

Inhibition of Mycobacteria by Garlic Extract (*Allium sativum*)

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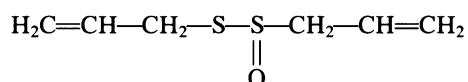
Received 21 September 1984/Accepted 10 January 1985

Thirty strains of mycobacteria, consisting of 17 species, were inhibited by various concentrations of garlic extract incorporated in Middlebrook 7H10 agar. The concentration required ranged from a low of 1.34 mg/ml to a high of 3.35 mg/ml of media. When there were multiple strains of a species, a mean inhibitory concentration was determined for that species. Six strains of *Mycobacterium tuberculosis* required a mean inhibitory concentration of 1.67 mg/ml of media.

Garlic has a long history of medicinal use, being mentioned more than 2,000 years ago in ancient Chinese medical literature (21). Several centuries ago the Egyptians used garlic to treat many disease entities (7). Aristotle and Hippocrates called attention to the healing powers of garlic, and Pasteur mentioned its medicinal and antibacterial properties (7). In addition to its well-documented antibacterial properties (11, 12, 15, 19, 20), garlic has also been found to be antifungal (2, 5, 6, 13, 14, 16, 18), antiprotozoal (3), and insecticidal (4).

Over the past decade there has been a proliferation of literature on the antimicrobial properties of garlic extract (7). The Chinese recently reported successful treatment of cryptococcal meningitis by oral, muscular, and intravenous administration of garlic (14). Caporaso et al. reported detection of antifungal activity in human serum against seven species of *Candida* and two species of *Cryptococcus* after ingestion of garlic (8).

The principal antimicrobial component of garlic oil is the sulfur compound diallyl thiosulfinate, which Cavallito and colleagues (9, 11) named allicin:



An intact garlic bulb does not contain allicin but rather its precursor, alliin, which is hydrolyzed to allicin, pyruvate, and ammonia by the phosphopyridoxal enzyme allinase when the tissue of the bulb is disrupted (10). Wills (20) reported that allicin is an inhibitor of sulfhydryl metabolic enzymes and suggests that its antimicrobial properties are due to specific interference with -SH groups.

The inhibitory effect of garlic on mycobacteria has been reported only on rare occasions (1). Around the turn of the century, W. C. Minchin, head of the tuberculosis ward at a Dublin hospital, wrote that garlic had a remarkable cure rate for tuberculosis. It was used as an inhalant, taken internally, and applied as a compress and as an ointment (7). M. W. McDuffie, at approximately the same time in New York City, compared garlic with 55 other treatments for tuberculosis and concluded that it was the most effective (7).

In the only previous study to quantitatively determine the concentration of garlic extract that inhibited *Mycobacterium tuberculosis*, Rao et al. (17), using only one strain of *M. tuberculosis*, found that 2 mg/ml was required to inhibit that

particular strain. To confirm their study and to determine the inhibitory concentration of garlic for several strains of *M. tuberculosis* as well as for 16 other species of mycobacteria, the following study was undertaken.

MATERIALS AND METHODS

Preparation of garlic extract. Ten bulbs of garlic (*Allium sativum*) were divided into separate cloves. The cloves were peeled and then ground in a Waring blender. Allicin was extracted by a modification of the procedure of Fromtling and Bulmer (13). The garlic pulp was agitated with 250 ml of sterile distilled water on a shaker for 60 min. The resulting paste was refrigerated for 2 h and then squeezed through gauze pads (Parke-Davis HRI 8071-507701) to remove the larger particles. Ca. 100 ml of oil was collected and centrifuged at 2,200 rpm for 15 min. The supernatant was passed through a Nalgene filter (0.45- μm pore size; Sybron/Nalge 245-0045). The filtrate was then sterilized by passage through a 0.22- μm -pore size filter (Sybron/Nalge 120-0020). Samples (1 ml) were dried in a 56°C oven to a constant weight and found to contain 134 mg (equivalent dry weight) of garlic extract per ml. The sterile extract was kept frozen at -22°C until used.

Preparation of test media. Six flasks, each containing 1 liter of Middlebrook 7H10 agar (Difco Laboratories) and 0.5% glycerol, were prepared and sterilized by autoclaving at 15 lb pressure for 10 min. After cooling to 55°C, 100 ml of Middlebrook OADC enrichment fluid (Difco) was added to each flask. Garlic extract was added to five of the flasks to give concentrations that ranged from 0.67 mg/ml in flask 1 to 3.35 mg/ml in flask 5. Flask 6 contained no garlic extract and was used to prepare 30 control plates. The contents of each flask containing garlic extract were poured in 20-ml amounts into 30 sterile petri dishes to form a solid agar.

Preparation of inocula. Each of the 30 strains of *Mycobacterium* spp. being tested was suspended in 5 ml of sterile deionized water and mixed thoroughly on a Vortex mixer (American Scientific Products no. S8223). The contents of each tube were diluted with sterile deionized water to a density equivalent to a no. 1 McFarland standard. One-tenth milliliter of each suspension was pipetted onto the petri dishes containing each concentration of garlic extract and then streaked with a loop. This procedure was repeated for each of the garlic-free control plates. All of the inoculated plates were incubated for 28 days at 36°C in an atmosphere of 7% CO₂. They were examined daily, and growth was recorded as it occurred.

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TABLE 1. Mean inhibitory concentrations of allicin for 30 strains of mycobacteria

Species	No. of strains	Mean inhibitory concn (mg/ml)	Time to growth for controls (days)
<i>M. avium</i>	3	3.13	6-10
<i>M. bovis</i>	1	1.34	11
<i>M. chelonae</i>	1	2.00	5
<i>M. fortuitum</i>	2	3.35	5
<i>M. flavescens</i>	1	3.35	5
<i>M. gastri</i>	1	2.00	5
<i>M. intracellulare</i>	1	3.35	9
<i>M. kansasii</i>	3	2.45	6-10
<i>M. malmoense</i>	1	2.00	5
<i>M. marinum</i>	2	2.00	5
<i>M. scrofulaceum</i>	2	3.01	5-10
<i>M. simiae</i>	2	3.35	5-10
<i>M. szulgai</i>	1	3.35	5
<i>M. terrae</i>	1	2.68	10
<i>M. triviale</i>	1	2.00	9
<i>M. tuberculosis</i>	6	1.67	7-16
<i>M. xenopi</i>	1	2.68	5

RESULTS

All 30 strains, comprising 17 species of mycobacteria, were inhibited by various concentrations of garlic extract, as measured by their failure to grow. The concentration required ranged from a low of 1.34 mg/ml to a high of 3.35 mg/ml. When there were multiple strains of a species, a mean inhibitory concentration was determined for that species.

M. bovis was the species most easily inhibited by the extract, requiring only 1.34 mg/ml. The six strains of *M. tuberculosis* required only slightly more extract, with a mean value of 1.67 mg/ml of media. Each garlic-free control culture grew well, requiring 5 to 16 days depending on the species characteristics (Table 1).

DISCUSSION

The inhibitory effect of garlic on *M. tuberculosis* has been mentioned in clinical reports for nearly 100 years; however, the only previous laboratory evaluation was performed by Rao et al. in 1946 for a single strain of *M. tuberculosis* (17). In this study we were able to confirm their quantitative determination of the concentration required to inhibit *M. tuberculosis* and to establish evidence of the inhibitory nature of garlic extract on 16 other species of mycobacteria.

Of the six strains of *M. tuberculosis* tested, four required 1.34 mg/ml, one required 2 mg/ml, and one required 2.68 mg/ml for inhibition. These data suggest that there is only a slight variation in the susceptibility of the strains to allicin.

Three strains of the commonly isolated photochromogenic pathogen *M. kansasii* required a mean inhibitory concentration of 2.45 mg/ml. *M. fortuitum*, *M. flavescens*, *M. intracellulare*, *M. simiae*, and *M. szulgai* required the most extract, 3.35 mg/ml. Overall, the concentration required to inhibit the 30 strains tested ranged from 1.34 to 3.35 mg/ml.

Whether garlic extract has any future in treating human mycobacterial infections remains to be evaluated. If the in vitro studies of the inhibitory power of garlic extract against

mycobacteria can be interpolated, it may be surmised that very high levels in serum would have to be achieved. These high levels could be toxic to the -SH groups of the animal or human being treated. Further studies in animals are indicated to determine achievable safe blood levels and overall toxicity. It is conceivable that smaller amounts or garlic extract along with other standard antituberculosis drugs may act synergistically against mycobacterial infections.

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