APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 12, 449–455 (1998)

Antimony Biomethylation by Mixed Cultures of Micro-organisms under Anaerobic Conditions

R. O. Jenkins,[1]* P. J. Craig,[2] D. P. Miller,[2] L. C. A. M. Stoop,[1] N. Ostah[2] & T.-A. Morris[2]
[1]Department of Biological Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK
[2]Department of Chemistry, De Montfort University, The Gateway, Leicester LE1 9BH, UK

The volatile antimony compound trimethylantimony (TMA) was detected in headspace gases over anaerobic soil enrichment cultures spiked with potassium antimony tartrate. The presence of TMA was variable (12 positives from 104 cultures) and dependent upon both the inoculum source (environmental sample) and enrichment culture conditions. Positives for TMA formation were obtained with variable frequency for four of the six soils tested and for three types of enrichment culture, designed to encourage growth of nitrate-reducing, methane-producing or fermentative bacteria. The identity of the volatile antimony compound produced in each of the three types of enrichment culture was confirmed by gas chromatography–mass spectrometry and gas chromatography–atomic absorption spectroscopy. There was no evidence of any other volatile antimony compound in the headspace gases. These data suggest that the capability to generate TMA is widely distributed in the terrestrial environment and is attributable to different metabolic types of micro-organisms.

Appl. Organometal. Chem. **12**, 449–455 (1998)

Keywords: trimethylantimony; biomethylation; anaerobic bacteria; inorganic antimony

Received 10 November 1997; accepted 19 December 1997

INTRODUCTION

Antimony (Sb) is found naturally in the environment in trace quantities, but widespread industrial utilization has redistributed this element so that it intermingles with biological food chains at high concentrations. Contemporary products that contain antimony include textiles, drugs, plastics, paper, wood, paints and fire-retardant systems.[1] Biovolatilization of antimony would have both environmental and health implications, since this process could move the element from one environmental compartment to another and could give rise to toxic chemical species.[2]

The biomethylation of elements (Ge, As, Se, Sn, Te) that literally surround antimony in the Periodic Table is well established (see, for example, Refs 3 and 4), whereas biomethylation of antimony has been observed in only a single instance.[5] Early studies[6,7] proposed that antimony could be mobilized in similar ways to inorganic arsenic compounds by certain moulds, although the analytical techniques used then (1947) were, inevitably, primitive and no real speciation attempts could be made. This tantalizing work was neglected until the discovery of methylantimony(V) species in natural waters at the nanograms/litre (ng dm^{-3}) level (see, for example, Ref. 1). The only antimony species thought to occur naturally in the environment are $CH_3SbO(OH)_2$ and $(CH_3)_2SbOOH$.[8,9] As methylantimony moieties arise via a hydride generation analytical process, it can be stated that CH_3Sb- and $(CH_3)_2Sb$- moieties exist in the environment. These methylantimony species have been found in various rivers in the USA, some German rivers, the Gulf of Mexico, and the Baltic Sea.[9] Hirner and coworkers[10,11] have shown that antimony compounds are volatilized from municipal waste dumps, but the species were not identified. A later paper from this group[12] used inductively coupled plasma–mass spectrometry (ICP–MS) to demonstrate the production of volatile antimony compounds from sediments. The authors speculated on the identity of the volatile species—based on calculated boiling points by the retention time only—and suggested that methyl- and ethyl-antimony compounds were produced. Compound-specific evidence was not

* Correspondence to: R. O. Jenkins, Department of Biological Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK.
E-mail: roj@dmu.ac.uk

 CCC 0268–2605/98/060449–07 $17.50

available. In 1996, Cullen and co-workers[13] reported the presence of organoantimony compounds in extracts of a freshwater plant; this was the first time that detection of such compounds in samples of biological origin was reported. These reports therefore constitute indirect evidence that biomethylation of antimony does occur, but no controlled laboratory biomethylation experiments had been carried out.

Trimethylantimony (TMA) production by a soil enrichment culture was described recently,[5] and represents the first characterization of a volatile antimony compound thought to arise biogenically from an inorganic antimony substrate. This study, however, was somewhat limited, both with regard to environmental sources of requisite biological activity (three soils tested) and culture incubation conditions (nitrate as electron acceptor for anaerobic respiration) required for antimony biomethylation. Furthermore, the study did not completely eliminate the possibility of transmethylation of antimony by other organometals and organometalloids that may have been present in the soil samples. Although one analytical screening technique (chemiluminescence) used by these authors was not element-specific, a mass spectrum was also obtained. The authors did not comment on the presence or absence of other volatile antimony compounds in headspace gases.

In this paper we present evidence, derived from both element- and compound-specific analysis, for biomethylation of a soluble antimony compound (potassium antimony tartrate) by various mixed cultures of micro-organisms. Since TMA is known to be rapidly oxidized in air to less volatile and non-volatile products,[14] the strategy used was to cultivate facultative and obligate anaerobic micro-organisms and to detect volatile antimony compounds in the anaerobic headspace of mixed-culture vials supplemented with an inorganic source of antimony. We present data for six types of mixed inocula (soils) and for three enrichment culture regimes designed to promote bacterial growth by anaerobic respiration (NO_3^- or CO_2 as terminal electron acceptor) or by fermentation.

MATERIALS AND METHODS

Soil samples used as culture inocula

Six soil samples from different environmental locations were collected into sterile glass bottles and stored in the closed vessels at room temperature. All soils were from UK locations: garden topsoil (5 cm depth) and pond (black sediment at 5 cm depth) from a public park in Leicester; chemically contaminated soils [5 cm (top) and 25 cm (bottom) depth] from a disused tannery works in Bolton; auto-garage soils [5 cm (top) and 25 cm (bottom) depth] from a long-term petrochemical-contaminated site in Lancashire.

Inductively coupled plasma–atomic emission spectrometry (ICP–AES) was used to determine the content of Sb, As, Sn, Pb and Hg in the soil samples. The soils were homogenized separately using a pestle and mortar and aliquots (1 g) were transferred to 5% nitric acid. Soil suspensions were digested by microwave heating (low power, 1.5 min), bath-sonication for 40 min and overnight agitation of the nitric acid solution. Digested samples were filtered (0.22 μm) and eluates were analysed using a Plasma 40 Atomic Emission Spectrometer (Perkin-Elmer, Beaconsfield, UK). Calibration standards (0.1–10 $\mu g\ cm^{-3}$) for each element were prepared from stock solutions of 1 $mg\ cm^{-3}$ (BDH, Poole, UK). Millipore Q water was used throughout.

Soil enrichment culture

Three culture media designed to enrich for methane-producing bacteria, nitrate-reducing bacteria or fermentative bacteria were used. The medium used for enrichment of methane-producing bacteria has been described previously.[15] A cooked meat medium (Oxoid, Basingstoke, UK) was used to enrich fermentative bacteria, particularly clostridia. The enrichment medium for facultatively anaerobic, nitrate-reducing bacteria contained (% w/v): KH_2PO_4, 0.7; K_2HPO_4, 0.3; glycerol, 0.5; $(NH_4)_2SO_4$, 0.1; sodium citrate, 0.05; $MgSO_4.7H_2O$, 0.01; KNO_3, 0.1 (pH 7.4). Aliquots (15 cm^3) of enrichment medium were placed in 20-cm^3 glass vials and autoclaved at 121 °C for 15 min. Ethyl alcohol was filter-sterilized and added to the methanogen medium after autoclaving. Immediately after cooling, sample vials were inoculated with 0.2 g of soil and supplemented with antimony to 10 $\mu g\ cm^{-3}$, added as soluble potassium antimony tartrate (PAT). Culture vials were sealed with PTFE-lined rubber septa and incubated in the dark at 25 °C except those for enrichment of methanogens, which were incubated in darkness at 30 °C. Three types of control vials [(i)–(iii)] were prepared in an identical manner except that (i) PAT was omitted, (ii) culture vials

were autoclaved after inoculation and (iii) a component of the medium was omitted (nitrate for the nitrate-reduction medium; ethanol for methanogen medium). Incubation was for 5–8 weeks.

Culture headspace analysis

Headspace gases from enrichment cultures were analysed routinely by purge-and-trap gas chromatography–atomic absorption spectrometry (GC–AAS).[16] Culture headspace gases were transferred under a flow of helium gas (40 cm^3 min^{-1}, 10 min). Constituent volatile compounds were cryofocused on a chromatographic column (50 cm glass, 4 mm i.d. OV-101 3%) immersed in liquid nitrogen. Following removal of liquid nitrogen, the column was heated electrothermally (125 °C for 5 min) and trapped compounds were eluted according to their boiling points. Detection of eluted compounds was by antimony-specific AAS [Perkin-Elmer PE3100; antimony lamp (S & J, Harlow, UK) at 217.6 nm in a quartz furnace heated to 850 °C]. Identification was by retention time in comparison with methylated antimony standards, produced by reduction of $(CH_3)_3SbCl_2$ (obtained from W. R. Cullen and I. Koch, University of British Columbia, Canada) with sodium borohydride. The standard reaction mix for reduction contained: glacial acetic acid, 3 cm^3; $(CH_3)_3SbCl_2$, 4.9 μg; sodium borohydride, 10 mg; Millipore water, 50 cm^3. Volatile antimony standards were purged from the solution using helium gas (40 cm^3 min^{-1}) onto the cold trap and analysed as for the culture headspace gases. The order of elution and the absolute identification of these compounds were confirmed by gas chromatography–mass spectrometry (GC–MS). Quantification of volatile antimony compounds detected by GC–AAS was achieved by correlating peak area of standards with amount of antimony mobilized from $(CH_3)_3SbCl_2$ [based on measurement of Sb, by ICP-MS, in reaction mixture pre and post reduction of $(CH_3)_3SbCl_2$].

GC-MS was also used to detect and identify unknown volatile compounds in culture headspace gases. Culture headspace gases were transferred under a flow of helium gas (40 cm^3 min^{-1}, 10 min) through Tenax®-TA (60/80 mesh, 6 cm × 5 mm i.d.) traps. Volatile compounds were released from the traps by thermal desorption at 200 °C for 10 min (TD4, Perkin-Elmer), and cryofocused at −40 °C. Subsequent transfer to the GC column was under a flow of helium (3 cm^3 min^{-1}) via a transfer line held at 180 °C. Separation of volatile compounds was achieved using a Carlo Erba 8000 Gas Chromatograph fitted with an OV1 column (30 m × 0.32 mm i.d.). Helium was used as carrier gas (2 cm^3 min^{-1}) and the injector was held at 150 °C for 3 min. The column was then heated from 40 to 100 °C at a rate of 10 °C min^{-1}. Identification of eluted compounds was achieved using a Fisons MD800 Mass Spectrometer (EI, 70eV). Identification was based on (a) eight peak index of mass spectra (Mass Spectra Data Center, Royal Society for Chemistry, UK, 3rd edition) and (b) the NIST (National Institute of Standards and Testing) library database of mass spectra. Identification of biogenic TMA was also based on comparison of retention times with those of volatile antimony standards.

RESULTS

Reduction of $(CH_3)_3SbCl_2$ with sodium borohydride dismutated the starting material into a mixture of SbH_3, CH_3SbH_2, $(CH_3)_2SbH$ and $(CH_3)_3Sb$, which were separated and detected by either antimony-specific GC–AAS (Fig. 1b) or GC–MS. GC–AAS was used to screen enrichment culture combinations (six soil types and three culture media) for the generation of volatile antimony compounds under anaerobic conditions. Trimethylantimony (TMA) was detected in culture headspace gases from each of the three types of enrichment culture media (Table 1). Overall, 12 culture vials were positive for TMA from a total of 104 analysed (all supplemented with PAT). The frequency of TMA detection varied between treatments and was influenced by both the source of culture inoculum (soil) and the type of enrichment culture medium. TMA was not detected in culture vials inoculated with the garden topsoil or auto-garage topsoil, i.e. these soil samples did not work. However, culture vials inoculated with pond sediment showed positives for TMA at a frequency of 50% and 22% for the methanogen and the cooked meat medium respectively. TMA was not detected in any other soil samples for the methanogen medium and, for the cooked meat medium, was found in only one other culture vial (chemical soil, bottom). The remaining positives for TMA were associated with nitrate-reducing medium at a frequency of 57% and 30% for chemical topsoil and garage bottom-soil respectively. The quantity of volatile elemental antimony detected was highly variable even for replicate samples: quantities from positive culture

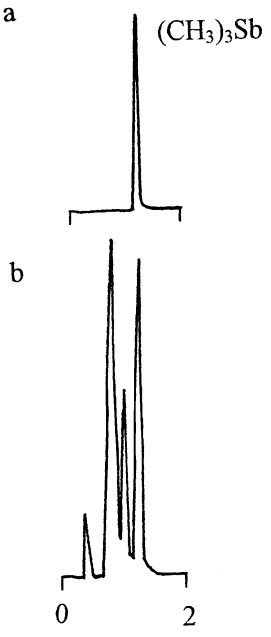

Figure 1 GC–AAS identification of TMA detected in culture headspace gases. Typical chromatograms of (a) volatile antimony compounds from culture headspace and (b) volatile antimony compounds (standards) produced by reduction of $(CH_3)_3SbCl_2$. Retention times (min) were: SbH_3, 0.41; CH_3SbH_2, 0.88; $(CH_3)_2SbH$, 1.14; $(CH_3)_3Sb$, 1.36.

vials were in the range 40–3450 ng, i.e. 0.03–2.30% of elemental antimony substrate. Chemical topsoil/nitrate-reduction medium was the most productive combination (1914 ± 690 ng volatile Sb). TMA was not detected in any control culture vial in which: (i) nitrate was omitted from the nitrate-reducing medium inoculated with chemical topsoil (seven replicates) or auto-garage bottom-soil (seven replicates); (ii) ethanol was omitted from the methanogen medium-inoculated with pond sediment (seven replicates). Similarly, TMA was not detected in any control culture vials (three replicated for each medium/soil combination) that had been autoclaved post-inoculation or had not been supplemented with PAT.

TMA was also detected in culture headspace gases by GC–MS analysis. Figure 2 compares a typical mass spectrum of TMA obtained for headspace gas analysis with the reference mass spectrum for this compound obtained from the NIST library database. TMA was found in headspace gases from each of the three types of enrichment culture media (Table 2) and no other volatile antimony compounds were detected. Volatile sulphur compounds (see also Ref. 5) and end-products of microbial fermentation were also detected in headspace gases (Table 2). Culture vials for the cooked meat medium produced a wide range of volatile sulphur compounds that probably reflected proteolytic decomposition of the meat by clostridia. The presence of ethanol, acetone, propanol, butanol and butanoate in these cultures is also consistent with fermentation by butyric acid bacteria, such as *Clostridium butylicum* and *Clostridium acetobutylicum.* Relatively low levels of some fermentation end-products were also found in cultures grown on the nitrate-reduction medium (Table 2), and are consistent with anaerobic growth in these culture vials. However, the principal mode of anaerobic metabolism in these vials was probably anaerobic respiration, with nitrate serving as terminal electron acceptor. Dimethyl suphide, carbon disulphide and dimethyl disulphide were also detected in headspace gases from the nitrate-reduction medium. Two of these sulphur gases (dimethyl sulphide, dimethyl disulphide), together with dimethyl trisulphide, have been reported previously to be produced by undefined mixed

Table 1 Frequency of detection of TMA in culture headspace gases by GC–AAS[a]

	Enrichment medium		
Source on inoculum	Nitrate-reduction	Methanogen	Fermentation
Garden topsoil	0/9	0/4	0/5
Pond sediment	0/6	2/4	2/9
Chemical soil (top)	4/7	0/4	0/4
Chemical soil (bottom)	0/8	0/4	1/5
Auto-garage soil (top)	0/8	0/4	0/4
Auto-garage soil (bottom)	3/10	0/4	0/4

[a] Values represent (number of culture vials that were positive for TMA)/(total number of vials analysed). Between 40 and 3450 ng of $(CH_3)_3Sb$ were produced in positive culture vials. All media were supplemented with antimony at 10 μg cm^{-3} (added as PAT). All positive results were confirmed by negative controls.

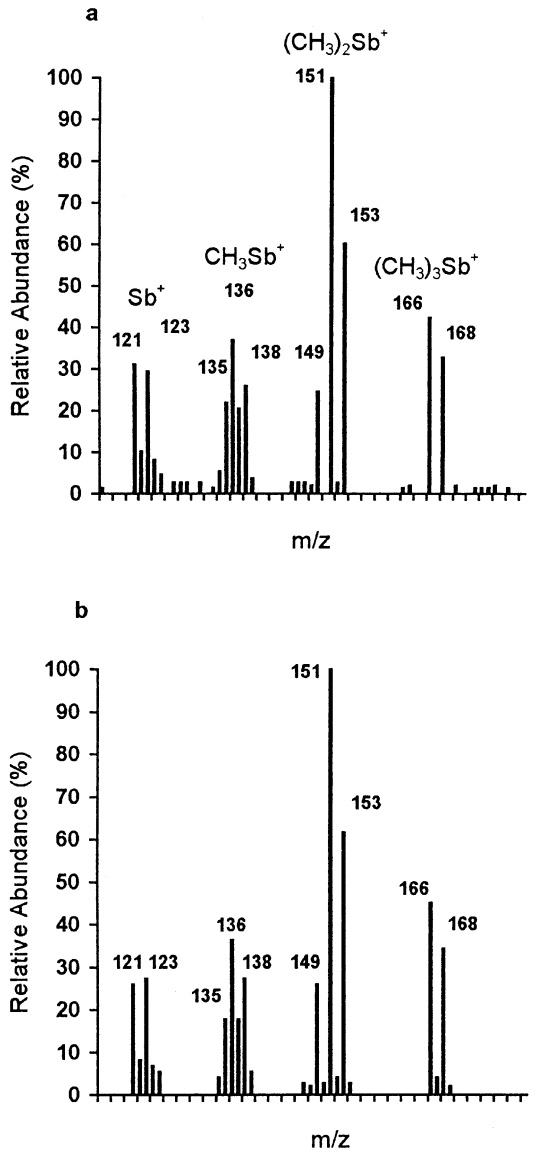

Figure 2 MS identification of TMA. (a) Typical mass spectrum of TMA from Tenax®-TA trap for culture headspace gases from methanogen medium supplemented with 10 μg cm^{-3} antimony (added as potassium antimony tartrate). (b) Mass spectrum (reference) of TMA from NIST library.

cultures growing anaerobically with nitrate as electron acceptor.[5] Volatile fermentation end-products were not detected following growth in the methanogen medium, which is consistent with provision of a non-fermentable carbon source (ethanol) in this medium.

To establish whether pre-enrichment of anti-mony biomethylation capability was likely to have occurred in soils, the levels of Sb and several other elements known to be biologically methylated (As, Sn, Pb, Hg) were determined for all soil samples (Table 3). The levels of Pb and As showed a seven- and five-fold variation between soil types respectively, whereas the levels for each of the other elements varied less than three-fold. Relatively high levels of both Pb and As were present in auto-garage (top and bottom) and garden topsoils. The garden topsoil also contained the highest level of Sb.

DISCUSSION

We have demonstrated formation of a volatile antimony compound, TMA, by soil enrichment cultures. The absence of TMA in control culture vials that lacked a component of the growth medium indicates that microbial growth was required for TMA formation. Generation of TMA by particular soil types under some, but not all, enrichment culture regimes is also consistent with biomethylation of inorganic antimony during culture incubation.

Unequivocal identification of TMA in culture headspace gases from all three enrichment culture regimes was obtained by GC–MS analysis. There was no evidence of volatilization of inorganic antimony by bioreduction (SbH_3 formation) or through formation of methyl hydrides, i.e. $(CH_3)_2SbH$ or $(CH_3)SbH_2$. These results suggest that TMA is the primary or sole form of volatile antimony produced by microbial action in the environment, and are consistent with $CH_3SbO(OH)_2$ and $(CH_3)_2SbOOH$ being the only antimony species thought to occur naturally,[8,9,13] in that they are the likely oxidation products of this unstable species.[14]

Our work provides independent confirmation (using different environmental samples and methods) of the observation of Gürleyük *et al.*[5] that TMA was produced during soil enrichment culture in which nitrate served as terminal electron acceptor for anaerobic respiration (nitrate-reduction medium). Detection of TMA in headspace gases from other types of enrichment culture, in which the principal modes of metabolism were fermentation or anaerobic respiration (CO_2 as electron acceptor), suggests that different metabolic types of bacteria can give rise to TMA in the absence of oxygen. It follows that in nature, distinct

Table 2 GC–MS analysis of culture headspace gases from enrichment cultures generating TMA

	Enrichment medium[a]		
Headspace gas	Nitrate-reduction	Methanogen	Fermentation
Volatile sulphur compounds			
Methyl sulphide	−	−	+
Dimethyl sulphide	+	−	+
Carbon disulphide	+	−	+
Dimethyl disulphide	+	+	+
Methyl ethyl disulphide	−	−	+
2,4-Dithiapentane	−	−	+
Methyl propyl disulphide	−	−	+
Dimethylbutane thioate	−	−	+
Dimethyl trisulphide	−	−	+
Dimethyl tetrasulphide	−	−	+
Volatile antimony compounds			
TMA [$(CH_3)_3Sb$]	+	+	+
Fermentation end-products			
Ethanol	+	+[b]	+
Acetone	+	−	+
Propanol	+	−	+
2-Butanol	−	−	+
Butanoate	−	−	+

[a] All media were supplemented with antimony at 10 μg cm^{-3} (added as PAT). +, Detected; −, not detected, i.e. < 5 ng Sb.
[b] Component of culture medium.

anaerobic environments—such as freshwater sediments, waterlogged soils or animal manure—may harbour the potential for antimony biovolatilization, as they do for phosphorus biovolatilization to phosphane.[17,18] Generation of TMA during enrichment for methane-producing bacteria is consistent with reports[10,11] of volatile antimony compounds in landfill and sewage gases.

Formation of TMA during enrichment culture was not correlated with high levels of Sb in the environmental samples, or with high levels of several other elements known to be biologically methylated (As, Sn, Pb, Hg). Indeed, the garden and garage topsoils, which showed no evidence of TMA formation under any of the enrichment conditions, contained relatively high levels of both As and Pb; the garden soil also contained the highest level of Sb. These results indicate that pre-enrichment of antimony biomethylation capability in soils through the presence of substrates for biomethylation, was probably *not* a prerequisite for TMA formation during anaerobic enrichment culture.

Reaction of TMA with oxygen is known to be rapid, with a rate constant around nine orders of magnitude greater than that for trimethylarsine.[14] This probably accounts for the scarcity of reports on methylantimony in the environment. However, the possibility of TMA formation by an aerobic micro-

Table 3 Elemental analysis of environmental soils used as inocula for enrichment cultures

	Element concentration [μ g (g wet wt soil)$^{-1}$][a]				
Soil type	Sb	As	Sn	Pb	Hg
Garden soil	2.13 ± 0.06	2.90 ± 0.09	2.37 ± 0.11	19.67 ± 0.22	1.03 ± 0.02
Pond sediment	1.70 ± 0.06	2.05 ± 0.05	1.65 ± 0.05	6.00 ± 0.13	1.18 ± 0.04
Chemical soil (top)	1.00 ± 0.03	1.30 ± 0.03	1.25 ± 0.03	2.75 ± 0.06	0.45 ± 0.02
Chemical soil (bottom)	0.85 ± 0.06	1.15 ± 0.06	1.20 ± 0.04	3.05 ± 0.06	0.50 ± 0.03
Garage soil (top)	1.43 ± 0.03	5.03 ± 0.10	1.77 ± 0.06	14.13 ± 0.28	0.70 ± 0.00
Garage soil (bottom)	1.85 ± 0.25	5.95 ± 0.75	2.25 ± 0.04	17.10 ± 0.32	0.90 ± 0.01

[a] ±, Standard deviations based on three replicate soil samples.

organism cannot be dismissed on this basis, the likely products of TMA oxidation, e.g. methylstibonic acids, should be taken into consideration in the experimental approach.[14] Residual oxygen in some of our culture vials, leading to oxidation of biogenic TMA to less volatile species, may account for the variability in detection of TMA in the replicate enrichment cultures. Soil sample inhomogeneity and natural variability associated with growth of mixed cultures may also have contributed directly to this variability, or indirectly by influencing residual oxygen concentrations in culture vials.

Research in the field of environmental antimony has focused on chemical speciation rather than the organisms responsible for biomethylation and, to date, a systematic search for micro-organisms able to volatilize antimony has not been reported. We note that the fungus most commonly suggested as a plausible agent for antimony volatilization,[19] *Scopulariopsis brevicaulis*, is a strong biomethylator of the closely related element arsenic.[3,20] The latter element is also known to be methylated by a wide range of bacteria, including members of the genera *Enterobacter*, *Pseudomonas*, *Methanobacterium* and *Bacillus*.[20] The work reported here suggests that, as for arsenic, different metabolic categories of prokaryotic organisms are able to methylate antimony and that this capability is widely distributed in the terrestrial environment. This is consistent with reports of detection of methylantimony species[8,9,13] or other volatile antimony species[10–12] in several environmental locations. Biological formation of TMA would be expected to influence the environmental chemistry of antimony greatly. It could, for example, enhance mobilization of antimony through volatilization or (in the presence of oxygen) lead to the formation of more water-soluble compounds, e.g. methylstibonic acids.

Acknowledgment We thank Professor W. R. Cullen and Dr I. Koch, University of British Columbia, Canada, for supplying $(CH_3)_3SbCl_2$. We also thank the British Council for several travel grants.

REFERENCES

1. S. Maeda, Safety and environmental effects. In: *The Chemistry of Organic Arsenic, Antimony and Bismuth Compounds*, Patai, S. (ed), Wiley, Chichester, 1994, p. 737.
2. R. Iffand, In: *Handbook on Toxicity of Inorganic Compounds*, Seiler, H. G., Sigel, H. and Sigel, A. (eds), Marcel Dekker, New York, 1988, p. 67.
3. W. R. Cullen, C. L. Froese, L. Lui, B. C. McBride, D. J. Patmore and M. Reimer, *Organometal. Chem.* **139**, 61 (1977).
4. T. Hamasaki, H. Nagase, Y. Yoshioka and T. Sato, *Crit. Rev. Environ. Sci. Technol.* **25**, 45 (1995).
5. H. Gürleyük, V. Van Fleet-Stalder and T. G. Chasteen, *Appl. Organometal. Chem.* **11**, 471 (1997).
6. K. Barnard, PhD Thesis, University of Leeds, UK, 1947, p. 9.
7. F. Challenger, *Chem. Rev.* **36**, 315 (1945).
8. M. O. Andreae, J.-F. Asmodé, P. Foster and L. Van't Dack, *Anal. Chem.* **53**, 1766 (1981).
9. M. O. Andreae and P. N. Froelich, *Tellus* **36B**, 101 (1984).
10. J. Feldmann and A. V. Hirner, *Int. J. Environ. Anal. Chem.* **60**, 339 (1995).
11. A. V. Hirner, J. Feldman, R. Goguel, R. Rapsomanikis, R. Fischer and M. Andreae, *Appl. Organometal. Chem.* **8**, 65 (1994).
12. E. M. Krupp, R. Grümping, U. R. R. Furchtbar and A. V. Hirner, *Fresenius J. Anal. Chem.* **354**, 546 (1996).
13. M. Dodd, S. A. Pergantis, W. R. Cullen, H. Li, G. K. Eigendorf and K. J. Reimer, *Analyst London* **121**, 223 (1996).
14. G. E. Parris and F. E. Brinckman, *Environ. Sci. Technol.* **10**, 1128 (1976).
15. R. Y. Stanier, E. A. Adelberg and J. L. Ingraham, (eds), *General Microbiology*, Macmillan, *London* 1977, p. 45.
16. P. J. Craig, D. Mennie, M. Needham and N. Ostah, *J. Organometal. Chem.* **447**, 5 (1993).
17. I. Devai and R. D. Delaune, *Org. Geochem.* **23**, 277 (1995).
18. G. Gassmann and D. Glindermann, *Angew. Chem., Int. Ed. Engl.* **32**, 761 (1993).
19. B. A. Richardson, *J. Forens. Sci. Soc.* **34**, 199 (1994).
20. W. R. Cullen and K. J. Reimer, *J. Chem. Rev.* **89**, 713 (1989).

