APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 2001, p. 475–480 Vol. 67, No. 1
0099-2240/01/$04.00+0 DOI: 10.1128/AEM.67.1.475–480.2001

Antimicrobial Properties of Garlic Oil against Human Enteric Bacteria: Evaluation of Methodologies and Comparisons with Garlic Oil Sulfides and Garlic Powder

Z. M. ROSS,[1] E. A. O'GARA,[2] D. J. HILL,[3] H. V. SLEIGHTHOLME,[4] AND D. J. MASLIN[2]*

St. George's University, St. George's, Grenada, West Indies,[1] and University of Wolverhampton, School of Health Sciences, Wolverhampton WV1 1DJ,[2] University of Wolverhampton, School of Applied Sciences, Wolverhampton WV1 1SB,[3] and West Midlands Regional Genetics Service, Birmingham Women's Hospital, Birmingham B15 2TG,[4] United Kingdom

Received 19 June 2000/Accepted 9 November 2000

The antimicrobial effects of aqueous garlic extracts are well established but those of garlic oil (GO) are little known. Methodologies for estimating the antimicrobial activity of GO were assessed and GO, GO sulfide constituents, and garlic powder (GP) were compared in tests against human enteric bacteria. Test methodologies were identified as capable of producing underestimates of GO activity. Antimicrobial activity was greater in media lacking tryptone or cysteine, suggesting that, as for allicin, GO effects may involve sulfhydryl reactivity. All bacteria tested, which included both gram-negative and -positive bacteria and pathogenic forms, were susceptible to garlic materials. On a weight-of-product basis, 24 h MICs for GO (0.02 to 5.5 mg/ml, 62 enteric isolates) and dimethyl trisulfide (0.02 to 0.31 mg/ml, 6 enteric isolates) were lower than those for a mixture of diallyl sulfides (0.63 to 25 mg/ml, 6 enteric isolates) and for GP, which also exhibited a smaller MIC range (6.25 to 12.5 mg/ml, 29 enteric isolates). Viability time studies of GO and GP against *Enterobacter aerogenes* showed time- and dose-dependent effects. Based upon its thiosulfinate content, GP was more active than GO against most bacteria, although some properties of GO are identified as offering greater therapeutic potential. Further exploration of the potential of GP and GO in enteric disease control appears warranted.

Garlic (*Allium sativum*) has traditional dietary and medicinal applications as an anti-infective agent (11, 17). In vitro evidence of the antimicrobial activity of fresh and freeze-dried garlic extracts against many bacteria (5, 16), fungi (1), and viruses (20) supports these applications.

Early steps involved in identifying the active constituents of garlic were the discovery that the compound allicin (allyl 2-propene thiosulfinate) is formed when garlic cloves are crushed (5, 6, 7) and that its formation depends upon the action of the enzyme alliinase of the bundle sheath cells upon the alliin of mesophyll cells (19). Methyl and allyl sulfide derivatives of allicin are formed by the steam distillation of mashed garlic (13) to produce garlic oil (GO), which is used in many medicinal garlic products.

The classic studies of Cavallito and coworkers (5, 6, 7) attributed the antibacterial properties of garlic clove homogenates to allicin. These properties were confirmed against *Escherichia coli* and *Staphylococcus aureus* for garlic clove homogenates plus related garlic compounds and commercial supplements (9). In Cavallito's studies, antimicrobial activity was found neither with aqueous garlic extracts lacking allicin nor following the addition of GO or diallyl sulfides (5) and no allyl sulfides were found in freshly prepared aqueous garlic clove extracts (6). Also, an early gas chromatographic study (4) indicated that the relatively rapid decomposition of allicin present in aqueous garlic extracts (14) involved transformation mainly to diallyl sulfides. For these reasons it was concluded that GO and its constituent sulfides lack antimicrobial activity. However, high-performance liquid chromatography (HPLC) applied to aqueous garlic extracts stored at room temperature for 24 h revealed that allicin transformation (14) was not to sulfides. Also, the use of agar plate methods (5), which provide free exposure of garlic compounds to the air and require good water solubility of test substances, might explain why antimicrobial activity was not detected for GO, which is both volatile and hydrophobic. The early conclusion that GO is not antimicrobial was influential, and investigations of the antimicrobial properties of GO and its sulfides have been few (1, 8). GO volatilization losses were avoided in a recent study (15) which revealed that GO and its major diallyl sulfides, as well as aqueous garlic extract (reconstituted freeze-dried garlic powder [GP]) and its major thiosulfinate product, allicin, each possess significant activity against *Helicobacter pylori.* The MIC of GO was found to lie between those of the most abundant GO constituents, diallyl disulfide (DADS) and diallyl trisulfide (DATS). Comparable findings for *S. aureus* and *E. coli* (17) also suggested that GO and GO sulfides have considerable antibacterial potency. Methyl sulfides of GO have not previously been studied.

The influence of test environments upon the activity of antimicrobial agents is well-recognized (3, 10), but possible influences of different culture media upon the antimicrobial activities of garlic components are little investigated. It is known that allicin reacts with cysteine (13) as do major diallyl sulfides of GO (DADS and DATS but not diallyl sulfide [DAS]) under simulated physiological conditions (13). Such reactions have potential importance for the interpretation of in vitro tests

* Corresponding author. Mailing address: University of Wolverhampton, School of Health Sciences, 62-68 Lichfield St., Wolverhampton WV1 1DJ, United Kingdom. Phone: 01902 321138. Fax: 01902 321161. E-mail: bs2910@wlv.ac.uk.

involving peptone-rich growth media and for the antimicrobial activity of garlic components in the gut.

In summary, the methodology of early experiments might explain the negative antimicrobial reputation of GO, while more recent findings indicate substantial activity against the few bacterial species that have been tested. Reevaluation of early methods and of the antibacterial properties of GO and GO sulfides with appropriate methodologies is therefore merited. The increasing incidence of bacterial food poisoning and evidence for considerable sensitivity of *H. pylori* to GO (15) indicated enteric bacteria as a suitable subject for this reevaluation.

The main objectives of this study were, therefore, to examine the influences of test environment on antimicrobial activities of GO and evaluate these activities upon a wide range of enteric bacteria, including pathogens. Additional objectives were comparison of antimicrobial activities of GO and GP upon enteric bacteria and assessment of the antimicrobial effects of diallyl and methyl sulfides of GO.

GO and GP were supplied and stored as before (15). A mixture of diallyl sulfides (supplied as DADS and named DASS) was obtained from Fluka Chemicals (Gillingham, United Kingdom). Dimethyl trisulfide (DMT) and dimethyl disulfide (DMD) were obtained from Pfaltz & Bauer (Waterbury, Conn.).

Garlic components were analyzed by HPLC analysis with established methods (12, 14) using an LKB Bromma HPLC System with a 2152 Controller, 2150 HPLC Pump, 2158 UVICORD SD (set at 254 nm), and a 2220 Recording Integrator. Separations were achieved using a Spherisorb ODS-2 column with previously described methods (15).

The compositions of GO and GP were similar to those of samples utilized previously (15). DMT was 99% pure and DASS included diallyl polysulfides at levels (33% DADS, 16.5% DATS, and 17.5% diallyl tetrasulfide [DATTS], balance DAS) broadly comparable to those of GO.

Cultures were from the University of Wolverhampton microbiological culture collection (mainly NCTC or NCIMB strains) or, in the case of clinical enteric isolates, from local hospitals (Queen Elizabeth Hospital, Birmingham, United Kingdom; West Park Hospital, Wolverhampton, United Kingdom). Confirmatory identification procedures were routinely applied to the latter. Freeze-dried cell cultures were revived by culture onto suitable agar media. *Lactobacillus acidophilus* was routinely cultured on Mann Rogosa Sharpe Agar (MRSA) (Oxoid, Basingstoke, United Kingdom). Campylobacter Selective Agar (CSA) (Oxoid) was used for *Campylobacter* spp., and all other bacteria were cultured on Tryptone Soya Agar (TSA) (Difco, BD Biosciences, Franklin Lakes, N.J.). *L. acidophilus* and *Bacteroides fragilis* were incubated at 37°C for 48 h in an anaerobic cabinet (Forma Scientific, Marietta, Ohio) under anaerobic conditions (10% H_2, 10% CO_2, 80% N_2). *Campylobacter* spp. were incubated at 37°C for 48 h under microaerophilic conditions generated using Oxoid CampyGen sachets in gas jars (Oxoid). All other bacteria were incubated at 37°C for 24 to 48 h, under aerobic conditions. Broth cultures were prepared by inoculating the respective liquid growth medium Mann Rogosa Sharpe broth (MRSB) (Oxoid) or Tryptone Soya broth (TSB) (Difco) and incubated at 37°C for 24 to 48 h under appropriate conditions.

Enumeration of viable cells was achieved by serial 10-fold dilutions in 1/4-strength Ringers solution (Oxoid) and spread-plating of 0.1 ml of diluted samples onto the appropriate agar medium. Colonies were counted after incubation for 24 to 48 h at 37°C.

TSA (Difco) plates used in antimicrobial assays poured to a depth of approximately 7 mm were surface inoculated by spreading 0.1 ml of culture over their surface and were allowed to air dry before storage at 4°C for 1 h. All tests using TSA plates involved incubation at 37°C for 48 h. Implementation of the agar radial-diffusion technique involved adding neat GO or GO diluted and emulsified in TSB to pre-cut 4-mm-diameter wells. Where direct contact of GO with the bacterial lawn occurred, bacterial growth was inhibited but no measurable zones of inhibition of growth for the several species of enteric bacteria tested (data not shown) were obtained. These results indicated that studies relying upon diffusion in agar were unsuitable for testing GO, presumably because of its hydrophobic nature. When a bacterial lawn was inverted over a 100-μl drop of undiluted GO placed inside the lid of a glass petri dish, growth inhibition was observed on the part of the lawn immediately adjacent to the GO, which apparently resulted from air diffusion of GO vapor. This indicated both antimicrobial activity by GO and likely underestimation of such activity in agar plate studies because of volatilization loss.

A 10% (wt/vol) GO suspension in TSB formed by shaking was initially homogeneous, but upon standing, GO (density = 1.037 g/ml) coalesced at the bottom of the vessel within 20 min. The clear upper fraction obtained failed in MIC tests against *Enterobacter aerogenes.* In contrast, samples collected from the turbid lower fraction yielded a MIC of 0.68 mg/ml, comparable to that of homogeneous 10% (wt/vol) GO suspensions sampled immediately after shaking.

To assist dispersal of GO, detergents were incorporated into the TSB medium, and MICs against *E. aerogenes* were determined. Dimethyl sulfoxide, dimethyl formamide, Tween 80, Triton X-100, and bile salts (Sigma-Aldrich Company Ltd, Poole, United Kingdom) at concentrations below their MICs (25% [vol/vol], 12.5% [vol/vol], 25% [vol/vol], 25% [vol/vol], 12.5% [vol/vol], respectively) all produced significant dispersal of GO. However, no influence upon MICs was observed, so detergents were not routinely used.

To produce a homogeneous emulsion without detergent, sterile GO, or GO sulfides, were first diluted 50-fold as 0.5 ml was added into 9.5 ml of the appropriate broth in a 25-ml Universal bottle. The bottle was tightly sealed, and the mixture was shaken vigorously for 3 min just before use.

In tests of incubation conditions, lower MICs and higher sulfide values were observed at 24 h (data not shown) under static conditions than those observed after shaking. Under static conditions, MICs did not differ when metal caps were compared with tight-sealing ground glass stoppers. Therefore, static incubation of broths was utilized routinely to minimize volatilization, and tubes with metal caps were used for convenience and low cost and to ensure a gaseous environment suitable for microbial growth.

MICs were determined by incubation at 37°C for up to 72 h of serial twofold dilutions of garlic substances in 5 ml of broth in 16-ml test tubes (for *Campylobacter* spp., 2-ml broth in bijoux bottles). MIC determinations were carried out in trip-

TABLE 1. MIC determinations of GO and GP against human enteric pathogens and commensals[a]

Bacterium	GO assay			GP assay		
	No. of strains tested	MIC (mg/ml)		No. of strains tested	MIC (mg/ml)	
		24 h	48 h		24 h	48 h
Human enteric pathogens						
Bacillus cereus	1	0.08	0.17			
Escherichia coli O55	1	5.5	5.5	1	12.5	25
Escherichia coli O128	1	2.75	2.75	1	12.5	25
Escherichia coli O112	1	2.75	5.5	1	3.13	3.13
Shigella boydii[b]	1	1.37	2.75	1	6.25	6.25
Shigella flexneri[b]	1	1.37	2.75	1	6.25	6.25
Shigella sonnei[b]	4	2.75	2.75–5.5	3	12.5	12.5
Vibrio fluvialis[b]	1	2.75	2.75			
Vibrio metschnikovii[b]	1	0.02	0.34			
Vibrio parahaemolyticus[b]	1	0.04	0.08			
Yersinia enterocolitica[b]	3	0.17–0.34	0.68	2	6.25	6.25
Listeria monocytogenes[b]	2	0.02	0.02–0.08	2	6.25	25
Salmonella enterica serovar Enteritidis[b]	2	5.5	5.5	3	6.25	12.5–25
Salmonella enterica serovar Infantis[b]	1	2.75	5.5	1	6.25	25
Salmonella enterica serovar Senftenberg[b]	2	5.5	5.5	1	12.5	25
Salmonella enterica serovar Typhimurium[b]	3	0.34–2.75	0.68–5.5	3	6.25–12.5	12.5–25
Campylobacter jejuni	4	0.16–0.32[c]	0.16–0.32[d]	4	3.13–12.5[c]	3.13–25[d]
Campylobacter coli	1	0.16[c]	0.49[d]	1	3.13[c]	6.25[d]
Campylobacter lari	1	0.16[c]	0.49[d]	1	1.59–3.13[c]	3.13[d]
Commensal enteric bacteria						
Bacteroides fragilis	2	0.02–0.04	0.04	1	6.25	6.25
Bacillus subtilus	3	0.17	0.17–0.68			
Enterobacter aerogenes	1	0.68	0.68	1	6.25	6.25
Enterococcus faecalis[b]	1	0.34	0.34			
Escherichia coli	13	0.34–2.75	0.34–5.5	4	3.13–12.5	6.25–25
Klebsiella aerogenes	1	0.04	0.17	1	12.5	12.5
Proteus vulgaris	1	1.37	2.74			
Lactobacillus acidophilus	5	0.17–2.75	0.34–2.75	2	6.25	12.5
Streptococcus faecalis[b]	2	0.34	0.34			
Streptococcus mutans	1	0.08	0.08			
Streptococcus pyogenes	1	0.02	0.04			

[a] Each MIC determination was performed in triplicate per bacterial isolate. Range values refer to MICs determined for the different enteric isolates tested.
[b] Enteric isolates.
[c] MICs were determined at 48 h.
[d] MICs were determined at 72 h.

licate, and consistent values were obtained for each bacterial isolate. Killing effects were confirmed by plating out onto TSA.

For a range of gram-positive and gram-negative enteric bacteria, including food-borne pathogens, MICs (Table 1) obtained for GO and GP indicated growth inhibition of all the bacteria tested. Incubation of MIC tubes for 48 h gave either the same or around twofold the 24-h MICs.

MICs varied widely (0.02 to 5.5 mg/ml) between different species and mostly within a 10-fold GO concentration range for different isolates of the same species. No obvious differences between the sensitivities of clinical and culture collection enteric isolates were observed. MICs for species commonly associated with food poisoning were similar to those observed for commensals, although with GO the latter tended to greater sensitivity than pathogens. However, with the human enteric pathogens *Campylobacter* spp., *Listeria monocytogenes*, and *Vibrio parahaemolyticus* and one clinical isolate of *Yersinia enterocolitica*, sensitivities to GO were many times greater than for the commensals *L. acidophilus* and *E. coli*.

Time course viability studies were carried out to reveal the effects of GO upon cell growth and viability. Serial twofold dilutions of GO or component sulfides, were prepared in 50-ml boiling tubes with TSB, sterile distilled water (SDW), or minimal salts medium (MSM) (composition of which [each value in grams/liter] was KH_2PO_4, 1.0; K_2HPO_4, 1.0; KNO_3, 1.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 0.1), producing final volumes of 20 ml. The tubes were inoculated with 40 μl of an overnight culture of *E. aerogenes*, producing initial viable cell populations of 10^6 CFU/ml, capped with aluminum foil, and incubated aerobically at 37°C for 24 h. Periodically, tubes were aspirated to homogenize the culture, and then 0.5-ml samples were removed for viable count determinations. Exposure to GO (Fig. 1A) and GP (Fig. 1B) of *E. aerogenes* grown in TSB revealed concentration-dependent capacities to kill or inhibit population growth. This pattern of effect replicates that of other time studies that we have performed upon other microorganisms with garlic components (reference 15 and unpublished observations).

With GO, an initial lag phase of 30 to 60 min occurred at concentrations of 0.34 to 2.75 mg/ml. Longer lag phases were observed with GP and were greater at lower concentrations (e.g., lag phases with GO were 30 min for 50 mg/ml and 400 min for 6.25 mg/ml). Lag phases may reflect delay in uptake of garlic molecules and/or of toxic effect upon metabolic pro-

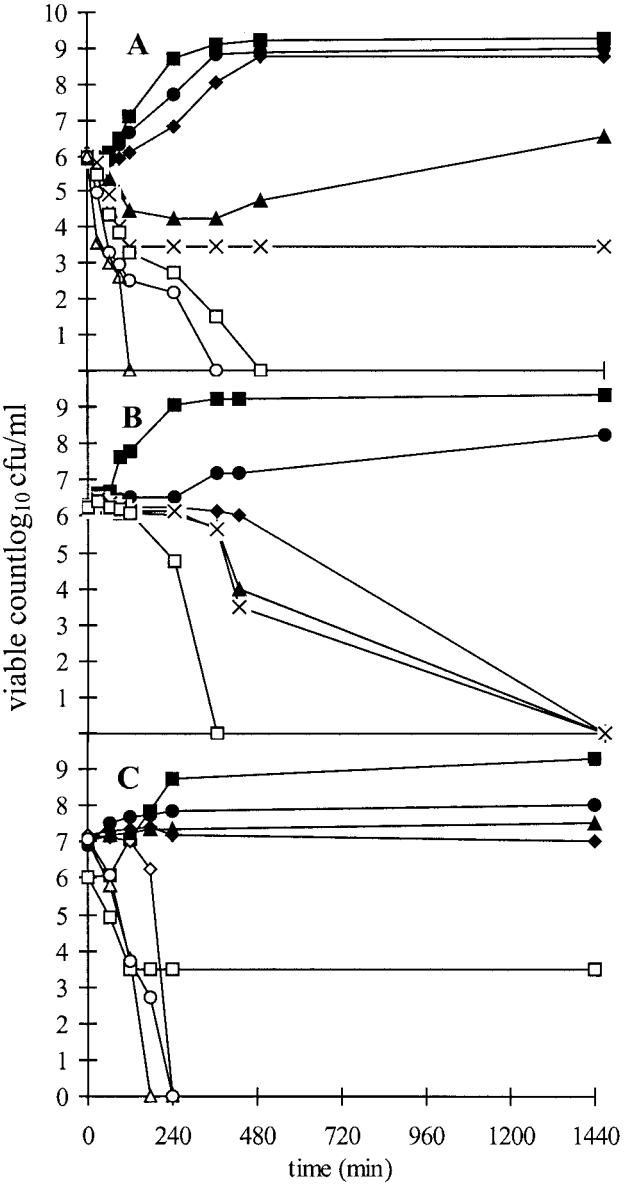

FIG. 1. (A) The effect of GO upon *E. aerogenes* viability with time. ■, 0 mg/ml (control); ●, 0.34 mg/ml; ◆, 0.68 mg/ml; ▲, 1.38 mg/ml; ×, 2.75 mg/ml; □, 5.5 mg/ml; ○, 11 mg/ml; △, 22 mg/ml. (B) The effect of GP upon *E. aerogenes* viability with time. ■, 0 mg/ml (control); ●, 3.13 mg/ml; ◆, 6.25 mg/ml, ▲, 12.5 mg/ml; ×, 25 mg/ml; □, 50 mg/ml. (C) The effect of a 2.75-mg/ml GO concentration upon *E. aerogenes* in SDW and MSM with and without a carbon source (glucose). ■, TSB; □, TSB + GO; ◆, SDW; ◇, SDW + GO; ▲, MSM; △, MSM + GO; ●, MSM + 1.0% glucose; ○, MSM + 1% glucose + GO.

cesses. However, at the higher concentrations used (5.5 to 22 mg/ml), and at lower concentrations in nutrient-deficient media (Fig. 1C), a potential for rapid bactericidal action of GO was indicated by complete loss of viability during the first 2 h. Comparable bactericidal effects were observed with GP concentrations of 6.25 to 50 mg/ml. At concentrations of 0.34 to 1.375 mg/ml, declines in viability were temporary and recovery of growth occurred. The latter ensued after shorter delays, at higher rates, and to greater levels the lower the GO concentration. This recovery of viability was not observed with GP.

At the intermediate GO concentration of 2.75 mg/ml, viability declined during the first 5 to 6 h and then levelled out, and HPLC analysis at this concentration showed that GO sulfide loss was greatest over this initial period when sampling-related agitation was greatest. Losses over 24 h were greatest for the smaller, lower-boiling-point sulfides (ca. 75% for DMT, 60% for DAS, 40% for DADS, and 20% for DATS and DATTS). Thus, volatilization is the most plausible explanation for these losses and could explain the recovery of *E. aerogenes* viability observed at concentrations of 0.34 to 2.75 mg of GO/ml. It could also explain why MICs were frequently higher at 48 h than at 24 h and why the MIC for *E. aerogenes* obtained in time studies was 2.75 mg/ml—considerably higher than the 0.68-mg/ml concentration obtained under static capped-tube conditions. With GP, the bactericidal concentration of 6.25 mg/ml obtained from the MIC study corresponded to that obtained in the time study, in which little evidence for recovery of viability was observed. This greater consistency of result probably results from the lesser volatility of the thiosulfinates of GP.

The time course study (Fig. 1C) of different microbial media indicates that medium composition is an important factor influencing antimicrobial activity of GO. Thus, at 2.75 mg of GO/ml, complete killing occurred within a few hours in each of SDW, MSM, and MSM plus glucose. In contrast, at the same GO concentration in TSB, after a steep initial drop, leveling-off of viability occurred, as was previously observed (Fig. 1A).

The role of tryptone in explaining this apparent protective effect of TSB was supported by positive correlation of *E. aerogenes* viability with levels of tryptone ranging from 0 to 15 g/liter of MSM (data not shown) in the presence of the potentially bactericidal concentration of 2.75 mg of GO/ml. Cysteine produced a similar effect as was shown by partial neutralization of the bactericidal effects of 2.75 mg of GO/ml in MSM in the presence of 10 mM cysteine and very substantial neutralization in the presence of 25 mM cysteine. As TSB and tryptones are likely sources of cysteine, protein components of other microbial growth matrices may produce similar reductions in antimicrobial effects of GP and GO. This means that the results of our own and previous studies of garlic materials may represent underestimates of their full antimicrobial potential.

MICs obtained for DMT and DASS were species dependent for a range of GO-sensitive bacteria (Table 2). The species most sensitive to GO were also those most sensitive to DMT and DASS (*L. monocytogenes*, *Y. enterocolitica*). For all five species tested, sensitivity to DMT was severalfold greater than that to DASS and similar or slightly greater to DMT than to

TABLE 2. Mean 24-h MICs of a selection of GO sulfides against six enteric bacteria

Enteric isolate	MIC (mg/ml)		
	GO	DASS	DMT
E. aerogenes	0.68	25	0.31
E. coli	0.68	25	0.31
S. enterica serovar Typhimurium	0.34	25	0.31
S. sonnei	2.75	10	0.02
L. monocytogenes	0.02	0.63	0.04
Y. enterocolytica	0.02	0.63	

TABLE 3. Comparison of potencies of GO and GP by using 24-h MICs against selected isolates

GP/GO ratio of MICs[a]	*E. coli* Cardiff W1485 K12W-T	*Salmonella enterica* serovar Typhimurium[c]	*L. monocytogenes*[c]	*B. fragilis* NCIB 9343
GP:GO	9.19	9.12	312.5	156.25
GP:GO, allowing for GP thiosulfinate content[b]	0.15	0.15	5.11	2.56

[a] Expressed on a weight-of-garlic-product basis.
[b] Total thiosulfinate content of GP = 16.367 ± 0.964 mg/g, $n = 3$.
[c] Enteric isolate.

GO. These similarities of ratio between MICs of DASS and DMT for each bacterium suggest a common antibacterial mode of action for GO sulfides.

The antimicrobial potency of DASS indicates that the major (i.e., diallyl) sulfide constituents of GO contribute significantly to the antimicrobial activity of GO. However, DASS exhibited MICs substantially higher than those of GO for all species tested so that diallyl sulfides do not fully explain GO activity. This result is consistent with the lower antimicrobial activities observed for diallyl sulfides separately when compared with GO (15, 17). These differences have been proposed to result from the synergistic activities (17, 18) of GO sulfides. However, our finding that DMT possesses high antimicrobial activity, and evidence that the antimicrobial potency of diallyl sulfides increases with each additional S atom (15, 17), raises the possibility of methyl sulfides and/or higher polysulfides explaining the high relative antimicrobial activity of GO.

As the considerable antimicrobial activity of GO and its sulfide constituents, several of which possess sulfhydryl (SH) reactivity (2, 21), was reduced in the presence of SH groups, an SH-reactive mode of action like that proposed for allicin is suggested. The greater reactivity of allicin (13) than GO sulfides with cysteine is associated with its sulfur-bound oxygen atom (17) and could explain its stronger antimicrobial effect (15, 17).

The actions of GP reported here against a number of enteric bacteria are consistent with broad-spectrum antibacterial effects previously evidenced (17) for aqueous garlic bulb extracts. The MIC results for GP over 24 h (Table 1) were comparable to those reported earlier (16).

The potency of GO was substantially greater than that of GP (Table 3) on a weight-of-garlic-product basis. However, when allowing for the dilution of alliin within the GP matrix and the consequent yield of allicin plus other thiosulfinates (1.6% of GP), the reverse was true for all species tested excepting for those most sensitive to GO (*L. monocytogenes*, *B. fragilis*). Thus, early findings (5) that the active constituents (i.e., allicin and other thiosulfinates) of garlic bulb extracts such as GP solution yield substantially greater antimicrobial activity than GO were confirmed. However, contrary to the early studies (5), which were negative for GO, our findings indicate that GO possesses substantial and broad-spectrum antibacterial activity against both gram-positive and -negative bacteria. For some isolates (*Vibrio metschnikovii* and *L. monocytogenes*), sensitivity to GO was as high as that recently reported for *H. pylori* (15). The far greater potency of GO on a unit weight of product basis reflects the much greater concentration of antibacterial substances present in GO than in water-soluble garlic bulb extracts such as GP. Whereas GP requires solubilization in a suitable aqueous medium (this excludes acidic gastric juice), so as to enable the enzyme-catalyzed formation of its active thiosulfinate components, the active components of GO are already present. Moreover, the observed reduction in the antimicrobial effect of GO by protein digestion products and cysteine, which are abundant in the gut, would probably be greater for GP-derived allicin, because of its higher cysteine affinity (13). These differences suggest that GO might have greater clinical potential than GP despite the usually greater antimicrobial potency of the latter. The potential of GO to combat human gut infection is supported by our initial observations of its significant antimicrobial effects within ex vivo samples of human digesta (data not shown).

In conclusion, the results suggest that earlier evaluation of the antimicrobial activity of GO sulfides using agar plate methods (5), which indicated negligible antimicrobial activity, failed to take account of the hydrophobic and volatile nature of GO. In contrast, our results show that GO exhibits considerable antimicrobial activity when tested in liquid cultures, especially when precautions to minimize volatilization loss are applied. Reduced antimicrobial activity in the presence of tryptone and cysteine suggests that GO activity may be underestimated in microbial culture media and reduced within the enteric environment and is compatible with evidence that the mode of action of garlic compounds depends upon SH reactions.

Both GO and GP revealed wide-spectrum antimicrobial activities. Molecule for molecule, these activities were greater with GP thiosulfinates than GO sulfides against most bacteria tested. However, the antibacterial activities of GO and GO sulfides were substantial against several pathogenic species, some of which are significant causes of food poisoning, so that further exploration of the potential of GO in enteric disease control is warranted.

Z. M. Ross was supported by the Science and Engineering Research Council (SERC) (United Kingdom) and Seven Seas Ltd., Hull, United Kingdom.

We thank David A. Roser MCIM, Director of the Garlic Research Bureau, Bury St. Edmunds, United Kingdom, for his help and encouragement.

REFERENCES

1. **Adetumbi, M., G. T. Javor, and B. H. S. Lau.** 1986. *Allium sativum* (Garlic) inhibits lipid synthesis by *Candida albicans.* Antimicrob. Agents Chemother. **30:**499–501.
2. **Bailey, J. H., and C. J. Cavallito.** 1948. The reversal of antibiotic action. J. Bacteriol. **55:**175–182.
3. **Brenner, V. C., and J. C. Sherris.** 1972. Influence of different media and bloods on results of diffusion antibiotic susceptibility tests. Antimicrob. Agents Chemother. **1:**116–122.
4. **Brodnitz, M. M., J. V. Pascale, and L. Van Derslice.** 1971. Thiopropanal S-oxide: a lachrymatory factor in onions. J. Agric. Food. Chem. **19:**269–272.
5. **Cavallito, C. J., and J. H. Bailey.** 1944. Allicin, the antibacterial principle of *Allium sativum.* I. Isolation, physical properties, and antibacterial action. J. Am. Chem. Soc. **66:**1950.
6. **Cavallito, C. J., J. H. Bailey, and J. Buck.** 1945. Allicin, the antibacterial principle of *Allium sativum.* III. Its precursor and "essential oil" of garlic. J. Am. Chem. Soc. **67:**1032.
7. **Cavallito, C. J., J. S. Buck, and C. M. Suter.** 1944. Allicin, the antibacterial principle of *Allium sativum.* II. Determination of the chemical structure. J. Am. Chem. Soc. **66:**1952–1954.
8. **Chung, J. G., G. W. Chen, L. T. Wu, H. L. Chang, J. G. Lin, C. C. Yeh, and T. F. Wang.** 1998. Effects of garlic compounds diallyl sulphide and diallyl disulphide on arylamine N-acetyltransferase activity in strains of *Helicobacter pylori* from peptic ulcer patients. Am. J. Chin. Med. **26:**353–364.
9. **Hughes, B. G., and L. D. Lawson.** 1991. Antimicrobial effects of *Allium*

sativum L. (garlic), *Allium ampeloprasum* (elephant garlic) and *Allium cepa* L. (onion), garlic compounds and commercial garlic supplement products. Phytother. Res. **5:**154–158.
10. **Jones, R. N.** 1996. Medium and supplement effects on the antimicrobial activity of Quinupristin/Dalfopristin tested by agar dilution and Etest methods. Diagn. Microbiol. Infect. Dis. **26:**99–102.
11. **Lawson, L. D.** 1998. Garlic: a review of its medicinal effects and indicated active compounds, p. 176–209. *In* L. D. Lawson and R. Bauer (ed.), Phytomedicines of Europe: their chemistry and biological activity. ACS Symposium Series, no. 691. American Chemical Society, Washington, D.C.
12. **Lawson, L. D.** 1991. Identification and HPLC quantitation of the sulphides and dialk(en)yl thiosulphinates in commercial garlic products. Planta Med. **57:**363–370.
13. **Lawson, L. D.** 1996. The composition and chemistry of garlic cloves and processed garlic, p. 37–107. *In* H. P. Koch and L. D. Lawson (ed.), Garlic. The science and therapeutic application of *Allium sativum* L. and related species–1996. Williams & Wilkins, Baltimore, Md.
14. **Lawson, L. D., S. G. Wood, and B. G. Hughes.** 1991. HPLC analysis of allicin and other thiosulfinates in garlic clove homogenates. Planta Med. **57:**263–270.
15. **O'Gara, E. A., D. J. Hill, and D. J. Maslin.** 2000. Activities of garlic oil, garlic powder, and their diallyl constituents against *Helicobacter pylori.* Appl. Environ. Microbiol. **66:**2269–2273.
16. **Rees, L. P., S. F. Minney, N. T. Plummer, J. H. Slater, and D. A. Skyrme.** 1993. A quantitative assessment of the antimicrobial activity of garlic (*Allium sativum*). World. J. Microbiol. Biotechnol. **9:**303–307.
17. **Reuter, H. D., H. P. Koch, and L. D. Lawson.** 1996. Therapeutic effects and applications of garlic and its preparations, p. 135–212. *In* H. P. Koch and L. D. Lawson (ed.), Garlic. The science and therapeutic application of *Allium sativum* L. and related species–1996. Williams & Wilkins, Baltimore, Md.
18. **Ross, Z. M., D. J. Maslin, and D. J. Hill.** 1993. The effect of steam distilled garlic oil on lactic acid and other enteric bacteria. 4th Symposium of European Microbiological Societies. FEMS Microbiol. Rev. **12**(G43)**:**137.
19. **Stoll, A., and E. Seebeck.** 1948. Allium compounds. I. Alliin the true mother compound of garlic oil. Helv. Chim. Acta **31:**189–210.
20. **Weber, N. D., D. O. Anderson, J. A. North, B. K. Murray, L. D. Lawson, and B. G. Hughes.** 1992. In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta Med. **58:**417–423.
21. **Wills, E. D.** 1956. Enzyme inhibition by allicin, the active principle of garlic. J. Biochem. **63:**514–520.