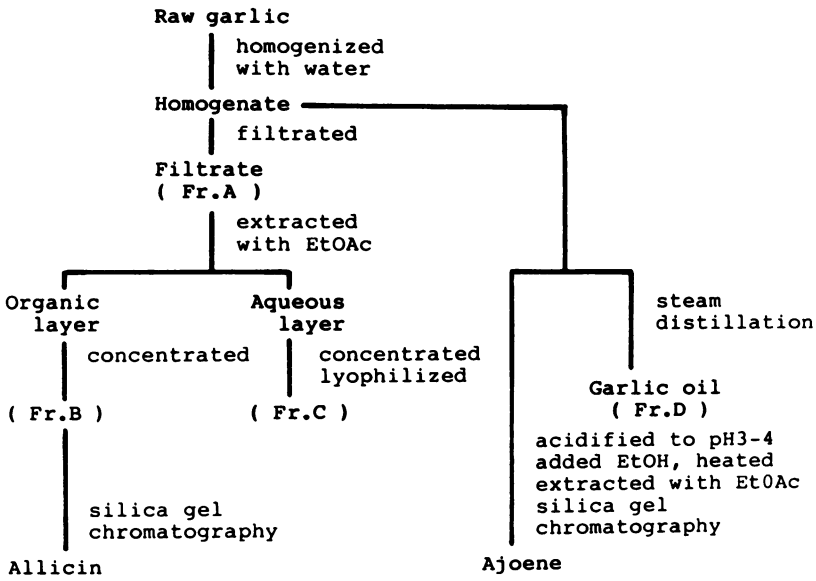


FIG. 1. Fractionation of garlic. Fr., Fraction.

Ajoene isolated in the present study was identified by comparison of the $^{1}H$- and $^{13}C$-nuclear magnetic and mass spectra with those of an authentic sample reported by Apitz-Castro et al. and Block and Ahmad (2, 3, 6). Its purity was 99.0%, checked by high-pressure liquid chromatography (column, TSK gel ODS-120A [3.9 mm by 30 cm]; solvent, 40% $CH_3CN$; detector, UV at 254 nm).

To check the antifungal activity of those fractions, two fungus strains, *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231, were obtained from the Institute for Fermentation, Osaka, Japan. For the test, spores of *A. niger* formed on Sabouraud agar medium and active *C. albicans* precultured in Sabouraud broth were cultured with or without varying concentrations of each fraction at 37°C with agitation for 3 days and 18 h, respectively. The activity of each fraction against *A. niger* was judged by measuring the dry weight of the fungus at the end of the culture. With *C. albicans*, evaluation was done by measuring the turbidity of the medium.

The results are shown in Table 1. The growth of both fungi was clearly inhibited by fractions A and B, allicin, and ajoene. The strong antifungal activity elicited by fractions A and B may be due to allicin. Interestingly, ajoene severely inhibited growth of the fungi. Although the activity was less than that of 5-fluorocytosine and amphotericin B (Sigma Chemical Co., St. Louis, Mo.), which were used as control drugs, ajoene was superior to allicin in antifungal activity.

To investigate how ajoene acts toward *A. niger* cultured for 24 h at 37°C in medium with ajoene at 20 μg/ml, which is

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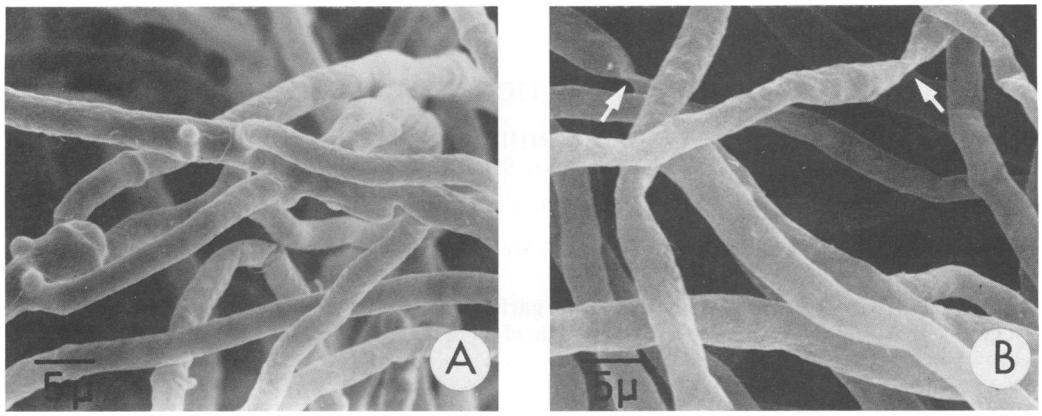


FIG. 2. Scanning electron microscopic observation of *A. niger*. (A) A normal hypha was characterized by thick ringlike septa and waxy crustlike reticulate surface ornamentation. (B) With ajoene treatment (20 μg/ml), hypha showed surface depression or flat ribbonlike structure (arrows). Magnification, ×1,600.

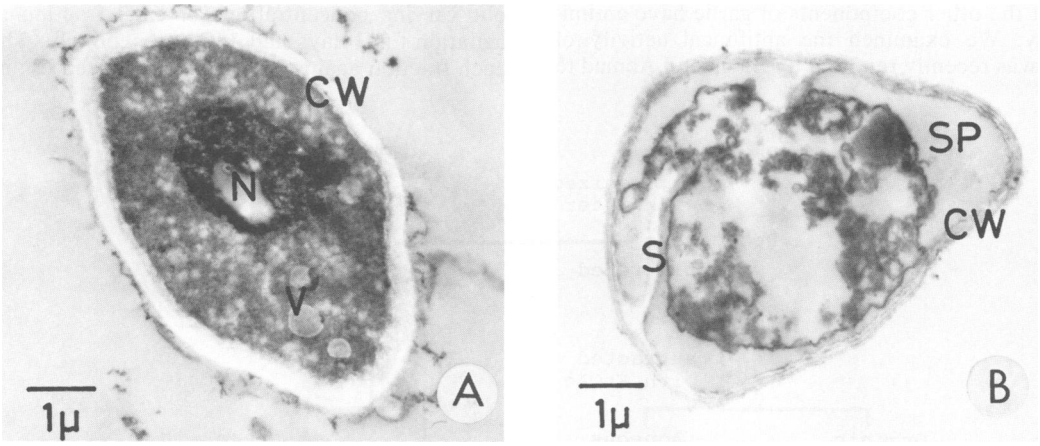


FIG. 3. Transmission electron microscopic observation of *A. niger*. (A) A normal hyphal cell showed highly dense cytoplasm and was enveloped with thick cell walls (CW) (400 nm thick). Cell wall and cell membrane were tightly attached. N, Nuclei; V, vesicle. (B) A hyphal cell treated with ajoene (20 μg/ml) showed cell enlargement. Detachment of cell membrane and cell wall (CW) occurred, and a wide space (SP) filled with amorphous material consequently appeared. The cell wall was reduced in thickness (to 200 nm). S, Septa. Magnification, ×9,000.

a completely inhibitory concentration, it was observed by both scanning and transmission electron microscopy. In comparing intact and ajoene-treated hyphae, severe damage (Fig. 2B and 3B) was observed. Morphological changes such as disappearance of surface ornaments, thickening of cell wall, and destruction of cell organella indicated that ajoene may act somewhat on the cell wall, as reported for other antifungal agents (11, 13).

In investigation of the activity against other fungal and bacterial strains, ajoene also strongly inhibited the growth of *Candida glabrata*, *C. tropicalis*, *Trichophyton mentagrophytes*, *Tricosporon beigelii*, and *Saccharomyces cerevisiae*. However, ajoene did not elicit antibacterial activity against gram-positive and gram-negative bacteria so far investigated, except *Staphylococcus aureus*.

In conclusion, ajoene had stronger antifungal activity than allicin. Although the mechanism is not clear, ajoene may

TABLE 1. Antifungal activity of components derived from garlic[a]

| Sample | 95% Inhibitory concn (μg/ml) | |
|---|---|---|
| | *A. niger* | *C. albicans* |
| Fraction A | 17.5 | 14.7 |
| Fraction B | 45.5 | 4.3 |
| Fraction C | >500.0 | >500.0 |
| Fraction D | 206.1 | 179.2 |
| Allicin | 30.9 | 17.3 |
| Ajoene | 16.6 | 7.6 |
| 5-Fluorocytosine | 5.0 | 7.3 |
| Amphotericin B | 2.6 | 0.2 |

[a] *A. niger* ($2.5 \times 10^5$ spores per 5 ml) and *C. albicans* ($10^6$ cells per 5 ml) were cultured in Sabouraud broth containing various concentrations of each sample for 3 days and 18 h, respectively, at 37°C.

damage the cell walls of fungi. Growth-inhibitory activity of ajoene toward bacteria was not expected, except for a special strain.

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