APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2001; **15**: 473–480
DOI: 10.1002/aoc.131

Antimony biomethylation by the wood rotting fungus *Phaeolus schweinitzii*

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The wood rotting fungus, *Phaeolus schweinitzii*, efficiently transforms the antimony(III) compounds potassium antimony tartrate and antimony trioxide to nonvolatile dimethylantimony and trimethylantimony species. The organoantimony species were detected in potato dextrose broth media samples by using hydride generation–gas chromatography–atomic absorption spectroscopy (HG–GC–AAS). The average concentrations of trimethylantimony species after 40 days incubation with potassium antimony tartrate were approximately 35 μg, 155 μg and 520 μg Sb/l, for substrate concentrations of 10 mg, 100 mg and 1000 mg Sb/l respectively. Thus, the maximum yield of trimethylantimony species was approximately 0.4%. When antimony trioxide (saturated solution, 4 mg Sb/l) was used as a substrate, the average concentration of trimethylantimony species was 150 μg Sb/l after 40 days. The HG–GC–AAS response for the dimethylantimony species was less than that for the trimethylantimony species; however, quantification was not possible because of the lack of an appropriate standard. In comparison, cultures of *P. schweinitzii* incubated with 1 mg As/l as sodium arsenite contained approximately 200 μg As/l as trimethylarsenic species, i.e. 20% yield. Biomethylation of antimony(V) was inefficient: cultures contained only 3 μg Sb/l as trimethylantimony species after incubation with 100 mg Sb/l as potassium hexahydroxyantimonate. No organoantimony species were detected in control cultures that contained only medium and inorganic antimony compounds. The identities of the organoantimony species were confirmed by using GC–Mass Spectrometry.

Keywords: *Phaeolus schweinitzii*; trimethylantimony; dimethylantimony; sudden infant death syndrome (SIDS); hydride generation–gas chromatography–atomic absorption spectroscopy

Received 9 April 2000; accepted 16 October 2000

INTRODUCTION

Recently, the biomethylation of antimony has attracted significant attention, mainly because of a hypothesis linking microbiological antimony volatilization to sudden infant death syndrome (SIDS).[1] A significant flaw in this hypothesis is that only a few microorganisms are known that can volatilize antimony, e.g. as trimethylstibine,[2–7] and most of these microorganisms are unidentified anaerobes.[4–6] Furthermore, only trace quantities of trimethylstibine are produced by these microorganisms.

The ability of the aerobic filamentous fungus *Scopulariopsis brevicaulis* to biomethylate antimony has received particular attention[2,3,6–12] because this fungus is historically well known for its ability to biomethylate arsenic,[13] the element above antimony in Group 15 of the periodic table. It was shown that *S. brevicaulis* can biomethylate antimony to produce nonvolatile dimethylantimony and trimethylantimony species,[10] and volatile trimethylstibine.[2,3] However, the yields of these

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antimony species are always significantly lower than the yields obtained for arsenic,[13–15] and the amounts of trimethylstibine produced are considered to be insignificant and unlikely to be a cause of SIDS.[16] However, *S. brevicaulis* has proven to be a useful model to study antimony biomethylation.[10]

Antimony biomethylation by *S. brevicaulis* is probably of little environmental significance. However, biomethylated antimony has been found in a variety of environmental compartments. For instance, trimethylstibine was detected in and above algae mats at a hot-spring,[17] and nonvolatile methylantimony species have been detected in various plants[18–20] and animals.[20] Fermentation gases, from landfills and sewage treatment plants,[21] contain trimethylstibine, which is very likely to have a biological origin. The environmental significance of antimony biomethylation is not clear, but biomethylation can be expected to alter the toxicity and mobility of antimony.

A variety of microorganisms have been identified that perform arsenic biomethylation.[14] Studies on *S. brevicaulis* indicate that the mechanism of antimony biomethylation is probably the same as that of arsenic,[10] so it is likely that microorganisms that biomethylate arsenic would also biomethylate antimony. With this in mind, we have been further examining some fungi that are known to biomethylate arsenic to see if these fungi can biomethylate antimony.

The filamentous fungus *Phaeolus schweinitzii* is well known by foresters as the cause of brown cubical butt rot, particularly in conifers, especially pine and Douglas fir. *P. schweinitzii* is an efficient biomethylator of arsenic, producing trimethylarsine. Pearce *et al.*[11] used this fungus in a study of arsenic and antimony volatilization in relation to SIDS. Arsenic trioxide was converted to trimethylarsine, and, in their study, Pearce *et al.*[11] found evidence of possible antimony volatilization by *P. schweinitzii*, but justifiably concluded that antimony is not volatilized by *P. schweinitzii*, because experimental repeatability was poor, the amount of antimony volatilized was just above their detection limit, and their method was not very specific.

Our previous studies of *S. brevicaulis* demonstrated that the most significant products of antimony biomethylation are nonvolatile dimethylantimony and trimethylantimony species found in the medium.[2,8–10] Volatile trimethylstibine is only a minor product.

In this paper, we describe the use of hydride generation–gas chromatography–atomic absorption spectroscopy (HG–GC–AAS) to determine nonvolatile dimethylantimony and trimethylantimony species produced by cultures of *P. schweinitzii* that were incubated with various inorganic antimony compounds. Also, for comparison, the amount of nonvolatile methylarsenic species produced by *P. schweinitzii* was determined. We conclude that the production of dimethylantimony and trimethylantimony species by *P. schweinitzii* may be a significant process in forests growing on antimony-contaminated soils.

MATERIALS AND METHODS

Materials

Potato dextrose broth (PDB) was obtained as a powdered solid from Difco Laboratories (Detroit, MI) and reconstituted according to the manufacturer's instructions. Minimal-salts/glucose medium[22] was prepared from reagent-grade chemicals. Media were sterilized at 121 °C, 19 psi (131 kPa) for 20 min. The antimony and arsenic substrates, potassium hexahydroxyantimonate (PHHA), antimony trioxide and sodium arsenite, were obtained from Aldrich. Potassium antimony tartrate (PAT) was obtained from Fisher Scientific.

Sodium borohydride (2%), citrate buffer (50 mM, pH 6), acetic acid (4 M) and ammonium carbonate buffer (50 mM, pH 12) were prepared from reagent-grade chemicals, as described in previous publications.[8,15] The standards, trimethylantimony dichloride and trimethylarsine oxide had been previously synthesized in-house.[23,24] An appropriate amount of solid was dissolved in distilled water to produce a 1000 ppm solution that was diluted to the working concentration of 1 ppm.

Cultures

P. schweinitzii was obtained from the American type culture collection (ATCC #10013). Submerged cultures were maintained in 500 ml or 1 l Erlenmeyer flasks that contained PDB (200 ml) or minimal-salts/glucose medium (400 ml). Antimony and arsenic compounds were added to cultures as sterile (0.2 μm syringe filtered) solutions, except for antimony trioxide, which was added directly in the solid form. The contents of the cultures are listed in Table 1.

The cultures were incubated on a rotary shaker ($\sim$135 rpm, 4.45 cm displacement) at 26 °C for the amount of time specified in Table 1. For the time-

Table 1 Experimental conditions and amounts of biomethylation products produced by cultures of *P. schweinitzii*

	Description of culture	Incubation time (days)	Concentration of methylmetalloid[e], species after incubation (μg E[e]/l)			Yield[f] (%)
			Dimethyl-metalloid	Trimethyl-metalloid	Total	
1a	10 mg Sb/l as PAT[a], 200 ml PDB[b], *P. schweinitzii*	40	30	30	60	0.6
b	10 mg Sb/l as PAT, 200 ml PDB, *P. schweinitzii*	40	30	40	70	0.7
c	100 mg Sb/l as PAT, 200 ml PDB, *P. schweinitzii*	40	80	160	240	0.2
d	100 mg Sb/l as PAT, 200 ml PDB, *P. schweinitzii*	40	90	150	240	0.2
e	1000 mg Sb/l as PAT, 200 ml PDB, *P. schweinitzii*	40	100	460	560	0.06
f	1000 mg Sb/l as PAT, 200 ml PDB, *P. schweinitzii*	40	90	580	570	0.06
g	0.2 g Sb_2O_3, 200 ml PDB, *P. schweinitzii*	40	60	200	260	6
h	0.2 g Sb_2O_3, 200 ml PDB, *P. schweinitzii*	40	70	100	170	4
i	1000 mg Sb/l as PAT, 100 ml PDB, control	40	<1	<1	<1	<0.0001
j	0.2 g Sb_2O_3, 100 ml PDB, control	40	<1	<1	<1	<0.03
k	0.2 g Sb_2O_3, 400 ml PDB, *P. schweinitzii*, time course	70	70	250	320	8
l	1000 mg Sb/l PAT, 400 ml PDB, *P. schweinitzii* time course	70	180	410	590	0.06
m	1 mg As/l as sodium arsenite, 200 ml PDB, *P. schweinitzii*	40	30	120	150	15
n	1 mg As/l as sodium arsenite, 200 ml PDB, *P. schweinitzii*	40	30	270	300	30
2a	100 mg Sb/l as PAT, 400 ml ms/g[c], *P. schweinitzii*	50	<1	30	30	0.03
b	100 mg Sb/l as PAT, 400 ml ms/g, *P. schweinitzii*	50	<1	40	40	0.04
c	1 mg As/l as sodium arsenite, 400 ml ms/g, *P. schweinitzii*	50	<1	60	60	6
d	1 mg As/l as sodium arsenite, 400 ml ms/g, *P. schweinitzii*	50	<1	20	20	2
3a	100 mg Sb/l as PHHA[d], 200 ml PDB, *P. schweinitzii*	40	10	4	14	0.01
b	100 mg Sb/l as PHHA, 200 ml PDB, *P. schweinitzii*	40	5	2	7	0.01
c	100 mg Sb/l as PHHA, 200 ml PDB, control	40	<1	<1	<1	<0.001
d	100 mg Sb/l as PHHA, 200 ml PDB, control	40	<1	<1	<1	<0.001

[a] PAT: potassium antimony tartrate.
[b] PDB: potato dextrose broth.
[c] ms/g: minimal-salts/glucose medium.
[d] PHHA: potassium hexahydroxyantimonate.
[e] Metalloid refers to arsenic or antimony; thus, E refers to As or Sb.
[f] Yield is defined as the total amount of organometalloid species relative to the total amount of metalloid species *in solution*.

course experiments, cultures were sampled aseptically on days 0, 6, 11, 19, 25, 35, 57 and 70 in a biological safety cabinet. The volume of each of the first four samples was 20 ml; the rest of the samples were 5 ml aliquots; samples were stored at −20 °C. It was not necessary to sterilize the media samples before handling and analysis, because *P. schweinitzii* is not a biohazard.

At the end of the experiments, the dry weights of the cultures were estimated by filtering off the biomass and drying the biomass at 150 °C to constant weight.

Analysis

Media samples, from cultures containing antimony compounds, were passed through solid phase extraction (SPE) columns, to remove inorganic antimony(III) compounds, as previously described.[8] Organoantimony species in the SPE eluate were determined by using semi-continuous flow HG–GC–AAS with 2% sodium borohydride and 50 mM citrate, pH 6.[8] Media samples, from cultures containing sodium arsenite (which were examined for comparison) were analyzed directly (SPE cleanup was not necessary because high concentrations of organoarsenic species were present) by using semi-continuous flow HG–GC–AAS with 2% sodium borohydride and 4 M acetic acid.[15] Trimethylantimony dichloride and trimethylarsine oxide were used to perform standard additions for quantification. The organometalloid species were identified on the basis of their retention times.

The identity of the organoantimony species was confirmed by using HG–GC–mass spectrometry:[9] media (5 ml) from cultures of *P. schweinitzii* was passed through an SPE cartridge and the eluate was collected in a 15 ml vial. The eluate in the vial was diluted with 5 ml of water and the vial was capped with a Teflon septum. Sodium borohydride (1 mL 2% $NaBH_4$) was injected into the vial. The vial was shaken vigorously, and then 1 ml of headspace gas was removed using a gas-tight syringe and the headspace gas was injected into the GC–MS apparatus.

RESULTS

There was good growth of *P. schweinitzii* in PDB, even when the antimony concentration was 1000 mg Sb/l. The final average dry weight of cultures **1a –1j, 1m** and **1n** was 0.54 g (range: 0.47–0.59 g) after 6 weeks of incubation in 200 ml of PDB. There was no correlation between antimony concentration and culture dry-weight, indicating that the antimony compounds used were not toxic to this fungus at the concentrations used. Growth was significantly less when *P. schweinitzii* was incubated in minimal-salts/glucose medium: the final dry-weights of cultures **2a** and **2b** were 0.06 and 0.12 g, respectively, after 6 weeks of incubation in 400 ml of medium.

Nonvolatile dimethylantimony and trimethylantimony species were detected in media samples when *P. schweinitzii* was incubated with the antimony(III) compounds, potassium antimony tartrate or antimony trioxide, for 40 days (Table 1). The concentrations of the dimethylantimony and trimethylantimony species were estimated by using HG–GC–AAS. The standard trimethylantimony dichloride was used to perform standard additions. The concentration of trimethylantimony species is only an estimate because of the assumption that the efficiency of hydride derivatization for the known trimethylantimony dichloride and the unknown trimethylantimony species determined are the same. The concentration of dimethylantimony species was estimated by assuming that the responses for dimethylantimony and trimethylantimony species are the same.

In Table 1, we also report the yield (%) of methylantimony species relative to the total amount of antimony in solution. However, these yields should be interpreted with care, and used only for approximate comparisons, because the most relevant, and useful, measure of yield is that based on the amount of *bioavailable* antimony in solution. Unfortunately, we do not know the bioavailability of antimony in our cultures, and this will also depend on the antimony compound used as a substrate (e.g. antimony trioxide compared with potassium antimony tartrate). Indeed, bioavailability may be the main reason why arsenic is so much more readily biomethylated than antimony. In Table 1, we report that the yield of methylantimony compounds, when antimony trioxide is a substrate, is approximately 10–50 times more than that obtained when potassium antimony tartrate is a substrate. This is most likely because, although for potassium antimony tartrate the antimony is completely solubilized, the antimony is complexed with tartaric acid and so is unlikely to be bioavailable.

The identities of the trimethylantimony and dimethylantimony species were confirmed by using GC–MS (ion trap). Trimethylantimony and di-

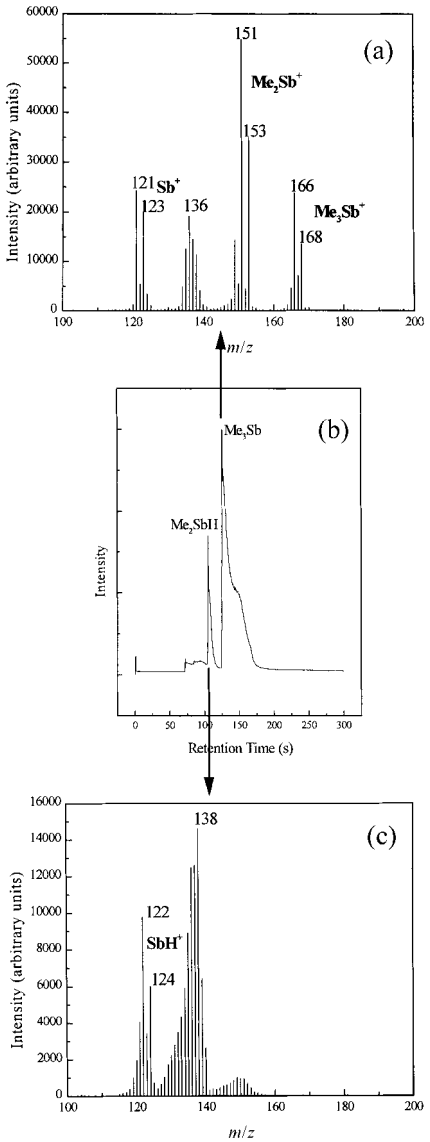

Figure 1 GC–MS TIC (b) of headspace gases from a media sample (**11**) that was derivatized using sodium borohydride. Mass spectra are plotted for the two peaks that correspond to trimethylstibine (a) and dimethylstibine (c).

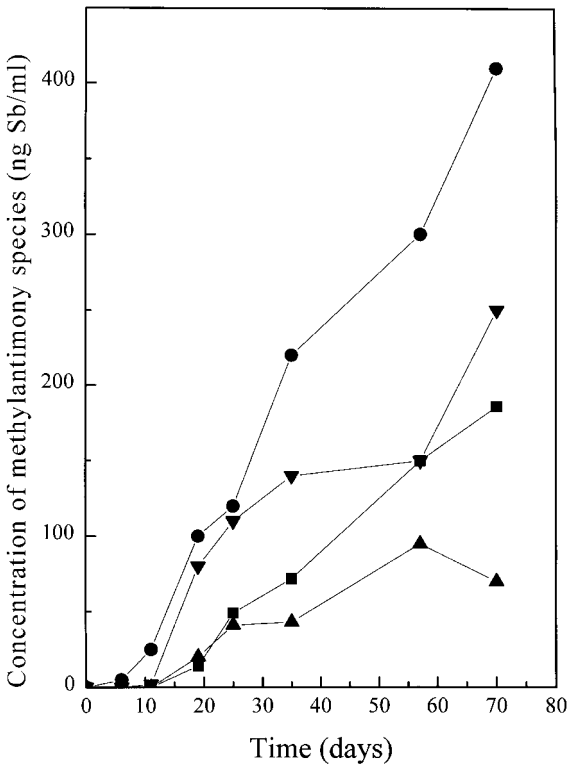

Figure 2 Time course for the production of trimethylantimony (▼) and dimethyantimony (▲) species in a culture that contained antimony trioxide, and time course for the production of trimethylantimony (●) and dimethyantimony (■) species in a culture that contained potassium antimony tartrate.

methylantimony species, in a sample of medium from culture **11**, were derivatized to the corresponding stibines in a septa-capped vial and the headspace gases were injected into the GC–MS apparatus. Two major peaks were evident in the total ion chromatogram (TIC; Fig. 1b). The mass spectrum of the second peak (Fig. 1a) is qualitatively equivalent to the mass spectrum, reported by NIST, for trimethylstibine. The mass spectrum of the first peak (Fig. 1c) in the TIC is less clear, but is consistent with the compound being dimethylstibine.

It is clear that the production of these antimony compounds is biogenic because dimethylantimony and trimethylantimony species were not detected in controls that contained inorganic antimony(III) compounds and PDB. Furthermore, in two cultures, samples were taken periodically to construct a time-course for antimony biomethylation. Antimony biomethylation proceeded slowly over the first 10 days, then proceeded much more rapidly over the next 20 days and then slowed (Fig. 2). Increases in concentration after 50 days are partially due to evaporation (which becomes more significant in later stages of the experiment because the volume of medium is less than at the start due to sampling).

When potassium antimony tartrate was used as a

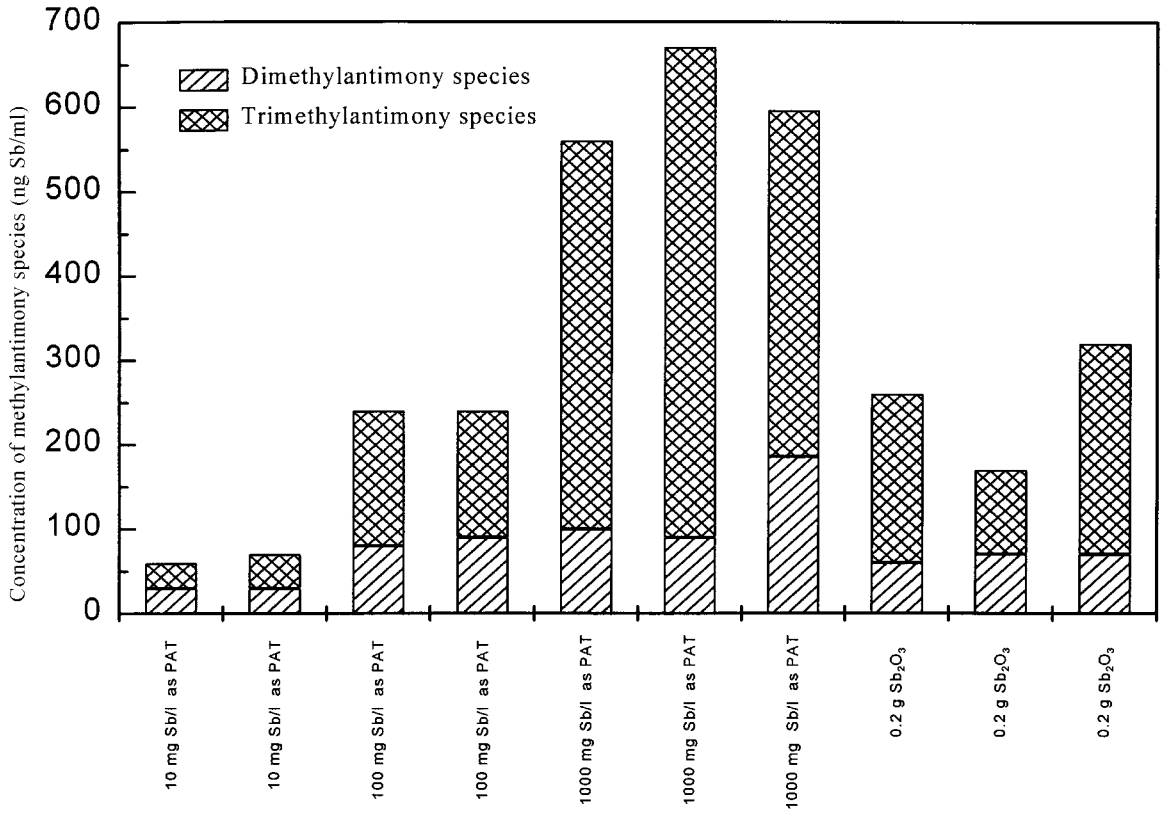

Figure 3 Concentrations of dimethylantimony and trimethylantimony species in *P. schweinitzii* cultures.

substrate for *P. schweinitzii* the quantity of dimethylantimony and trimethylantimony species produced increased with increasing concentration of substrate antimony (Fig. 3).

For comparison, media samples, from cultures containing sodium arsenite (1 mg As/l) were analyzed after 40 days and were found to contain 195 μg As/l as trimethylarsenic species.

When the antimony(V) compound, potassium hexahydroxyantimonate was used as a substrate for *P. schweinitzii*, only trace amounts of dimethylantimony and trimethylantimony species were produced. No methylantimony species were detected in the corresponding controls. In this experiment, the media samples were also examined by using HG–GC–AAS to see if there was any reduction of antimony(V) to antimony(III). The concentration of antimony(III) in active cultures was not significantly greater than the concentration in the controls. If antimony biomethylation proceeds via the same mechanism as for arsenic, then reduction of antimony(V) to antimony(III) must occur before biomethylation can occur; however, this could occur entirely within the cell, and so would not be detected in these experiments, where only the medium was examined.

DISCUSSION

The wood decay fungus *P. schweinitzii* was found to be very effective at producing nonvolatile dimethylantimony and trimethylantimony species from inorganic antimony(III) compounds. This is only the second report of a fungus capable of antimony biomethylation, the first being *S. brevicaulis*.[2,3,7–10,12,25] However, *P. schweinitzii* is one to two orders of magnitude more efficient at antimony biomethylation than *S. brevicaulis*. For example, when *P. schweinitzii* was incubated with 100 mg Sb/l as PAT in minimalsalts/glucose medium, the concentration of trimethylantimony species after 50 days incubation was 35 μg Sb/l,

even though growth was poor, whereas 3 μg Sb/l as trimethylantimony species is typically found in *S. brevicaulis* cultures,[8] which grow extremely well, in minimal-salts/glucose medium. Under the above conditions *P. schweinitzii* and *S. brevicaulis* both produce ~40 μg As/ml as trimethylarsenic species.

Some other significant differences were found in the behavior of *S. brevicaulis* and *P. schweinitzii* towards antimony. For example, in cultures of *S. brevicaulis* there was little relation between the amount of potassium antimony tartrate used as a substrate and the amount of methylantimony species produced, whereas for *P. schweinitzii* a trend was much more evident (Fig. 3). Furthermore, *S. brevicaulis* typically produced amounts of dimethylantimony species greater than, or equal to, the amounts of trimethylantimony species, but *P. schweinitzii* usually produced significantly more trimethylantimony species than dimethylantimony species. This was particularly noticeable when the amount of organoantimony species produced increased (Fig. 3). For both microorganisms, it is likely that when the dimethylantimony species reaches a threshold concentration the dimethylantimony species is further methylated to the trimethylantimony species, and this threshold concentration is more rapidly reached by *P. schweinitzii*; such a process would be consistent with antimony biomethylation proceeding via the same mechanism as arsenic biomethylation.

The most significant similarities between *P. schweinitzii* and *S. brevicaulis* are: (1) neither *P. schweinitzii* nor *S. brevicaulis* significantly reduce antimony(V) to antimony(III); (2) neither *P. schweinitzii* nor *S. brevicaulis* efficiently biomethylate antimony(V); (3) neither *P. schweinitzii* nor *S. brevicaulis* produce significant quantities of monomethylantimony species. These facts are consistent with the mechanism of antimony biomethylation for *P. schweinitzii* and *S. brevicaulis* being the same as the mechanism of arsenic biomethylation, a possibility that is further reinforced by other studies of antimony biomethylation that we have performed.[8–10] The differences in the efficiency of antimony biomethylation may be due either to differences in rates of antimony transport into the cell, or to differences within the cell.

In these experiments, we examined only nonvolatile products of antimony biomethylation. It is likely that *P. schweinitzii* also produces significant amounts of volatile trimethylstibine from inorganic antimony(III) and, indeed, Pearce *et al.*[11] found some evidence to support this contention. Based on results for *S. brevicaulis* (which typically volatilizes ~5% of the organoantimony species produced), it is likely that a 400 ml culture of *P. schweinitzii* will volatilize a detectable amount (~50 ng Sb) of trimethylstibine per day.

P. schweinitzii is found in large quantities in forests, in association with wood rot, making its ability to perform antimony biomethylation particularly significant. The interaction of this fungus with wood that contains inorganic antimony compounds may result in significant biotransformation. Furthermore, some plants, such as *Silene vulgaris* (Bladder Campion), can hyper-accumulate antimony (up to 1160 mg Sb/kg was found in the shoots)[26] and the use of such plants to phytoremediate contaminated soils has received much attention. Composting is a proposed method of reducing the volume of biomass after phytoremediation. Clearly, the presence of *P. schweinitzii*, or related fungi, in such compost is likely, and the possibility of significant antimony biotransformation, or even biovolatilization, must be considered.

Because *P. schweinitzii* is more efficient at antimony biomethylation, it might prove to be a more useful model microorganism in the study of antimony biotransformation. For example, there is a need to know the structure of the dimethylantimony and trimethylantimony species detected in the medium, and the high concentrations of these species in the media from cultures of *P. schweinitzii* should facilitate this process. Also, the question has arisen as to whether more complex organoantimony species, such as stibiobetaine, might be produced in the environment; other workers have detected arsenobetaine in some arsenic-accumulating terrestrial fungi.[27] It might be worth examining *P. schweinitzii* to see if such species are present.

Acknowledgements Funding for this work was provided by NSERC, Canada.

REFERENCES

1. Richardson BA. *J. Foren. Sci. Soc.* 1994; **34**: 199.
2. Andrewes P, Cullen WR, Polishchuk E. *Appl. Organomet. Chem.* 1999; **13**: 659.
3. Craig PJ, Jenkins RO, Dewick R, Miller DP. *Sci. Tot. Environ.* 1999; **229**: 83.
4. Gurleyuk H, Vanfleetstalder V, Chasteen TG. *Appl. Organomet. Chem.* 1997; **11**: 471.
5. Jenkins RO, Craig PJ, Miller DP, Stoop LCAM, Ostah N, Morris TA. *Appl. Organomet. Chem.* 1998; **12**: 449.
6. Gates PN, Harrop HA, Pridham JB, Smethurst B. *Sci. Tot. Environ.* 1997; **205**: 215.

7. Jenkins RO, Craig PJ, Goessler W, Irgolic KJ. *Hum. Exp. Toxicol.* 1998; **17**: 231.
8. Andrewes P, Cullen WR, Feldmann J, Koch I, Polishchuk E. Reimer K. *J. Appl. Organomet. Chem.* 1998; **12**: 827.
9. Andrewes P, Cullen WR, Feldmann J, Koch I, Polishchuk E. *Appl. Organomet. Chem.* 1999; **13**: 681.
10. Andrewes P, Cullen WR, Polishchuk E. *Chemosphere* 2000; **41**: 1717.
11. Pearce RB, Callow ME, Macaskie LE. *FEMS Microbiol. Lett.* 1998; **158**: 261.
12. Jenkins RO, Craig PJ, Goessler W, Miller D, Ostah N, Irgolic KJ. *Environ. Sci. Technol.* 1998; **32**: 882.
13. Challenger F. *Chem. Rev.* 1945; **36**: 315.
14. Cullen WR, Reimer KJ. *Chem. Rev.* 1989; **89**: 713.
15. Cullen WR, Li H, Hewitt G, Reimer KJ, Zalunardo N. *Appl. Organomet. Chem.* 1994; **8**: 303.
16. Limerick S (Chair). *Toxic Gas Hypothesis: Report of Expert Group to Investigate Cot Death Theories*. Department of Health: Wetherby, UK, May 1998.
17. Hirner AV, Feldmann J, Krupp E, Grumping R, Goguel R, Cullen WR. *Org. Geochem.* 1998; **29**: 1765.
18. Craig PJ, Forster SN, Jenkins RO, Miller D. *Analyst* 1999; **124**: 1243.
19. Dodd M, Pergantis SA, Cullen WR, Li H, Eigendorf GK, Reimer KJ. *Analyst* 1996; **121**: 223.
20. Koch I, Wang L, Feldmann J, Andrewes P, Reimer KJ, Cullen WR. *Int. J. Environ. Anal. Chem.* 2000; **77**: 111.
21. Feldmann J, Koch I, Cullen WR. *Analyst* 1998; **123**: 815.
22. Cox DP, Alexander M. *Appl. Microbiol.* 1973; **25**: 408.
23. Morgan GT, Davies GR. *Proc. R. Soc., Ser. A* 1926; 523.
24. Nelson JC. Ph.D. Thesis, University of British Columbia, 1993.
25. Andrewes P, Cullen WR, Polishchuk E. *Environ. Sci. Technol.* 2000; **34**: 2249.
26. Baroni F, Boscagli A, Protano G, Riccobono F. *Environ. Pollut.* 2000; **109**: 347.
27. Byrne AR, Slejkovec Z, Stijve T, Fay L, Gossler W, Gailer J, Irgolic KJ. *Appl. Organomet. Chem.* 1995; **9**: 305.

