S-Methyl-L-Cysteine Sulfoxide as the Precursor of Methyl Methanethiolsulfinate, the Principal Antibacterial Compound in Cabbage

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ABSTRACT

S-Methyl-L-cysteine sulfoxide (SMCSO) was present in several cultivars of cabbage (*Brassica oleracea*) at 690–1,120 ppm. SMCSO was not inhibitory to *Leuconostoc mesenteroides*, but its hydrolysis product, methyl methanethiolsulfinate (MMTSO), was inhibitory. MMTSO was formed in fresh cabbage juice (CJ) and in a model system containing SMCSO and fresh cabbage (pH 4.0) precipitate, perhaps by cysteine sulfoxide lyase. Growth inhibition by 1 mM MMTSO was reversed by 5 mM dithiothreitol, cysteine, or reduced glutathione, but not by oxidized glutathione or thiamine.

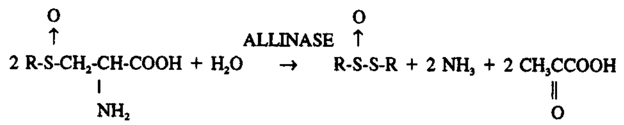
Key Words: S-Methyl-L-cysteine sulfoxide, methyl methanethiolsulfinate, cabbage, lactic-acid bacteria, glutathione

INTRODUCTION

THE PRESENCE of antibacterial activity in cabbage has been confirmed by many workers (Pederson and Fisher, 1944; Little and Grubaugh, 1946; Dickerman and Liberman, 1952; Yildiz and Westhoff, 1981; Liu et al., 1986; Kyung and Fleming, 1993) following the initial demonstration of activity in 1936 by Sherman and Hodge. The antibacterial substance of cabbage was reported to be destroyed by heating (Sherman and Hodge, 1936; Pederson and Fisher, 1944). Yildiz and Westhoff (1981), however, reported that heating caused cabbage extract to become inhibitory. Kyung and Fleming (1993) reported inhibitory activity in fresh, unheated juice of several cultivars (cvs) of cabbage. Heating the cabbage before juice extraction prevented formation of the inhibitor(s) in some cvs, but not others. The growth inhibitory substance of fresh cabbage was suggested to be carbohydrate in nature and of a low molecular weight (Dickerman and Liberman, 1952; Liu et al., 1986). However, identity of the inhibitory compound(s) has not been elucidated.

Glucosinolate hydrolysis products (GHPs) have been found in cabbage (Van Etten et al., 1976, 1980) and inhibit a wide range of bacteria and fungi (Virtanen, 1962; Zsolnai, 1966). However, cabbage juice (CJ) was reported to be more inhibitory to Gram-negative than to Gram-positive bacteria (Pederson and Fisher, 1944). Our interests in factors influencing sauerkraut fermentation and the confusion in published reports on antibacterial activity in cabbage led us to search for an alternative antibacterial compound(s) in cabbage.

S-Methyl-L-cysteine sulfoxide (SMCSO), a nonprotein sulfur amino acid, has been reported in *Cruciferae*, including cabbage (Synge and Wood, 1956; Morris and Thompson, 1956; Arnold and Thompson, 1962; Pederson and Albury, 1969; Gustine, 1985; Marks et al., 1992), and it is structurally similar to alliin (S-allyl-L-cysteine sulfoxide) (Fig. 1), another nonprotein sulfur amino acid commonly found in the *Lilliaceae* family which includes garlic and onion. Garlic and onion have long been known for their antibacterial activity when their tissue is injured (Walton et al., 1936; Saleem and Al-Delaimy, 1982). Cavallito and Bailey (1944) isolated an antimicrobial compound from garlic (*Allium sativum*) and named it 'allicin'. The main antimicrobial compound in crushed garlic was reported to be allyl 2-propenyl-1-thiolsulfinate (allicin), which was formed from its precursor, alliin, in the presence of allinase (Cavallito et al., 1944; Stoll and Seebeck, 1951a). According to Stoll and Seebeck (1951a), this reaction can be illustrated as:



$$2\,R\text{-}\overset{\overset{O}{\uparrow}}{S}\text{-}CH_2\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH + H_2O \xrightarrow{\text{ALLINASE}} R\text{-}\overset{\overset{O}{\uparrow}}{S}\text{-}S\text{-}R + 2\,NH_3 + 2\,CH_3\underset{\underset{O}{\|}}{C}COOH$$

SMCSO was also hydrolyzed by the same enzyme in *Brassica* to generate methyl methanethiolsulfinate (MMTSO) (Marks et al., 1992), having the same functional group as allicin (Fig. 1) and antibacterial activity (Small et al., 1947). Since cabbage contains SMCSO and its hydrolase (Mazelis, 1963; Pederson and Albury, 1969; Hall and Smith, 1983; Marks et al., 1992), and MMTSO is produced as a result of the enzyme reaction in a *Brassica* (Marks et al., 1992), the functionality of SMCSO was tested as a bacterial growth inhibitor in cabbage. SMCSO appears to be important in cabbage flavor (Dateo et al., 1957; Chin and Lindsay, 1993a) and to cause haemolytic anemia in livestock grazing kale (Barry et al., 1984).

Our objective was to test the hypothesis that SMCSO is the precursor of antibacterial activity in unheated CJ, and that MMTSO generated from SMCSO inhibits the growth of *Leuconostoc mesenteroides*, an important bacterium in the fermentation of cabbage to sauerkraut.

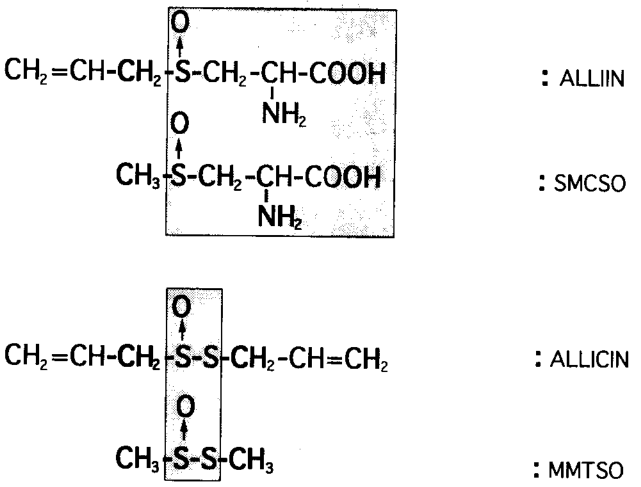


Fig. 1—Structural similarities between alliin and SMCSO, and between allicin and MMTSO (those in boxes are common parts).

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MATERIALS & METHODS

CABBAGE cvs Brutus, Galaxy, and Bentley were obtained from Castle Harvester Co., Inc. (Seneca Castle, NY) and Cecile cv. was obtained from Shiocton Kraut Co., Inc. (Shiocton, WI).

Glutathione (reduced, GSH, and oxidized, GSSG, forms), cysteine, thiamine, pyridoxal 5-phosphate, *o*-phthaldialdehyde (OPA), hydrogen peroxide, and S-Methyl-L-cysteine sulfoxide were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol, acetonitrile, dimethyl disulfide, peracetic acid, dithiothreitol (DTT), tertbutylthiol, dichloromethane, allyl isothiocyanate (NCS), allyl cyanate (CN), phenylethyl NCS, and benzyl NCS were purchased from Aldrich Chemical Co. (Milwaukee, WI). Other GHPs (Table 1) were gifts from Dr. M. E. Daxenbichler through Dr. G. F. Spencer (USDA-ARS, Peoria, IL). Dimethyl trisulfide (Eastman Kodak Co., Rochester, NY) was provided by Dr. R. C. Lindsay, Department of Food Science, University of Wisconsin-Madison (Madison, WI).

Bacterial strain and culture condition

L. mesenteroides C33 (Stamer et al., 1971) was obtained from the culture collection of our lab. It was stored at −84°C in MRS broth (Difco Laboratories, Detroit, MI) containing 16% glycerol. A stock culture was streaked onto MRS agar. An isolated colony was transferred to filter-sterilized, heated CJ and subcultured for 16 hr before each experiment. Aliquots of CJ or MRS broth (10 mL) were dispensed into 16 mm × 150 mm glass culture tubes with caps and statically incubated at 30°C after inoculation. The inoculum was 100 μL of undiluted or appropriately diluted culture.

Preparation of CJ

Juice from fresh or heated cabbage was extracted by an electrical centrifuge-type juice extractor (Braun, Germany) as described by Kyung and Fleming (1993). Heated CJ was made by quartering cabbage and steaming (≈ 100°C) it in an autoclave at atmospheric pressure for 10 min before extraction.

Bacterial count

Viable cell numbers were counted as colony-forming units (CFU)/mL by spiral plating (Spiral Biotech, Bethesda, MD) onto MRS agar plates and incubating aerobically at 30°C for 48 hr.

Growth inhibition by GHPs

Stock solutions for each of the 12 GHPs were made by dissolving 100 times the concentration needed in 95% ethanol. Then, 0.5 mL of this concentrate was added to 4.5 mL of heated CJ. Gentle warming was necessary for complete dissolution of 4-methylsulfinylbutyl NCS and CN. These solutions were then filter-sterilized (0.2 mm, Costar bottle filter, Costar Corp., Cambridge, MA). Filter-sterilized stock solutions (1 mL) were added to 9 mL of CJ before inoculation of *L. mesenteroides*. The control medium contained 0.95% ethanol. Growth was followed by measuring optical density of the broth at 600 nm (Spectronic 20, Bausch and Lomb, Inc., Rochester, NY). Myrosinase (0.5 mg/mL, Sigma) was added into heated CJ for growth inhibition studies.

Table 1—GHPs tested for inhibition of L. mesenteroides *C33*

GHPs[a]	Concentration (ppm) in cabbage[b]
Allyl NCS	10.4 - 48.5
Allyl CN	(7.0 - 32.8)
3-Methylsulfinylpropyl NCS	23.7 - 76.1
3-Methylsulfinylpropyl CN	(19.0 - 61.2)
4-Methylsulfinylbutyl NCS	8.6 - 92.6
4-Methylsulfinylbutyl CN	(7.0 - 75.9)
4-Methylsulfonylbutyl NCS	3.7 - 10.7
4-Methylsulfonylbutyl CN	(3.2 - 8.9)
4-Methylthiobutyl CN	(0 - 1.1)
5-Vinyloxazolidine-2-thione	0.6 - 10.7
Phenylethyl NCS	1.4 - 2.4
Benzyl NCS	0.2 - 1.4

[a] NCS, isothiocyanate; CN, nitrile.

[b] Concentrations reported only as NCS (Van Etten et al., 1976, 1980). Numbers in parentheses are calculated values from corresponding NCS.

Preparation of pH 4.0 precipitate and its pre-treatment

pH 4.0 precipitate was obtained from fresh CJ of Brutus cv. cabbage by adjusting the juice pH to 4.0 with HC1, followed by centrifugation and washing of the precipitate with distilled water. The precipitate was treated and used as described by Kyung and Fleming (1993).

SMCSO preparation

S-Methyl-L-cysteine was oxidized by hydrogen peroxide to SMCSO by the method of Lepp and Dunn (1955) for methionine sulfoxide preparation. The (+) diastereoisomer was separated from the (−) form by fractional crystallization from an acetone/water mixture or water/ethanol mixture (Stoll and Seebeck, 1951b; Woolfson et al., 1987). Partially (76%) separated (+) SMCSO was used as a standard compound.

SMCSO was confirmed by mass spectrometry (JEOL, HX-110) using fast atom bombardment with glycerol as the matrix. Mass spectra gave prominent peaks at 136 $(M + H)^+$ for S-methylcysteine and 152 $(M + H)^+$ for SMCSO.

Sample preparation for SMCSO analysis

Fresh cabbage heads were separated into outer leaves (mostly green), inner leaves (mostly white), and core and cut into 2.5 × 5 cm pieces. Cut cabbage was immediately homogenized by a Waring Blendor with 5 volumes of 50% methanol/50% water (Ziegler and Sticher, 1989). After centrifugation (Eppendorf centrifuge 5415, Brinkmann Instruments, Westbury, NY) of the homogenate, the juice was analyzed. Heated CJ samples for the SMCSO analysis were the same as the heated CJ for cell cultivation. Heated CJ was diluted to 2 to 5% concentration with the methanol/water mixture before analysis for SMCSO.

HPLC analysis of SMCSO in CJ

SMCSO was analyzed as the pre-column derivative of OPA by the isocratic HPLC analysis method of Ziegler and Sticher (1989), except for modification of flow rate of the mobile phase (0.7 mL/min). The mobile phase was composed of 24% acetonitrile in 50 mM potassium phosphate buffer (pH 7.0). OPA derivatives were separated at 30°C on a 100 mm × 4.6 mm column (Spherisorb ODS II, 3 μm, Alltech Associates, Inc., Deerfield, IL) connected to a Waters 510 solvent delivery system and a spectrophotometric detector (Varian-Aerograph, VUV-10, Varian Associates Inc., Sunnyvale, CA). The OPA derivative of SMCSO was monitored at 337 nm.

SMCSO-pH 4.0 precipitate model system

Precipitate obtained from 200 mL of fresh CJ (as described in the pH 4.0 preparation section) was added to 100 mL of 0.1M phosphate buffer (pH 6.5) solution containing 700 ppm of SMCSO and 10 μM of pyridoxal 5-phosphate. The mixture was incubated at 30°C for 24 hr and volatile compounds were extracted with dichloromethane and analyzed by GC/MS.

Identification of volatile sulfur compounds

Mass spectra of the isolated compounds were obtained by combination GC/MS (model HP 5985B mass spectrometer and RTE VI data system, Hewlett-Packard, Palo Alto, CA). MMTSO in centrifuged and filter-sterilized fresh CJ and a model SMCSO-enzyme system were qualitatively analyzed over a 48-hr period. Aliquots of 50 mL of CJ were extracted with 5 mL of dichloromethane, and 2 μL portions of dichloromethane extract were injected for GC/MS analysis. Dichloromethane extracts were stored at −83°C, when held before analysis. The GC column (30m capillary, J & W Scientific, Inc., Folsom, CA) was coated with DB-5 (0.25 μm thickness), and the oven temperature was programmed from 40 to 200°C at 5°C/min with the initial temperature held 1 min. Carrier gas was He (1.5 mL/min). Samples were injected in the splitless mode. The injector port and detector temperatures were both at 150°C. Electron impact ionization, (potential 70 ev) was used and the mass range scanned was 40–200 daltons. The molecular weight was confirmed by positive ion methane chemical ionization. The pseudo molecular ion (M + H, M/Z 100) was the base peak. The scan range used was 80–400 daltons.

MMTSO preparation

MMTSO was synthesized by oxidizing dimethyl disulfide with peracetic acid according to the method of Moore and O'Connor (1966). It was purified by vacuum distillation at 2 mm Hg, and the fraction boiling at 65°C was collected (Chin and Lindsay, 1993a). It was 96% pure as analyzed by GC-MS. Prepared MMTSO was stored at −83°C (Revco Cabinet Freezer, Revco Scientific Inc., Asheville, NC).

Growth inhibition by MMTSO

A stock solution of 1000 ppm MMTSO was made by dissolving it in MRS broth. The solution was filter-sterilized and diluted to appropriate concentrations (0.5, 2, 10, 50, 100, 200, 300 ppm) with filter-sterilized MRS broth before bacterial inoculation and incubation at 30°C. Fresh MMTSO stock solutions were prepared in each inhibition or analytical study. Growth of the bacterium was followed by measuring viable counts on MRS agar plates using the spiral plating system.

Antagonistic effect of SH compounds on antibacterial activity of MMTSO

Stock solutions (500 mM each) of DTT, GSH, cysteine, and thiamine were made in distilled water. A stock solution of GSSG was made at 250 mM. Stock solutions of 20 or 100 μL were separately added into 10 mL of MRS broth containing 1 mM MMTSO, resulting in 1 or 5 mM of compounds, except GSSG which was 0.5 or 2.5 mM. Test solutions in MRS broth were inoculated with *L. mesenteroides* at 4×10^5 CFU/mL and incubated at 30°C. Growth was followed by estimating viable cells.

RESULTS & DISCUSSION

Effect of GHPs on growth

Glucosinolates were found up to 1,000 ppm in cabbage (Van Etten et al., 1976, 1980) and GHPs such as NCS, SCN, and CN were known to have antibacterial activity (Virtanen, 1962; Zsolnai, 1966). Thus, GHPs were tested in heated Cecile cv. CJ (noninhibitory) against *L. mesenteroides*. GHPs were not growth inhibitory to the bacterium at 100 ppm (data not shown), which was higher than concentrations reported in cabbage (Table 1; Van Etten et al., 1976, 1980). Myrosinase, which converts glucosinolates into NCS, SCN, and CN (Cole, 1976; Daxenbichler et al., 1977), did not restore the inhibitory activity in the heated CJ (data not shown), also indicating that GHPs were not responsible for the growth inhibition of CJ against *L. mesenteroides*. The previously reported antimicrobial activity of GHPs has been suggested to be due to their binding to SH groups of proteins essential to cellular metabolism (Zsolnai, 1966). Thus, NCS was considered to be a sulfhydryl inhibitor (Zsolnai, 1966).

Tang (1974) proposed a mechanism to explain the inhibition of papain by benzyl NCS. Papain has one active cysteine moiety (Barron, 1951). NCS and SCN were found to be growth inhibitory primarily against Gram-positive bacteria and fungi, but inactive against Gram-negative bacteria (Zsolnai, 1966).

Effect of SMCSO on growth in fresh CJ

As the amount of added laboratory-synthesized SMCSO was increased (Fig. 2) growth inhibition became more prominent. When 400–500 ppm of SMCSO was added, the viable number of bacteria was reduced after about 4 hr, in contrast to a further increase in growth at lower concentrations.

Both SMCSO and alliin are hydrolyzed by the same enzyme. Hydrolysis of SMCSO generates MMTSO, a compound with the same functional group as allicin (Fig. 1). Thus SMCSO, when hydrolyzed in the same manner as alliin, may also be antimicrobial. An earlier report indicated that synthetic MMTSO was antimicrobial to Gram-positive and Gram-negative bacteria and fungi (Small et al., 1947).

SMCSO content of cabbage

SMCSO contents of cabbage cvs (Table 2) were higher ($P \leq 0.0001$) in juice from heated than from unheated cabbage. Perhaps SMCSO was partially destroyed during the blending and extraction of fresh cabbage tissue with methanol. Possibly a precursor compound(s) of SMCSO in cabbage was thermally decomposed to form SMCSO during the heating of cabbage. Also, heating may have facilitated release of SMCSO from its location in cabbage tissue by thermally disrupting the integrity of the cellular or subcellular structure. Concentration of SMCSO in juice of heated cabbage was not higher because of water loss during heating and cooling, since cabbage did not lose weight during heating and cooling. The highest concentration of SMCSO was in the core ($P \leq 0.0001$), and inner and outer leaves had about the same concentrations. Reported SMCSO contents of cabbage cvs. have been 185 to 2,218 ppm, fresh weight basis (Synge and Wood, 1956; Morris and Thompson, 1956; Bradshaw and Borzucki, 1982; Marks et al., 1992). Mae et al. (1971) reported that Chinese cabbage contained up to 6 micromoles (786 ppm) of SMCSO per g of fresh vegetable, and they presumed that SMCSO was important in sulfur metabolism, acting as a soluble pool for organic sulfur. Growth inhibitory activity of cabbage (Kyung and

Table 2—SMCSO content (ppm) of cabbage juice[a]

	Parts of fresh CJ			Parts of heated CJ		
Cultivar	CF	IL	OL	CR	IL	OL
Brutus	800	700	690	1120	940	920
Galaxy	—	—	—	—	720	—
Bentley	—	—	—	—	700	—
Cecile	—	—	—	—	910	—

[a] CR, core; IL, inner leaves; OL, outer leaves; -, not determined. Data are averages of duplicate analyses. The systematic error for duplicates was 24 ppm.

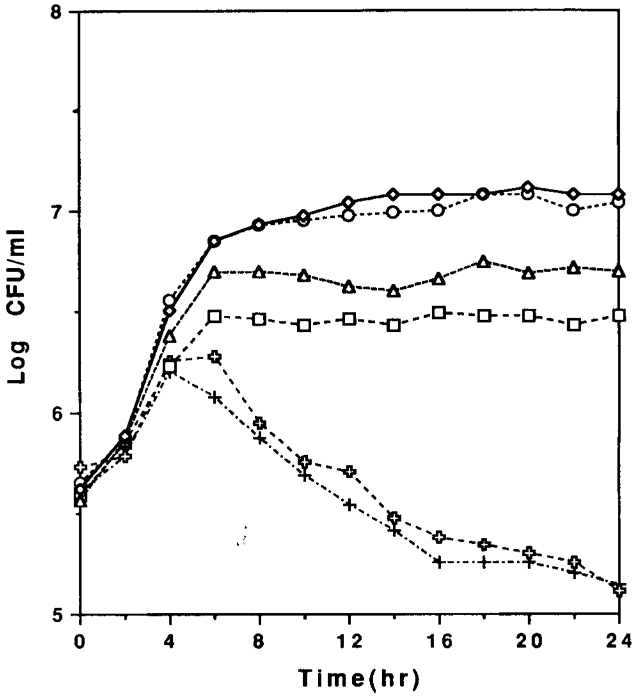

Fig. 2—Effect of SMCSO added into fresh juice of Brutus cv. cabbage on the growth of L. mesenteroides. Concentrations of added SMCSO were: -◇-, 0; -○-, 100; -△-, 200; -□-, 300; -✥-, 400; and -+-, 500 ppm.

Fleming, 1993) appeared to be related to SMCSO content (Brutus > Galaxy > Bentley) (Table 2).

Antibacterial effect of SMCSO in MRS broth

The generation of antibacterial activity from SMCSO in a system other than CJ was studied. SMCSO (700 ppm) was added to MRS broth along with pH 4.0 precipitate from fresh CJ. *L. mesenteroides* grew similarly in MRS broth control, MRS broth with SMCSO only, or MRS broth with pH 4.0 precipitate only (Fig. 3). However, growth inhibition with the same pattern as in fresh CJ occurred when both SMCSO and pH 4.0 precipitate had been added. The pattern of growth inhibition was the same as that of fresh CJ.

This clearly suggested that SMCSO was the inactive precursor of the growth inhibitory compound of cabbage and was activated by a factor in fresh CJ which was shown to be inactivated upon heating (Kyung and Fleming, 1993).

Volatile sulfur compounds in fresh CJ

The presence of MMTSO, as well as other volatile sulfur compounds, DMDS, DMTS, and an unknown were found in fresh CJ (Fig. 4). The unknown compound (no. 4) had a molecular weight of 99 and was tentatively identified as 1-cyano-2,3 epithio propane based on the mass spectrum. Our mass spectrum was very similar to that of Springett and Adams (1988) who found this compound in a model system from Brussel sprouts. Allyl NCS is known to be generated in cabbage homogenate (Chin and Lindsay, 1993b), but was eliminated based on GC and MS data. The mass spectrum of MMTSO (Fig. 5) was identical to that published previously (Marks et al., 1992). MMTSO is a known antibacterial compound (Small et al., 1947) with a minimum inhibitory concentration of 5 ppm for many bacterial species. MMTSO concentration reached maximum at 24 hr and declined thereafter when centrifuged and filter-sterilized fresh CJ was stored at 30°C (Fig. 4). Generation of MMTSO in the enzyme-

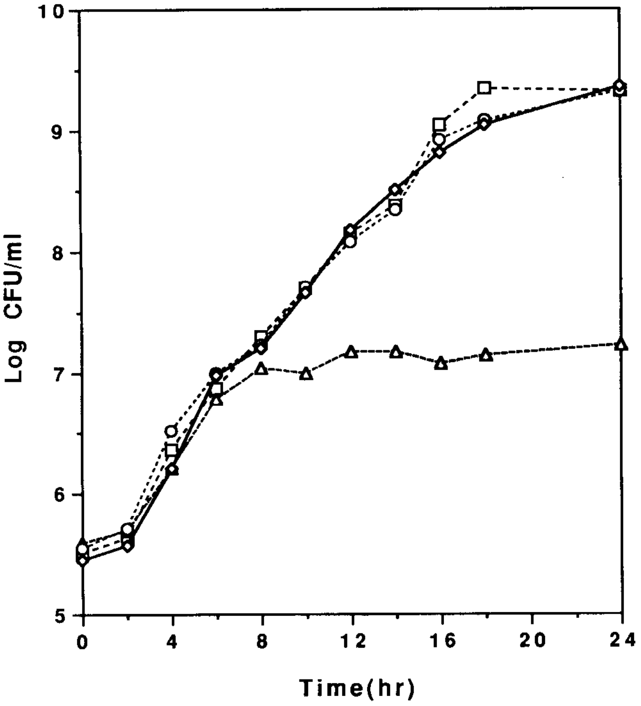

Fig. 3—Effect of SMCSO and pH 4.0 precipitate in MRS broth on the growth of *L. mesenteroides*. Additions to the MRS broth included: -◇-, none (control); -○-, SMCSO only; -□-, precipitate only; and -△-, SMCSO + precipitate.

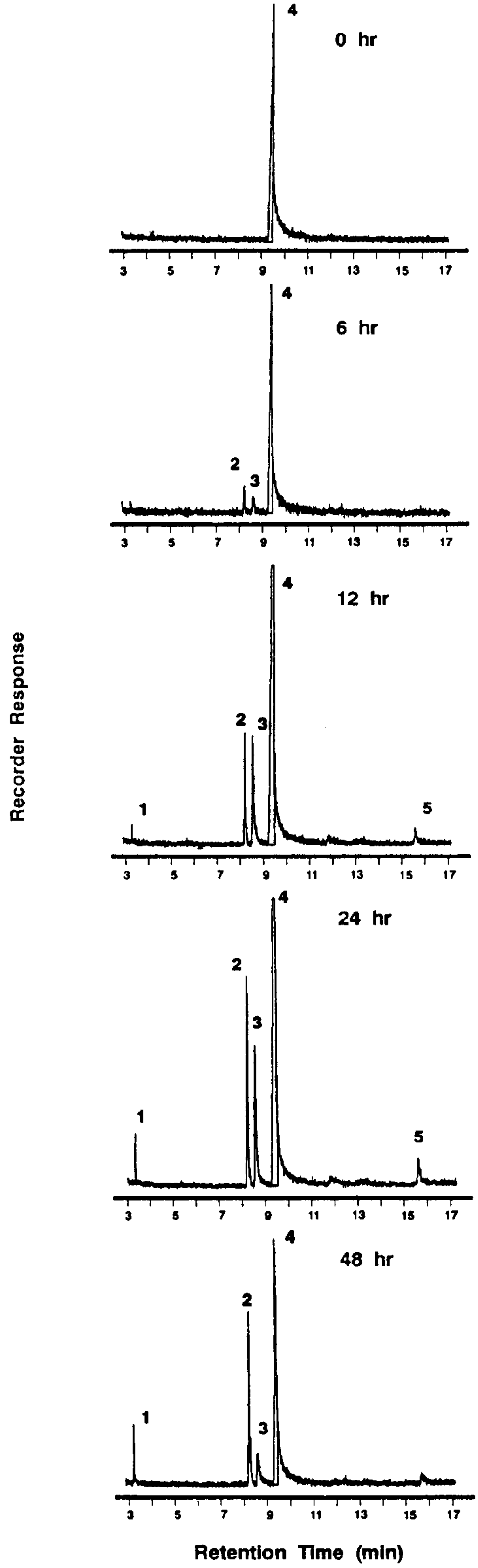

Fig. 4—GC chromatogram of volatiles in centrifuged and filter-sterilized fresh juice of Brutus cv stored at 30°C. 1, DMDS; 2, DMTS; 3, MMTSO; 4, 1-cyano-2,3-epithiopropane; 5, unidentified (tentatively assigned as DMDS tetraoxide).

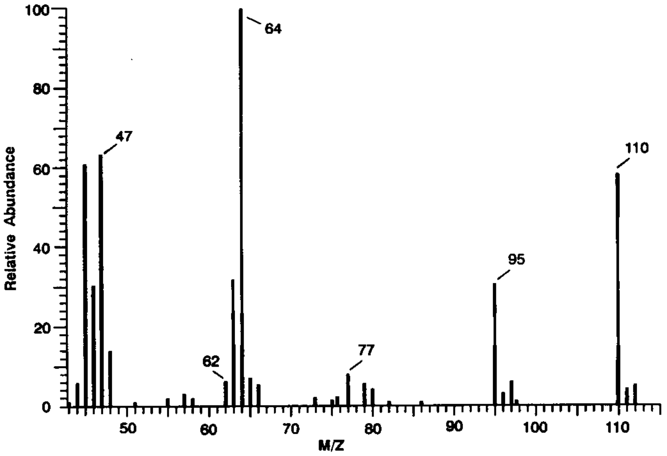

Fig. 5—Mass spectrum of MMTSO (extracted by dichloromethane from centrifuged and filter-sterilized fresh juice of Brutus cv cabbage incubated at 30°C).

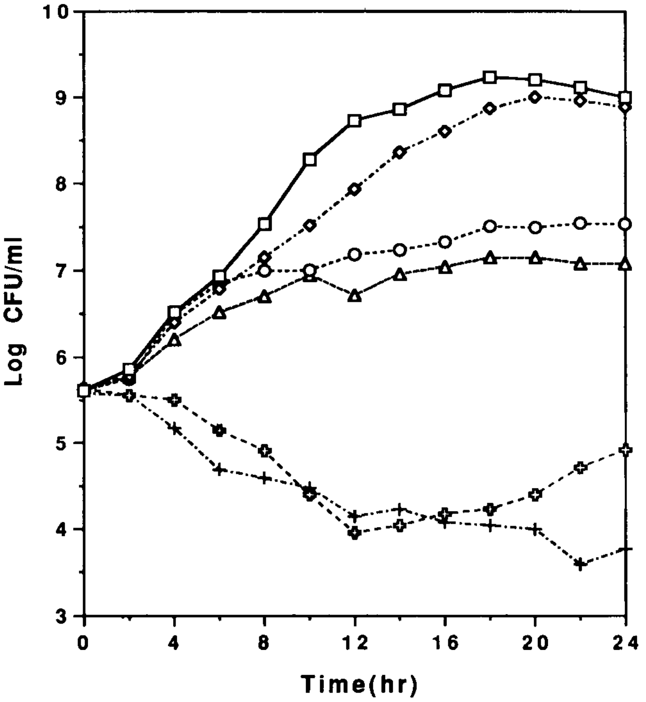

Fig. 6—Effect of MMTSO added into MRS broth on the growth of L. mesenteroides. *Concentrations of added MMTSO were: -□-, 0; -◇-, 2; -○-, 10; -△-, 100; -✙-, 200; and -+-, 300 ppm.*

SMCSO system at pH 6.5 was also observed (data not shown). Marks et al. (1992) previously demonstrated the existence of MMTSO in Brussels sprouts extract and in an enzyme model system at pH 8.4. MMTSO is unstable (Small et al., 1949; Moore and O'Connor, 1966) and spontaneously decomposes under mild conditions (pH 6.3 buffer) by disproportionation to give products such as dimethyl sulfide (DMS), DMDS, and DMTS (Chin and Lindsay, 1993a). DMS, however, was not found in our CJ samples.

The fact that MMTSO was found in fresh CJ and in the SMCSO-pH 4.0 precipitate model system were evidence that the pH 4.0 precipitate from fresh CJ contained cysteine sulfoxide lyase. The enzyme in garlic has an isoelectric point of 4.0 (Stoll and Seebeck, 1951b). The pH 4.0 precipitate of garlic had alliinase activity (Fujiwara et al., 1958). Thus, the activating principle of SMCSO, which was found to be heat-labile, pH-dependent, and precipitate at pH 4.0 (Kyung and Fleming, 1993), appeared to be cysteine sulfoxide lyase.

Antibacterial effect of MMTSO

The growth of *L. mesenteroides* in MRS broth with varying amounts of MMTSO (Fig. 6) showed a pattern of inhibition the same as that of fresh CJ. At 100 ppm, bacterial growth neared its maximum at about the 6th hr of incubation, with a viable cell population of about 10^7 CFU/mL (see control, Fig. 2). At higher concentrations of MMTSO (200 and 300 ppm), the population began to decline from the beginning of incubation. From the experiments with fresh CJ (Fig. 2) and MMTSO in heated CJ (Fig. 6), we deduced that fresh CJ contained 10 to 100 ppm MMTSO. Inhibition of *L. mesenteroides* was characterized by reduction of growth rate and final cell populations.

The antibacterial activity of thiolsulfinates, including allicin and MMTSO, has been explained as a reaction between thiolsulfinates and SH groups of essential cellular proteins (Cavallito et al., 1944; Small et al., 1947, 1949). Small et al. (1947) mentioned that -S (O)-S- was responsible for the antibacterial activity and that it reacted readily with cysteine to yield mixed disulfides. Fujiwara et al. (1958) showed essentially the same reaction between allicin and thiamine. The general reaction, as proposed by Small et al. (1947),

$$R\text{-}\overset{\overset{O}{\uparrow}}{S}\text{-}S\text{-}R' + HS\text{-}R'' \rightarrow R'\text{-}S\text{-}S\text{-}R'' + RSOH$$

can apply to reactions where thiosulfinates are involved, and the reaction is believed to be the common mechanism of antibacterial activity of thiosulfinates and fresh CJ. Species of lactic acid bacteria responded differently toward the antibacterial activity of cabbage (Kyung and Fleming, 1993). Variability in sensitivity of microorganisms to thiosulfinates was shown by Small et al. (1947). They hypothesized that more sensitive organisms may contain more lipoprotein type SH enzymes than do less sensitive ones, or more total cellular lipids, and that essential SH groups in sensitive organisms may be more superficially located in the enzyme. Barron (1951) divided SH groups of proteins into three types, depending upon their reactivity—freely reacting, sluggish, and masked. The nature of SH groups of proteins is probably also important in the antibacterial activity of MMTSO.

Reversal of antibacterial activity of MMTSO by SH compounds

Compounds with free SH groups (e.g. DTT, cysteine, GSH) and other compounds such as GSSG and thiamine were tested in MRS broth containing 1 mM MMTSO for reversal of antibacterial activity (Table 3). Those compounds with free SH groups (5 mM each of DTT, cysteine, and GSH) completely reversed the antibacterial activity of 1 mM MMTSO, while those not having free SH groups (GSSG and thiamine) did not. These findings support the hypothesis that MMTSO imparts its antibacterial action by reacting with SH groups of biologically important proteins (Cavallito et al., 1944; Small et al., 1947, 1949). Thiamine was reported to react with allicin (Fujiwara et al., 1954) to make an allicin-thiamine adduct. However, thiamine did not have any reversal effect on antibacterial activity of MMTSO in MRS broth. Thus, thiamine did not appear to react with MMTSO. Cysteine was reported to react with thiolsulfinates (Cavallito et al., 1944; Small et al., 1947, 1949) and to inactivate antibacterial activity of thiolsulfinates (Ostermayer and Tarbell, 1960) and garlic (Cavallito and Bailey, 1944). It is clear (Table 3) that thiolsulfinates react with biological molecules having free SH groups to influence the growth of bacteria.

Table 3—Reversal effect of SH compounds on the antibacterial activity of MMTSO[a]

	Time (hr)			
Compounds	0	6	12	24
Control	5.60	6.63	6.85	6.95
GSSG 0.5 mM	5.58	6.32	6.88	6.98
0.25 mM	5.58	6.38	6.79	6.92
Thiamine 1 mM	5.65	6.59	6.72	7.15
5 mM	5.65	6.62	6.91	7.40
DTT 1 mM	5.63	6.61	6.88	7.43
5 mM	5.63	6.65	8.51	9.23
Cysteine 1 mM	5.59	6.66	6.85	7.38
5 mM	5.59	6.86	8.91	9.30
GSH 1 mM	5.62	6.62	7.00	7.43
5 mM	5.62	6.94	8.95	9.23

[a] Log CFU/mL after incubation at 30°C for the indicated time. Control, MRS broth with 1 mM MMTSO; GSSG, glutathione, oxidized; DTT, dithiothreitol; GSH, glutathione, reduced.

CONCLUSION

MMTSO generated by cysteine sulfoxide lyase from SMCSO is the principal inhibitory compound against *L. mesenteroides*. Molecules in cabbage such as amino acids, peptides, and biologically nonessential proteins containing SH groups may modify the antibacterial activity of MMTSO. Factors influencing the generation of MMTSO may be important in regulating the fermentation of sliced cabbage to sauerkraut. This is the first report that MMTSO is a major antibacterial compound in cabbage.

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