

## Antifungal Activity of Ajoene Derived from Garlic

SUSUMU YOSHIDA, SHIGEO KASUGA, NORIHIRO HAYASHI, TSUYOSHI USHIROGUCHI,  
HIROMICHI MATSUURA, AND SHIZUTOSHI NAKAGAWA\*

Central Research Laboratories, Wakunaga Pharmaceutical Company, Ltd., Takata-gun Hiroshima 729-64, Japan

Received 3 November 1986/Accepted 25 November 1986

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 alliinase (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

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

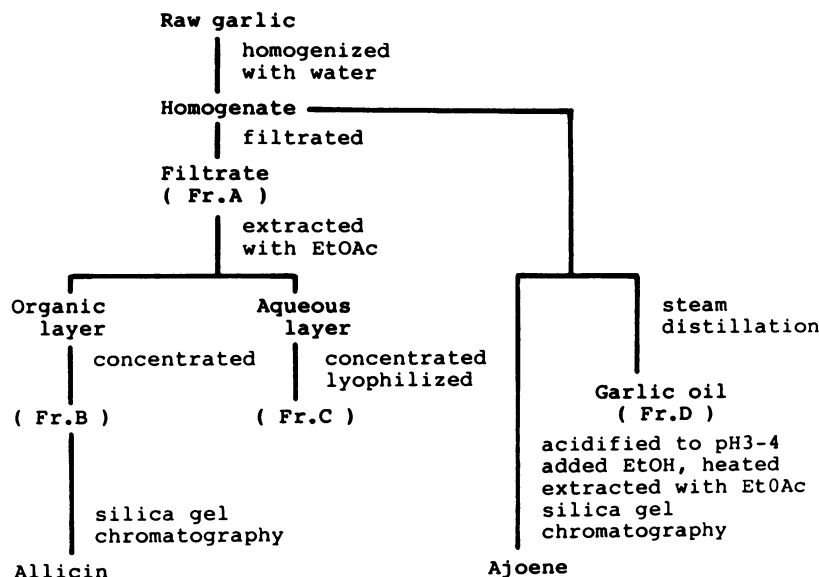


FIG. 1. Fractionation of garlic. Fr., Fraction.

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).

Ajoene isolated in the present study was identified by comparison of the <sup>1</sup>H- and <sup>13</sup>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<sub>3</sub>CN; detector, UV at 254 nm).

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

\* Corresponding author.

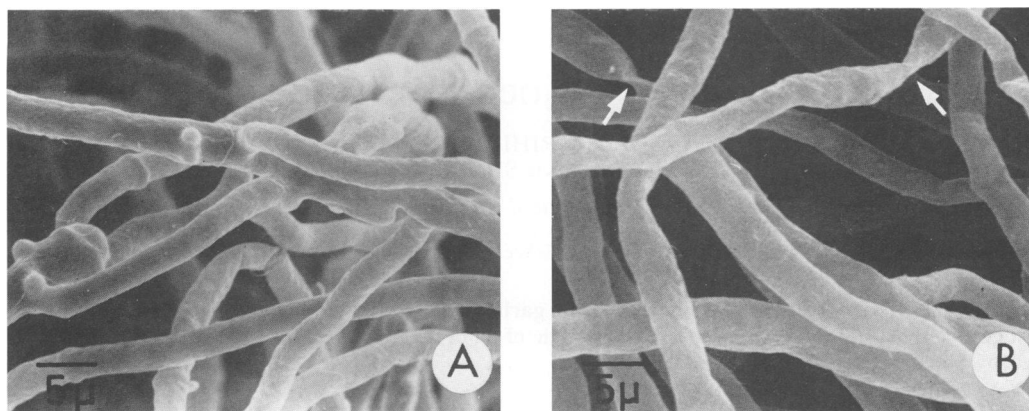


FIG. 2. Scanning electron microscopic observation of *A. niger*. (A) A normal hypha was characterized by thick ringlike septa and waxy crustlike reticulate surface ornamentation. (B) With ajoene treatment (20  $\mu\text{g/ml}$ ), hypha showed surface depression or flat ribbonlike structure (arrows).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

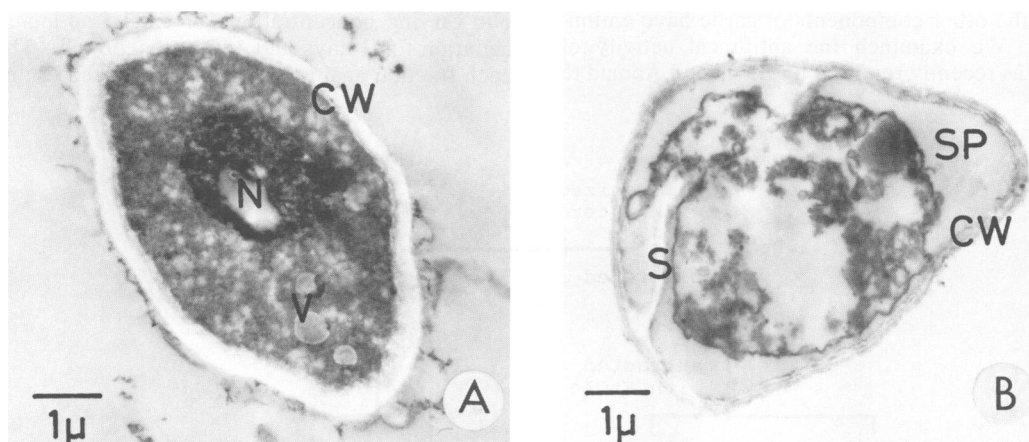


FIG. 3. Transmission electron microscopic observation of *A. niger*. (A) A normal hyphal cell showed highly dense cytoplasm and was enveloped with thick cell walls (CW) (400 nm thick). Cell wall and cell membrane were tightly attached. N, Nuclei; V, vesicle. (B) A hyphal cell treated with ajoene (20  $\mu\text{g/ml}$ ) 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,  $\times 9,000$ .

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 beigeli*, 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<sup>a</sup>

Sample	95% Inhibitory concn ( $\mu\text{g/ml}$ )	
	<i>A. niger</i>	<i>C. albicans</i>
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

<sup>a</sup> *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.

We are grateful to Yoshiyasu Matsuo, an emeritus professor at Hiroshima University School of Medicine, for useful advice.

#### LITERATURE CITED

1. Adetumbi, M. A., and B. H. S. Lau. 1983. *Allium sativum* (Garlic)—a natural antibiotic. *Med. Hypoth.* **12**:227–237.
2. Apitz-Castro, R., S. Cabreva, M. R. Cruz, E. Ledezma, and M. K. Jain. 1983. Effect of garlic extract and of three pure components isolated from it on human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure. *Thromb. Res.* **32**:155–169.
3. Apitz-Castro, R., J. Escalante, R. Vargas, and M. K. Jain. 1986. Ajoene, the antiplatelet principle of garlic, synergistically potentiates the antiaggregatory action of prostacyclin, fopskolin, indomethacin and dypiridamole on human platelets. *Thromb. Res.* **42**:303–311.
4. Appleton, J. A., and M. R. Tansey. 1975. Inhibition of growth of 200 pathogenic fungi by garlic extract. *Mycologia* **67**:882–885.
5. Arunachalam, K. 1980. Antimicrobial activity of garlic, onion and honey. *Geobios* **7**:46–47.
6. Block, E., and S. Ahmad. 1984. (E,Z)-Ajoene: a potent antithrombotic agent from garlic. *J. Am. Chem. Soc.* **106**:8295–8296.
7. Cavallito, C. J., J. S. Buck, and C. M. Suter. 1944. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *J. Am. Chem. Soc.* **66**:1950–1951.
8. Delaha, E. C., and V. F. Garagusi. 1985. Inhibition of mycobacteria by garlic extract (*Allium sativum*). *Antimicrob. Agents Chemother.* **27**:485–486.
9. Esanu, V., 1981. Recent advances in the chemotherapy of herpes virus infections. *Rev. Roum. Med. Virol.* **32**:57–77.
10. Esanu, V., and E. Prahoveanu. 1983. The effect of garlic, applied as much or in association with NaF, on experimental influenza in mice. *Rev. Roum. Med. Virol.* **34**:11–17.
11. Jacob, Z., and O. P. Serivastava. 1983. Scanning electron microscopic study of the effects of tolcliate and clotrimazole on *Trichophyton rubrum*. *Indian J. Med. Res.* **78**:518–523.
12. Johnson, M. G., and R. H. Vaughn. 1969. Death of *Salmonella typhimurium* and *Escherichia coli* in the presence of freshly reconstituted garlic and onion. *Appl. Microbiol.* **17**:903–905.
13. Masperi, P., G. Dall'olio, A. Calefano, and G. L. Vannini. 1984. Autophagic vacuole development in *Trichophyton mentagrophytes* exposed *in vitro* to miconazole. *Sabouraudia* **22**: 27–35.
14. Moore, G. S., and R. D. Atkin. 1977. The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi. *Mycologia* **69**:341–348.
15. Nagai, K. 1973. Experimental studies on the preventive effect of garlic extract against infection with influenza virus. *Jpn. J. Infect. Dis.* **47**:321–325.
16. Prasad, G., and V. D. Sharma. 1981. Antifungal property of garlic (*Allium sativum* Linn.) in poultry feed substrate. *Poult. Sci.* **60**:541–545.
17. Tansey, M. R., and J. A. Appleton. 1975. Inhibition of fungal growth by garlic extract. *Mycologia* **67**:409–413.
18. Upadhyay, M. P., K. L. Manandhar, and P. B. Shrestha. 1980. Antifungal activity of garlic against fungi isolated from human eyes. *J. Gen. Appl. Microbiol.* **26**:421–424.