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Widespread Occurrence of Bacterial Thiol Methyltransferases and the Biogenic Emission of Methylated Sulfur Gases

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A majority of heterotrophic bacteria isolated from soil, water, sediment, vegetation, and marine algae cultures methylated sulfide, producing methanethiol. This was demonstrated (i) with intact cells by measuring the emission of methanethiol with a sulfur-selective chemiluminescence detector, and (ii) in cell extracts by detection of sulfide-dependent thiol methyltransferase activity. Extracts of two *Pseudomonas* isolates were fractionated by gel-filtration and ion-exchange chromatography, and with sulfide as the substrate a single peak of thiol methyltransferase activity was seen in each case. Extracts of several bacterial strains also contained thiol methyltransferase activity with organic thiols as substrates. Thus, *S*-adenosylmethionine-dependent thiol methyltransferase activities are widespread in bacteria and may contribute to biogenic emissions of methylated sulfur gases and to the production of methyl thioethers.

It is well established that microorganisms—especially bacteria in soils, sediments, and aquatic systems—play a significant role in the atmospheric sulfur cycle (1–3, 5, 12, 25). Reduction of sulfate to the level of sulfide and degradation of sulfur amino acids are two major microbial processes that lead to the production of volatile sulfur species, such as hydrogen sulfide, carbonyl sulfide, carbon disulfide, methanethiol, dimethyl sulfide, and dimethyl disulfide. We have been working to understand the biochemical origin of methyl groups in the methylated sulfur species and to characterize the enzymes that are responsible for their production by bacteria.

Microbial production of methanethiol has been described by a variety of investigators, primarily in studies of the degradation of methionine. The microflora of soil, wastewater, sediments, and freshwater ponds produce methanethiol when incubated with methionine (12, 20, 21, 29). Similarly, decaying vegetation and algal mats and spoiled food produce methanethiol from protein-derived methionine (16, 29). Pure cultures of various bacteria, actinomycetes, and fungi carry out this transformation (21). The detection of dimethyl disulfide along with methanethiol in many of these studies is probably due to rapid oxidation of the thiol to the disulfide in aerobic systems. Presumably, methanethiol is produced by the action of the enzyme L-methionine γ-lyase, an enzyme that catalyzes the cleavage of the β-γ C-S bond of methionine (23). The same enzyme, studied in detail from *Pseudomonas* and *Aeromonas* strains, catalyzes the cleavage of *S*-methylcysteine to produce methanethiol (23).

The microflora in the habitats mentioned above are also exposed continuously to another source of sulfur, namely, sulfide, both from the reduction of sulfate by anaerobes and from the release of sulfide from the degradation of sulfur amino acids such as cysteine and homocysteine. We have been investigating the possibility that aerobic bacteria isolated from soils and freshwater sediment can emit methanethiol after exposure to sulfide. The rationale for this idea stem from results of studies by Weisiger et al. (28), who demonstrated that the methyltransferase in mammalian tissues can detoxify hydrogen sulfide, and from our own similar findings in the ciliate *Tetrahymena* (A.-M. Drotar and R. Fall, manuscript in preparation).

Experiments aimed at measuring the methylation of sulfide are difficult to perform because of the analytical difficulties in detecting methanethiol in the presence of an excess of hydrogen sulfide. In gas chromatographic measurements with a sulfur-selective detector, such as the flame photometric detector, tailing from an abundant hydrogen sulfide peak would obscure the detection of methane thiol. With the advent of a new sulfur-selective chemiluminescence detector (18), it has become possible to carry out this analysis routinely, because this gas chromatographic detector responds to organosulfur compounds but not to hydrogen sulfide.

In this report we provide evidence that many aerobic bacteria, isolated from a variety of sources, are capable of the enzymatic methylation of sulfide to produce methanethiol. As a result, it is possible that they contribute to the biogenic emission of methylated sulfur gases from terrestrial and marine environments.

MATERIALS AND METHODS

Chemicals. [*methyl*-^{3}H]S-adenosyl-L-methionine (15 mCi/mmol) was obtained from New England Nuclear Corp. (Boston, Mass.). *S*-Adenosyl-L-methionine and dimethyl sulfide were purchased from Sigma Chemical Co. (St. Louis, Mo.). Ammonium sulfide was obtained through Fisher Scientific Co., (Denver, Colo.), and methanethiol was obtained through Scientific Gas Products (Denver, Colo.). Dimethyl disulfide and 2-mercaptobenzothiazole were obtained from Aldlrich Chemical Co. (Milwaukee, Wis.). All other chemicals were of reagent grade quality.

Bacterial strains. Bacterial isolates were obtained from samples taken from several sites. Sediment or soil samples were collected at the site in sterile plastic bottles and transported on ice to the laboratory. Each sample was serially diluted 10-fold in cold 0.06 M potassium phosphate buffer (pH 7.5). Fractions of each dilution were spread on casein-peptone-starch (CPS) agar (13). The plates were incubated at room temperature for 7 days. Individual colo-

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nies were randomly picked and purified by repetitive transfer. Identification of isolates was accomplished by using routine microbiological and biochemical tests (22).

Some bacteria were isolated from marine algae cultures obtained from Carolina Biologicals (Burlington, N.C.), including cultures of the diatom *Thalassiosira* sp., the dinoflagellate *Pyrocystis* sp., and the golden brown alga *Coccolithophora* sp. Samples from the algae were plated onto marine agar (Difco Laboratories, Detroit, Mich.), and individual bacterial colonies were picked and maintained on marine agar plates. The growth of each isolate was tested for dependence on seawater by transferring cells to broth (Standard Methods; BBL Microbiology Systems, Baltimore, Md.) prepared with either 3% (wt/vol) NaCl or seawater (Carolina Biologicals). Only those isolates that showed a seawater dependence were retained and categorized as marine strains (10).

Analysis of methanethiol production. For the analysis of methanethiol production, bacterial cultures were grown overnight with shaking (150 rpm) to the late log phase at room temperature in yeast extract-peptone-starch (YPS) broth. YPS broth contained Bacto-Peptone (Difco) and yeast extract at 2 g/liter, starch at 0.5 g/liter, and K_2HPO_4 at 0.2 g/liter (pH 7.4). They were harvested by centrifugation and washed in 10 mM potassium phosphate (K buffer; pH 7.4). Washed cells were suspended in K buffer and dispensed in 10-ml fractions into 25-ml Erlenmeyer flasks. The flasks were sealed with rubber serum stoppers after ammonium sulfide was added to a final concentration of 1 mM. Control flasks contained either cells without added ammonium sulfide or boiled cells plus ammonium sulfide. A 1-ml headspace sample was removed between 1 and 2 h with a gas-tight syringe and analyzed by gas chromatography as described below. The final cell density was determined by viable cell counts on replicate CPS spread plates.

Methanethiol was detected by using a packed glass column (1.8 m by 2 mm [inner diameter]) of 3% SP 2100 on 100/120 mesh Supelcoport (Supelco, Bellefonte, Pa.). The gas chromatograph was model 5710A obtained from Hewlett-Packard Co. (Palo Alto, Calif.). A chemiluminescence detector with selectivity for reduced sulfur compounds was used. The detector monitored the chemiluminescence resulting from the reaction of the column effluent with molecular fluorine at reduced pressures. The chemiluminescence was detected with a cooled, red-sensitive photomultiplier tube, and this signal was detected by photon-counting components. The selectivity was produced by using an optical filter that passed wavelengths centered at 706.5 nm with a 10-nm bandwidth. The detector and instrumentation have been described previously (18). A standard curve was generated by using methanethiol-helium gas calibration mixtures. A dimethyl sulfide standard curve was generated similarly from dilute solutions in hexane. Calibration checks were routinely made with dimethyl sulfide, and the methanethiol curve was adjusted accordingly.

Production of hydrogen sulfide from sulfate. Production and emission of hydrogen sulfide was identified by the darkening of a strip of filter paper coated with lead acetate (22). A 10-ml culture of each strain was grown overnight in minimal medium (7) with 0.5% glycerol as a carbon source, or on broth (Standard Methods; BBL) made up in seawater for the isolates from marine algae cultures. The cultures were harvested by centrifugation, washed twice with 20 mM phosphate buffer (pH 7.4), and then suspended in 10 ml of the same buffer. Cell suspensions were pipeted in 5-ml fractions into 25-ml Erlenmeyer flasks. Control flasks were sealed with serum stoppers fitted with plastic cups containing a strip of Whatman no. 1 filter paper (Whatman, Inc., Clifton, N.J.) soaked in saturated lead acetate and dried (22). Experimental flasks were set up identically, except that they contained sodium sulfate added to a final concentration of 25 mM. The filter paper was checked for a positive black precipitate of lead sulfide at 2, 5, and 20 h and scored qualitatively.

Confirmation of methylation of endogenous sulfide. To test the production of methanethiol in an isolate capable of generating hydrogen sulfide from sulfate, a 50-ml culture of the isolate SEIA was grown overnight as described above, harvested by centrifugation, and washed twice with 20 mM phosphate buffer (pH 7.4). The bacteria were suspended in 50 ml of buffer (approximately 10^9 cells per ml), and the suspension was divided into two equal portions and placed into 250-ml polycarbonate bottles. Sodium sulfate at a concentration of 25 mM was added to one bottle. Both bottles were sealed with Teflon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) film secured with a rubber band. The film was punctured so that the carrier gas and sampling Teflon tubing could be inserted. Both samples were analyzed by using a gas chromatograph coupled to a flame photometric detector; this system, which has been described in detail elsewhere (P. Goldan and W. Kuster, manuscript in preparation), was routinely calibrated with the following sulfur gases (with typical retention times): hydrogen sulfide (2.0 min), carbonyl sulfide (3.4 min), methanethiol (6.5 min), dimethyl sulfide (8.9 min), carbon disulfide (9.2 min), and dimethyl disulfide (13.3 min).

Assay of thiol methyltransferase activity in cell extracts. To test for the enzymatic methylation of sulfide, cell extracts of each bacterial isolate were assayed for thiol methyltransferase activity by using a radiometric assay (27). This assay measures the transfer of a ^{3}H-labeled methyl group from [*methyl*-^{3}H]*S*-adenosylmethionine to an acceptor substrate; in this case it was sulfide. Cultures of each bacterial isolate were grown overnight in 35 ml of YPS media, or for the marine algae isolates, in broth (Standard Methods; BBL) made up in seawater. The cells were harvested by centrifugation, washed twice with K buffer, and suspended in 10 ml of K buffer. Each suspension was passed through a cold French pressure cell at 16,000 lb/in^2 and then centrifuged at 4°C at 27,000 × *g* for 20 min; the resulting supernatant was used as the crude extract. For the assay, crude extract or a column fraction was dispensed into 0.5-ml microcentrifuge tubes. Ammonium sulfide was added to a final concentration of 4 mM. The reaction was started by adding 0.1 mM [*methyl*-^{3}H]*S*-adenosylmethionine (5.5 mCi/mmol), and the tubes were capped before they were incubated at 25°C for 20 or 40 min. The final volume was 0.5 ml. The reaction was stopped by pipeting the reaction mixture into screw-cap tubes containing 0.5 ml of 2 N HCl. The acidified mixture was extracted with 6 ml of toluene, and 4.5 ml of the organic layer was counted in a scintillation counter. Background counts from control reactions containing (i) no enzyme and (ii) no thiol substrate were subtracted from this number to obtain the enzymatic rate (9). Each crude extract was assayed in duplicate at two time points and two enzyme levels. The amount of protein in each crude extract was determined by the method described by Bradford (4), with bovine serum albumin used as the standard.

Gel-filtration and ion-exchange chromatography of *Pseudomonas* thiol methyltransferase. A 1-liter culture of either strain PF4 or PF12 was grown overnight in YPS broth. The cells were harvested and washed by centrifugation, and 20

ml of a crude extract was prepared as described above. All subsequent manipulations were carried out at 4°C. A portion of the extract (15 ml) was applied to a column of Sephacryl S-200 (3 by 50 cm; Sigma) which was previously equilibrated in 10 mM Tris–0.5 mM EDTA (pH 7.4; TE buffer). The column was eluted with TE buffer, and fractions were collected and assayed for thiol methyltransferase activity. The central two-thirds of the peak of enzyme was pooled and applied to a column of DEAE-Sephacel (2 by 6 cm; Sigma). The column was washed with 100 ml of TE buffer and then eluted with a linear salt gradient from 0 to 0.5 M NaCl in TE buffer. Fractions were collected and assayed for thiol methyltransferase activity.

RESULTS AND DISCUSSION

We have been considering the possibility that one source of atmospheric methanethiol could be from the microbial methylation of sulfide. Therefore, a variety of bacteria, including laboratory strains and isolates from soil, sediments, agricultural crops, and marine algae cultures were screened for the ability to produce methanethiol when exposed to external sulfide.

Before mass screening of our collection of bacterial strains and isolates was attempted, the optimal pH for whole-cell methylation of sulfide was determined for strains PF4 and SO1 by using K buffer at pH 5 to 9. The optimum pH for the appearance of methanethiol in the headspace in each case was 7.4. The amount of detectable methanethiol at pH 6 and 9 was only 50% of the maximum amount seen at pH 7.4, and it dropped further to 12% at pH 5.0. All other whole-cell incubations were therefore run at pH 7.4.

Cell suspensions of the remainder of our bacterial strains and isolates were screened for the ability to release methanethiol into the headspace after exposure to ammonium sulfide. The rate of appearance of methanethiol in the headspace normalized to cell density is shown in Table 1. It is notable that all cultures produced methanethiol, although the rate varied over 2 orders of magnitude. Methanethiol was not detected in any of the control flasks that either lacked sulfide or contained boiled cells. During these analyses we also looked for the appearance of dimethyl sulfide and dimethyl disulfide in the chromatographic profiles. In general, they were not present as major products.

The cultures most active in methanethiol production were *Rhizobium trifolii* R8021, SK4-1 (a gram-positive rod), and *Flavobacterium* sp. strain SE15C. The lowest activities were seen in the following isolates: *Aeromonas* sp. strain 7966, *Erwinia* spp. strains GrA and P141C, and *Pseudomonas* spp. strains PF5 and PF6. Methanethiol production in the genera *Pseudomonas* ranged from 8.1 (PF5) to 60 (T2304) pmol min^{-1} (ml of headspace)$^{-1}$ (10^{10} cells)$^{-1}$.

To determine if added sulfide was toxic to bacteria under these conditions the viability of some strains was tested. When the four strains SE1A, SO1A, PF4, and PF12 were exposed to 1 mM $(NH_4)_2S$ for 3 h under conditions identical to those of the methylation experiment, the viable cell count was identical to those for cultures not exposed to the ammonium sulfide. Therefore, the added sulfide was nontoxic in these cases, and methanethiol production was not a result of cell death and decomposition.

To verify bacterial methanethiol production from sulfide by a standard sulfur-detection technique, we turned to using gas chromatography system with a flame photometric detector. Because excess hydrogen sulfide interferes with the detection of methanethiol, we searched for a bacterial strain that was capable of generating endogenous sulfide when exposed to external sulfate. All the strains shown in Table 1 were screened for the ability to produce hydrogen sulfide when exposed to excess sulfate. Of the 40 strains, 9 tested positive. Two of the positive strains were isolated from soil (SO1A and SO1B), two from grape leaves (GrA and GrE), one from sediment (SE1A), and three from marine algae cultures (MCO-1, PY-1, and T-1); one was a laboratory strain, YE. None of the pseudomonads or rhizobia strains tested positive.

The sediment isolate SE1A was chosen for further study. Analysis of the flux of sulfur gases from cell suspensions was accomplished by using a gas chromatographic system that was routinely calibrated to detect a variety of biogenic sulfur gases. No hydrogen sulfide or methanethiol was detectable in the gas phase above flasks containing either washed cells with no added sulfate or boiled cells with 25 mM sulfate. Viable cells exposed to sulfate produced methanethiol and hydrogen sulfide in readily detectable amounts, however. These results suggest that sulfide is generated in the cells via sulfate reduction and methylated to yield methanethiol.

The generally accepted mechanism for the bacterial production of methanethiol is through the enzymatic cleavage of methionine (23). Our isolate SE1A formed methanethiol from methionine at a rate of 0.51 nmol min^{-1} (mg of cell protein)$^{-1}$ when measured by the procedure described by Law and Sharpe (16). The methionine concentration in these studies was 12.5 mM. In our sulfide methylation studies, however, to account for the production of methanethiol by this mechanism, the cells would have had to degrade their own free methionine pools. Methionine pools in bacteria are small and range from less than 0.1 mM in stationary-phase cells to 1 mM in fast-dividing, log-phase cells (11). These concentrations are small enough that we would not be able to detect the amount of product formed from them, which was our observation for the control reactions that were run. If the sulfide that we added functioned to induce the breakdown of cellular protein, however, the methionine pool could expand, thereby increasing the rate of methanethiol formation through cleavage of methionine. Resolution of this problem requires analysis of the origin of the sulfur and methyl groups of the emitted methanethiol by using isotopic tracer techniques.

To test for the enzymatic methylation of sulfide in crude extracts of each of our bacterial isolates, a radiometric assay was run. This assay measured ^{3}H-labeled methyl group transfer from [*methyl*-^{3}H]*S*-adenosylmethionine to sulfide. Methylation of sulfide occurred in crude extracts of all species that were examined (Table 1). The specific activity ranged over 2 orders of magnitude, with the highest activity, 0.204 nmol min^{-1} (mg of protein)$^{-1}$, being in strain R128. Seven different marine bacteria also showed significant thiol methyltransferase activity (Table 1).

The specific activities of thiol methyltransferase in cell extracts did not correlate with the amounts of methanethiol detected in the headspace analysis of whole-cell incubations. This lack of correlation could result from the enzymatic reaction not being the limiting step during the whole-cell reaction. For example, the entry of sulfide into the cell could be rate limiting. It is also possible that other metabolic pathways in which sulfide is utilized could be operating to greater or lesser extents in the isolates examined. The products of these pathways would not necessarily have been detected. The specificity of a competing metabolic reaction in the cell could change the amount of sulfide available to the methyltransferase enzyme and, thus, the amount of

TABLE 1. Methylation of sulfide in vivo and in vitro by a variety of heterotrophic bacteria

Isolate and bacterial strain	Identity	Source or reference	Whole-cell methanethiol production (pmol/min per ml of headspace per 10^{10} cells)[a]	Thiol methyltransferase activity (nmol/min per mg of protein)[b]
Soil isolates				
SO1A	*Corynebacterium* sp.	University of Colorado surface soil	35.8, 38.8	0.027
SO1B	Gram-positive diplococcus	University of Colorado surface soil	52.1	0.024
SO4B	Gram-negative rod	University of Colorado surface soil	17.4	0.007
SO6B	Gram-negative coccobacillus	University of Colorado surface soil	18.5	0.041
SO7B	Gram-negative coccobacillus	University of Colorado surface soil	15.9, 26.2	0.078
Sediment isolates				
SE1A	*Corynebacterium* sp.	University of Colorado Varsity Pond, surface sediment	40.0	0.083
SE3A	Gram-positive coccobacillus	University of Colorado Varsity Pond, surface sediment	9.1	0.022
SK4-1	Gram-positive rod	Skeleton Creek sediment, Enid, Okla.	89.0	0.032
SK4-6	Gram-variable coccobacillus	Skeleton Creek sediment, Enid, Okla.	14.4	0.088
SE15C	*Flavobacterium* sp.	University of Colorado Varsity Pond surface sediment	300[c]	0.013
SK7-5	Gram-negative rod	Skeleton Creek sediment, Enid Okla.	23.3	0.033
BL1-6	Gram-negative coccobacillus	Blue Lake no. 2 sediment, Mount Blanca Colo.	10.0	0.079
BL2-7	Gram-variable coccobacillus	Blue Lake no. 2 sediment, Mount Blanca, Colo.	15.0	0.072
CO9	Unidentified	Como Lake sediment, Mount Blanca, Colo.	16.8	0.127
LL1	*Aeromonas hydrophila*	Lake Lavon sediment, Texas	13.1	0.100
Laboratory strains				
7966	*Aeromonas hydrophila*	ATCC 7966	7.1	0.010
GrA	*Erwinia herbicola*	Grape, California (19)	7.7	0.010
GrE	*Erwinia herbicola*	Grape, California (19)	12.4	0.013
P141C	*Erwinia herbicola*	Clover, Georgia (19)	6.7	0.006
PF4	*Pseudomonas fluorescens*	ATCC 17575	24.8, 64.0	0.109
PF5	*Pseudomonas fluorescens*	ATCC 17467	8.1	0.053
PF6	*Pseudomonas fluorescens*	ATCC 17574	8.7	0.184
PF7	*Pseudomonas fluorescens*	ATCC 17573	47.3	0.101
PF12	*Pseudomonas fluorescens*	Fresh water (17)	10.2, 35.2	0.095, 0.163
FB1024	*Pseudomonas fluorescens*	Marine water (10)	13.5, 37.9	0.073, 0.104
T2304	*Pseudomonas syringae*	Oats, Wisconsin; S. Hirano (19)	60.0	0.034
R128	*Rhizobium leguminosarum*	M. McNeil, University of Colorado	13.9	0.204
R127	*Rhizobium phaseoli*	M. McNeil, University of Colorado	17.2	0.033
LPR$^+$	*Rhizobium trifolii*	M. McNeil, University of Colorado	38.1	0.198
LPR$^-$	*Rhizobium trifolii*	M. McNeil, University of Colorado	24.6	0.161
R843	*Rhizobium trifolii*	M. McNeil, University of Colorado	30.4	0.020
R845	*Rhizobium trifolii*	M. McNeil, University of Colorado	16.5	0.020
R8021	*Rhizobium trifolii*	M. McNeil, University of Colorado	99.6	0.020
YE	*Yersinia enterocolitica*	R. Weaver, strain B5725	9.0	R. Weaver, strain B5725
Marine strains				
MCO-1	Gram-negative coccobacillus	*Coccolithophora* sp. culture	ND	0.061
MCO-3	Gram-negative fusiform rod	*Coccolithophora* sp. culture	ND	0.065
PY-1	Gram-negative pleiomorphic rod	*Pyrocystis* sp. culture	ND	0.069
PY-2	Gram-negative fusiform rod	*Pyrocystis* sp. culture	ND	0.031
T-1	Gram-negative pleiomorphic rod	*Thalassiosira* sp. culture	ND	0.062
T-2	Gram-negative thin rod	*Thalassiosira* sp. culture	ND	0.005

[a] Each value represents a separate experiment; ND, not determined.
[b] Each assay represents the average of eight determinations.
[c] This value is artificially high due to poor plating efficiency of this strain.
[d] See the text.

TABLE 2. Thiol methyltransferase activity with various thiols

Bacterial strain	Activity (nmol min^{-1} mg of protein^{-1}) with [a]:				
	$(NH_4)_2S$ (4mM)	PCBT (0.2 mM)[b]	2-NBT (0.2 mM)	2-MBT (0.2 mM)	6-MP (0.2 mM)
Corynebacterium sp. strain SO1A	0.048	0.014	0.128	0.105	0.011
Pseudomonas sp. strain:					
PF4	0.104	0.020	0.119	0.034	0
PF12	0.045	0.013	0.046	0.176	0
FB1024	0.043	0.015	0.087	0.027	0.008
E. coli HB101	0.004	0	0	0.002	0.001

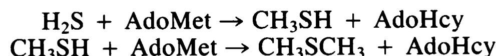
[a] Abbreviations: PCBT, pentachlorobenzenethiol; 2-NBT, 2-nitrobenzenethiol; 2-MBT, 2-mercaptobenzothiazole; 6-MP, 6-mercaptopurine.
[b] Assay included 0.5% Triton X-100 and 1 mM dithiothreitol.

methanethiol produced. As discussed above, another possibility is that sulfide itself is not being methylated but only triggers the breakdown of a metabolic intermediate, such as methionine, to produce methanethiol.

The finding of thiol methyltransferase activity in virtually all bacteria tested is in marked contrast to the results of Larsen (15), who found no detectable activity in the gastrointestinal and mixed populations of bacteria he tested. The two major differences between the results of this study and those of Larsen were the thiol substrate employed and the type of bacteria tested. We used ammonium sulfide while Larsen used 2-mercaptobenzothiazole as the thiol substrate. Our bacteria were aerobes of facultative anaerobes, while Larsen tested only obligate anaerobes. To determine if some of our isolates were capable of methylating organic thiols, crude extracts were assayed for the *S*-adenosylmethionine-dependent methylation of the thiols 2-mercaptobenzothiazole, 6-mercaptopurine, pentachlorobenzenethiol, and 2-nitrobenzenethiol; the last two thiols were previously shown to be substrates for eucaryotic thiol methyltransferases (8, 9). For comparison, each extract was assayed with sulfide as the thiol substrate (Table 2). Crude extracts from four bacterial isolates methylated these organic thiols at rates generally comparable to those observed with sulfide as the substrate (Table 2). Extracts from *Escherichia coli* HB101, however, did not methylate two of the organic thiols (pentachlorobenzenethiol and 2-nitrobenzenethiol) and only weakly methylated sulfide and the other two organic thiols (6-mercaptopurine and 2-mercaptobenzothiazole).

The enzymatic nature of the methylation activity seen in bacterial extracts was verified by fractionating extracts from two different pseudomonads (strains PF4 and PF12) on a gel-filtration column of Sephacryl S-200 (3 by 50 cm) eluted with TE buffer at 4°C. In both cases a single peak of thiol methyltransferase activity with ammonium sulfide as substrate was detected; the results for strain PF4 are shown in Fig. 1. The column was calibrated with molecular weight standards, and the molecular weight of the enzyme was estimated at 87,000 for PF4 and 76,000 for PF12. When the enzyme peak from the column was pooled and chromatographed on an ion-exchange column of DEAE-Sephacel, the enzyme from strain PF4 was retained and could be eluted with a linear salt gradient as a single peak at 0.325 M NaCl. The profile for the enzyme from PF4 is shown in Fig. 1. Similar results were obtained with the enzyme from PF12. For PF4 these two purification steps yielded 26% of the original activity and a 15-fold increase in the specific activity of the enzyme. These results indicate that at least two of our bacterial isolates contain a distinct *S*-adenosylmethionine-dependent thiol methyltransferase. The specificity for the methyl acceptor and the methyl donor of any of these methyltransferases remains to be determined.

The major organosulfur compound released by biological sources is dimethyl sulfide (2, 5). The biochemical pathways for dimethyl sulfide production in terrestrial systems are not well defined. One possibility is the following sequence, first described in a mammalian system (28), where AdoMet is *S*-adenosylmethionine and AdoHey is *S*-adenosylhomocysteine:



$$H_2S + AdoMet \rightarrow CH_3SH + AdoHcy$$
$$CH_3SH + AdoMet \rightarrow CH_3SCH_3 + AdoHcy$$

In this sequence hydrogen sulfide is successively methylated to methanethiol and then dimethyl sulfide. In the bacterial systems described here dimethyl sulfide production was not clearly established. If the second methylation reaction has a K_m of the same order of magnitude as the first, however, the high concentration of sulfide could out compete methanethiol for binding to the active site, and the second step would, therefore, not be seen. It remains to be determined if this methylation mechanism significantly contributes to the production of dimethyl sulfide by bacteria.

In summary, the ability to produce methanethiol from sulfide appears to be characteristic of aerobic bacteria from

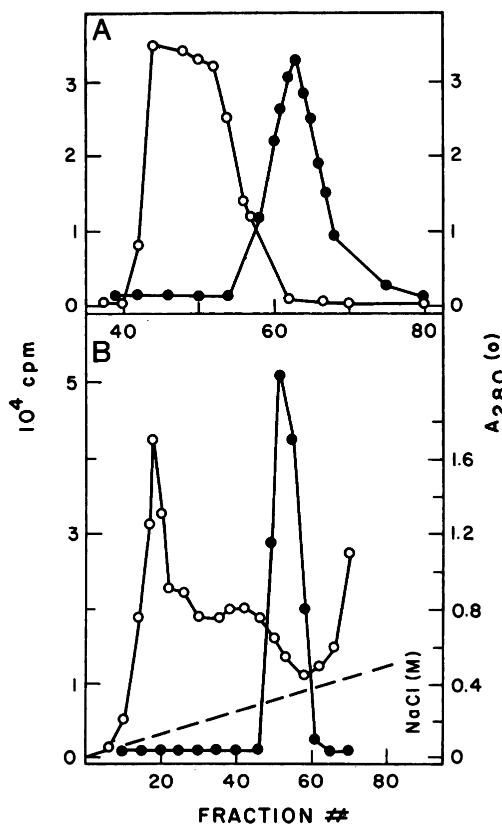


FIG. 1. Gel-filtration and ion-exchange chromatography of thiol methyltransferase activity from *Pseudomonas fluorescens* PF4. (A) A cell extract from a 1-liter culture was applied to a column of Sephacryl S-200. Fractions were collected dropwise and assayed for thiol methyltransferase activity by using the radiolabel assay. (B) The center of the peak of thiol methyltransferase activity was pooled, and the pool was applied to a column of DEAE-Sephacel. Symbols: ○, Total protein; ●, thiol methyltransferase activity.

numerous sources. This biotransformaton appears to be mediated by a thiol methyltransferase. This enzyme system appears to be widely distributed in nature. It has been detected in an alga, *Euglena gracilis* (8); a yeast, *Candida lipolytica* (A. M. Drotar, Ph.D. thesis, University of Colorado, Boulder, 1982); a protozoan, *Tetrahymena thermophila* (9); higher plants (14); and mammals (24, 26, 27). The methylation of hydrogen sulfide by a variety of bacteria and other organisms could be one source of the methylated sulfur gases released into the atmosphere from both terrestrial and marine environments (1, 2, 6).

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LITERATURE CITED

1. **Adams, D. F., S. O. Farwell, E. Robinson, M. R. Pack, and W. L. Bamesberger.** 1981. Biogenic sulfur source strengths. Environ. Sci. Technol. **15**:1493–1498.
2. **Andreae, M. O., W. R. Barnard, and J. M. Ammons.** 1983. Biological production of dimethylsulfide in the ocean and its role in the global atmospheric sulfur budget. Environ. Biogeochem. Ecol. Bull. **35**:167–177.
3. **Bechard, M. J., and W. R. Rayburn.** 1979. Volatile organic sulfides from freshwater algae. J. Phycol. **15**:379–383.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
5. **Bremner, J. M., and C. G. Steele.** 1978. Role of microorganisms in the atmospheric sulfur cycle. Adv. Microbiol. Ecol. **2**: 155–201.
6. **Cantoni, G. L., and D. G. Anderson.** 1956. Enzymatic cleavage of dimethylpropiothetin by *Polysiphonia lanosa*. J. Biol. Chem. **222**:171–177.
7. **Carlton, B. C., and B. J. Brown.** 1981. Gene mutation, p. 222–242. *In* P. Gerhardt (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
8. **Drotar, A. M., and R. R. Fall.** 1985. Methylation of xenobiotic thiols by *Euglena gracilis*: characterization of a cytoplasmic thiol methyltransferase. Plant Cell Physiol. **26**:847–854.
9. **Drotar, A. M., and R. Fall.** 1986. Characterization of a xenobiotic thiol methyltransferase and its role in detoxication in *Tetrahymena thermophila*. Pest. Biochem. Physiol. **25**:396–406.
10. **Fall, R., and R. C. Schnell.** 1985. Association of an ice nucleating pseudomonad with culture of the marine dinoflagellate, *Heterocapsa niei*. J. Mar. Res. **43**:257–265.
11. **Heinrich, W. L., and K. Ring.** 1976. Regulation of amino acid transport in growing bacteria. Arch. Microbiol. **109**:229–235.
12. **Kadota, J., and Y. Ishida.** 1972. Production of volatile sulfur compounds by microorganisms. Annu. Rev. Microbiol. **26**:127–143.
13. **Koch, A. L.** 1981. Growth measurements, p. 179–207. *In* P. Gerhardt (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
14. **Lamoureux, G. L., and D. G. Rusness.** 1980. In vitro metabolism of pentachloronitrobenzene to pentachloromethylthiobenzene by onion: characterization of glutathione S-transferase, cysteine C-S lyase, and S-adenosylmethionine methyl transferase activities. Pest. Biochem. Physiol. **14**:50–61.
15. **Larsen, G. L.** 1985. Distribution of cysteine β-lyase in gastrointestinal bacteria and in the environment. Xenobiotica **15**:199–209.
16. **Law, B. A., and M. E. Sharpe.** 1978. Formation of methanethiol by bacteria isolated from raw milk and cheddar cheese. J. Dairy Res. **45**:267–275.
17. **Maki, L. R., and K. J. Willoughby.** 1978. Bacteria as biogenic sources of freezing nuclei. J. Appl. Meteorol. **17**:1049–1053.
18. **Nelson, J. K., R. H. Getty, and J. W. Birks.** 1983. Fluorine induced chemiluminescence detector for reduced sulfur compounds. Anal. Chem. **55**:1767–1770.
19. **Phelps, P., T. H. Giddings, M. Prochoda, and R. Fall.** 1986. Release of cell free ice nuclei by *Erwinia herbicola*. J. Bacteriol. **167**:496–502.
20. **Pohl, M., E. Bock, M. Rinken, M. Ayclin, and W. A. Konig.** 1984. Volatile sulfur compounds produced by methionine degrading bacteria and the relationship to concrete corrosion. Z. Naturforsch. **390**:240–243.
21. **Segal, W., and R. L. Starkey.** 1969. Microbial decomposition of methionine and identity of the resulting sulfur compounds. J. Bacteriol. **98**:908–913.
22. **Smibert, R.M., and N. R. Krieg.** 1981. General characterization, p. 409–443. *In* P. Gerhardt (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
23. **Soda, K., H. Tanaka, and N. Esaki.** 1983. Methionine γ-lyase. Trends Biochem. Sci. **8**:214–217.
24. **Teotmeier, F., and G. Brunner.** 1983. Solubilization characteristics of pig liver S-methyltransferase. Enzyme **30**:185–195.
25. **Wakeham, S. G., B. L. Howes, and J. W. H. Dacey.** 1984. Dimethyl sulfide in a stratified coastal salt lake. Nature (London) **310**:770–772.
26. **Weinshilboum, R. M., S. Sladek, and S. Klumpp.** 1979. Human erythrocyte thiol methyltransferase: radiochemical micro-assay and biochemical properties. Clin. Chim. Acta **97**:59–71.
27. **Weisiger, R. A., and W. B. Jakoby.** 1979. Thiol S-methyltransferase from rat liver. Arch. Biochem. Biophys. **196**:631–637.
28. **Weisiger, R. A., L. M. Pinkus, and W. B. Jakoby.** 1980. Thiol S-methyltransferase: suggested role in detoxication of intestinal hydrogen sulfide. Biochem. Pharmacol. **29**:2885–2887.
29. **Zinder, S. H., W. N. Doemel, and T. D. Brock.** 1977. Production of volatile sulfure compounds during the decomposition of algal mats. Appl. Environ. Microbiol. **34**:859–860.