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Death of *Salmonella typhimurium* and *Escherichia coli* in the Presence of Freshly Reconstituted Dehydrated Garlic and Onion

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The kinetics of the decline of populations of *Salmonella typhimurium* inoculated into freshly reconstituted dehydrated onion and garlic powders was studied. Measurable bactericidal activity was observed for onion and garlic concentrations of 1 and 5% (w/v), respectively, with maximal death rates occurring for concentrations of 5 and 10%. At these concentrations, the decimal reduction times were 1.1 and 1.2 hr, respectively, for resting cell cultures and 1.8 and 2.1 hr, respectively, for growing cultures. Of the major volatile aliphatic disulfide compounds of onions, *n*-propyl allyl and di-*n*-propyl, at concentrations of 0.1%, showed a comparable activity against resting cells but only a bacteriostatic effect toward actively growing cultures, which overcame this effect in 2 to 6 hr. At comparable concentrations, growing cultures of *Escherichia coli* were as susceptible to garlic, but apparently more resistant to onion, than were those of *S. typhimurium*.

Since the time of Pasteur (2), the antibacterial properties of onion and garlic have been observed and recorded. Although there is an extensive medical and practical qualitative literature dealing with the antimicrobial properties of garlic and onion, there is little quantitative information available detailing the kinetics of decline of bacterial cultures in the presence of these two materials. The preliminary work reported here, with dehydrated garlic and onion powders, is an attempt to answer this need. The general literature has been reviewed by Vaughn (Food Technol., *in press*).

In this study, freshly reconstituted dehydrated powders and the major volatile aliphatic disulfide compounds (1) detectable from these and the fresh products were examined for their bactericidal activity against *Salmonella typhimurium* and *Escherichia coli*.

MATERIALS AND METHODS

Inoculum. *S. typhimurium* (mouse strain; from W. W. Sadler, University of California at Davis) and *E. coli* 9002 were grown in nutrient broth as shake cultures at 37 C for 14 hr. Such cultures were then either diluted into 0.9% NaCl for resting-cell studies or into fresh nutrient broth for growth experiments.

Resting-cell experiments. In screw-cap test tubes (25 by 150 mm), the appropriate dilution of the test organisms was well mixed with onion or garlic powder by hand shaking and incubated at 37 C. Periodically 1.0-ml samples were removed and serially diluted in 0.9% NaCl; appropriate duplicate samples were poured with half-strength M-Bismuth Sulfate Broth (Difco) hardened with 1.5% agar (Difco). After incubation for 72 hr at 37 C, black colonies representing the cells of *S. typhimurium* remaining from the original inoculum were counted. Uninoculated onion and garlic powders in no instance yielded such colonies when this medium was used.

Growing-cell experiments. Since the natural bacterial flora of powdered onion and garlic would interfere in growth experiments, 100-g lots of these products were first gas-sterilized with propylene oxide. This treatment reduced the resident microbial load to less than 10 organisms per g recoverable on nutrient agar (Difco). Suitable concentrations of these powders were mixed with 100 ml of sterile nutrient broth in 500-ml Erlenmeyer flasks and incubated as shake cultures at 37 C. At intervals, 10-ml samples were removed, serially diluted in 0.9% NaCl, and pour-plated with nutrient agar.

Volatile disulfide compounds. The di-*n*-propyl, *n*-propyl allyl, methyl allyl, methyl-*n*-propyl, and diallyl disulfide compounds were kindly supplied by Richard A. Bernhard of this department. Of these compounds, di-*n*-propyl disulfide and *n*-propyl allyl disulfide showed the greatest activity against resting cells of *S. typhimurium* and were therefore selected for further study. These compounds were tested by the procedures described above for the garlic and onion powder.

RESULTS AND DISCUSSION

Figure 1 shows the lethality of garlic and onion for resting-cell populations of *S. typhimurium*. The threshold concentrations of onion and garlic at which detectable death occurred were 1 and 5% (w/v), respectively. As the concentrations were increased to 2.5 and 7.5%, the decimal reduction times (D = time required for 90% reduction in the number of survivors) were reduced to 7 and 4 hr, respectively. Maximum death rates, i.e., minimum D values of 1.1 and 1.2 hr, resulted when 5% onion and 10% garlic, respectively, were used.

The *p*H values of the various concentrations of dehydrated garlic and onion powders freshly reconstituted in 0.9% NaCl are given in Table 1.

The fact that greater concentrations of garlic and onion decreased the *p*H values by only 0.1 unit, from 6.1 to 6.0 and from 5.3 to 5.2, respectively, strongly suggests that *p*H is not the factor responsible for the lethal activity observed at higher concentrations. The lowest values observed were still well above those reported as being generally bactericidal to *Salmonella* (3).

The volatile aliphatic disulfide compounds, tested at 0.1% concentration, were not all bactericidal to resting cells of *S. typhimurium*. However, *n*-propyl allyl disulfide, di-*n*-propyl disulfide, and diallyl disulfide were quite active, yielding D values of 0.8, 1.1, and 3.2 hr, respectively (Fig. 2). These first two values represent death rates as rapid as those for 5% onion and 10% garlic.

The next question was whether the effect exerted by the above materials was as great on growing cultures of *S. typhimurium* as on resting cells. Figure 3 shows the results of a typical experiment. The compounds *n*-propyl allyl

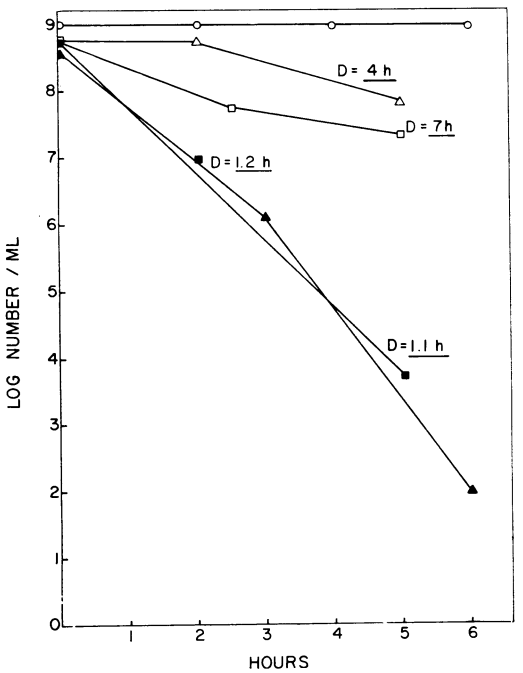

FIG. 1. *Death of resting-cell cultures of S. typhimurium in garlic and onion powders freshly reconstituted in 0.9% NaCl. Cells were recovered on bismuth sulfite-agar. Symbols: ○, control, 0.9% NaCl; △, 7.5% garlic; ▲, 10% garlic; □, 2.5% onion; ■, 5% onion.*

TABLE 1. *The pH values of garlic and onion powders freshly reconstituted in 0.9% NaCl*

Concn of garlic (w/v) in 0.9% NaCl	*p*H	Concn of onion (w/v) in 0.9% NaCl	*p*H
%		%	
1.0	6.1	1.0	5.3
2.5	6.1	2.5	5.2
5.0	6.05	5.0	5.2
7.5	6.0	7.5	5.2
10.0	6.0	10.0	5.2

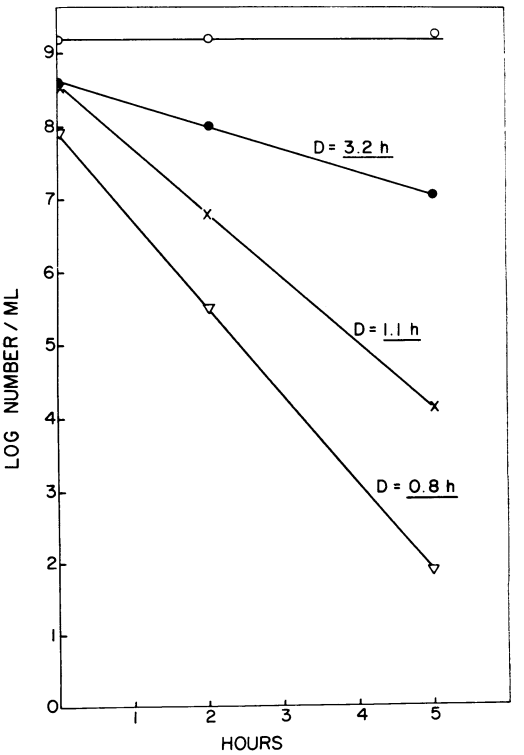

FIG. 2. *Death of resting-cell cultures of S. typhimurium in the presence of 0.1% concentrations of volatile aliphatic disulfide compounds in 0.9% NaCl. Cells recovered on bismuth sulfite-agar. Symbols: ○, control, 0.9% NaCl; ●, diallyl disulfide; ×, di-n-propyl disulfide; ▽, n-propyl allyl disulfide.*

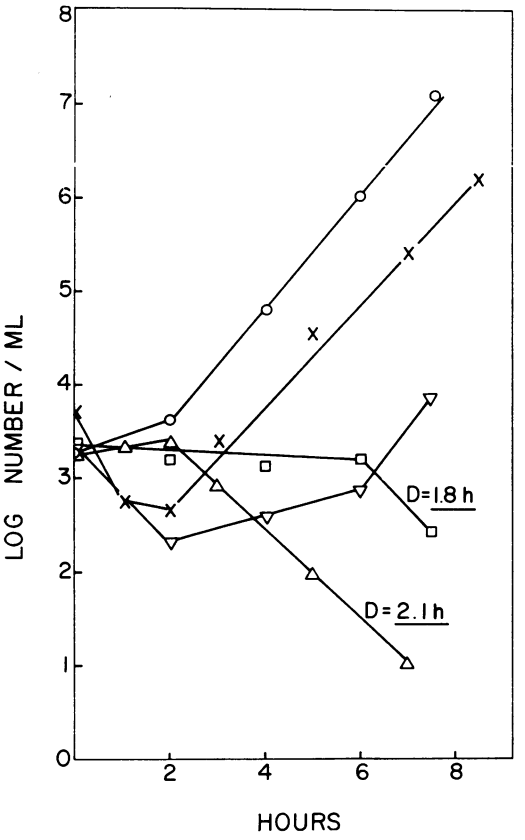

FIG. 3. *Response of growing cultures of S. typhimurium to garlic and onion powders freshly reconstituted in nutrient broth and to 0.1% concentrations of di-n-propyl disulfide and n-propyl allyl disulfide in nutrient broth. Cells recovered on nutrient agar. Symbols: ○, control, nutrient broth; △, 10% garlic; □, 5% onion; ×, di-n-propyl allyl disulfide; ▽, n-propyl allyl disulfide.*

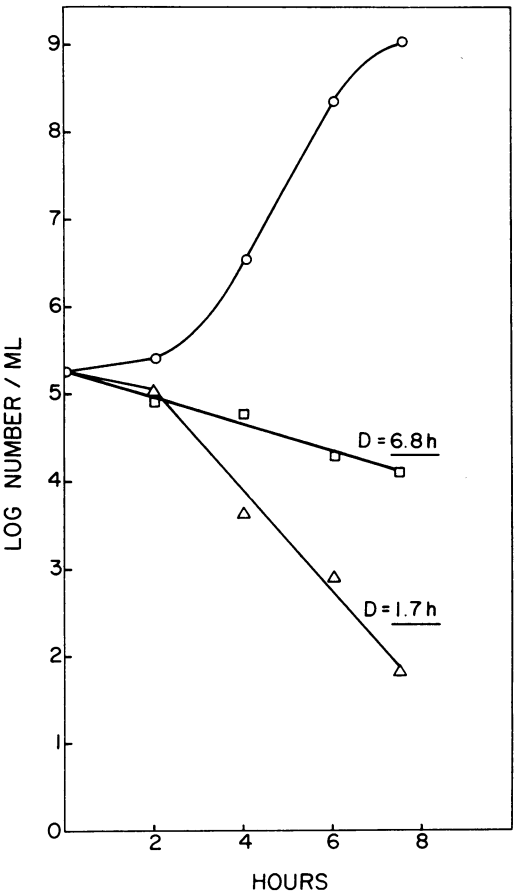

FIG. 4. *Response of growing cultures of E. coli to garlic and onion powders freshly reconstituted in nutrient broth. Cells recovered on nutrient agar. Symbols: ○, control, nutrient broth; △, 10% garlic; □, 5% onion.*

disulfide and di-*n*-propyl disulfide only exerted a bacteriostatic influence, which was overcome in 2 to 6 hr when normal rates of growth resumed. Although garlic and onion were initially only bacteriostatic, they caused death in 2 to 6 hr. The *D* values were 1.8 and 2.1 hr, respectively, for 5% onion and 10% garlic. The richness of the growth medium with the growing cultures versus that for the resting-cell experiments probably accounts for these somewhat higher *D* values.

Figure 4 shows the lethal effect of garlic and onion on growing populations of *E. coli*. This organism seemed as susceptible as *S. typhimurium* to 10% garlic (D = 1.7 hr) but perhaps is more resistant to 5% onion (D = 6.8 versus 1.8 hr for *S. typhimurium*).

Although none of the individual volatile aliphatic disulfide compounds tested prevented the eventual growth of *S. typhimurium*, combinations of them might. The characteristics of survivors from populations of *S. typhimurium* treated with garlic or onion resembled those of untreated cells. In preliminary tests, the progeny of such survivors showed the same susceptibility to garlic and onion as the parental type, suggesting that these survivors were not resistant mutants. These two possibilities should be further investigated.

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