Production of Trimethylarsine Gas from Various Arsenic Compounds by Three Sewage Fungi

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The production of volatile arsenic compounds by biological action was observed over a century ago, but experimental evidence linking fungal metabolism to the formation of an arsenic gas was first obtained by GOSIO (1893). He isolated a strain of Scopulariopsis brevicaulis and several fungi from arsenic-containing potato mash cultures which were producing a garlic-like odor. He erroneously concluded, however, that the gas produced by these organisms was diethylarsine (GOSIO 1901) and it was not until 1932 that CHALLENGER et al. (1933) correctly identified the arsenic-containing substance as trimethylarsine. Challenger and coworkers extensively studied the ability of S. brevicaulis to methylate organic and inorganic forms of arsenic and other metalloids (CHALLENGE 1945). They partially established the mechanism of methylation of arsenic (CHALLENGER et al. 1954) and postulated metabolic pathways for the formation of trimethylarsine, dimethylselenide and dimethyltelluride (CHALLENGER 1945). Subsequently, several workers have noted the ability of a Trichophyton species (ZUSSMAN et al. 1961) an two species of wood-rotting fungi (MERRILL and FRENCH 1964) to produce "garlic odors" in the presence of inorganic arsenic compounds. A renewed interest in biological methylation of metals and metalloid has resulted in the discovery that a Methanobacterium can produce dimethylarsine from arsenite (McBRIDE and WOLFE 1971) under conditions similar to those in which the same organism was previously shown to methylate inorganic mercury compounds (WOOD et al. 1968).

The results of these studies imply that the release of arsenic compounds in industrial and agricultural wastes provides a potential source of toxic gases if microorganisms possessing this metabolic capability are widespread. Thus, we began searching for microorganisms in soil and sewage which could form trimethylarsine when cultured in the presence of various arsenic compounds. The compounds were chosen on the basis of their use as pesticides and their being intermediates in Challenger's proposed pathway.

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MATERIALS AND METHODS

Chemicals-All chemicals used were reagent grade. The purity of the trimethylarsine (TMA) standard (Ventron Corp., Beverly, Mass.) and that which was produced in culture was confirmed by gas chromatography on two different columns. The structures of the standard gas and that produced in culture were confirmed by mass spectral analysis, and both of these gases exhibited identical parent peaks at 120 m/e.

Chromatography-Gas chromatography was performed on a Varian Model 1700 chromatograph (Varian Aerograph, Walnut Creek, Calif.) equipped with a flame ionization detector. The stainless steel column was 78 cm in length, had an inner diameter of 2mm and contained either 5% FFAP (w:w) coated Chromosorb G (used routinely) or Chromosorb 101. The injector and detector temperatures were 200C. The column temperatures were 75C for the FFAP column and 150C for the Chromosorb 101 column. The flow rate of the carrier gas was 100 ml/min.

Mass Spectrometry-The mass spectral analyses were performed on a Perkin-Elmer (Norwalk, Conn.) No. 270 mass spectrometer with an ionization voltage of 70 eV and an accelerating voltage of 2000 eV.

Isolation Media-The isolation media were buffered over a pH range of 4 to 9. The six buffers used were 0.05M potassium succinate, pH 4 and 5; 0.05M potassium phosphate, pH 6 and 7; and 0.05M sodium borate, pH 8 and 9. The following additions were made to 50 ml of each buffer to these final concentrations: $CaCl_2.2H_2O$, 0.015%; $MgSO_4.7H_2O$, 0.025%; Bacto casamino acids, 0.1% and Bacto peptone, 0.1%. Monobasic potassium phosphate was added to a final concentration of 0.025% to all but the phosphate buffers.

Culture Media-The culture media were identical to the isolation media with the exception that glucose was added to each culture to a final concentration of 1.0%.

Isolation of Arsenic-metabolizing Microorganisms-A 50 ml volume of isolation medium in a 150 ml dilution bottle was mixed with each of the following compounds to a final concentration of 100 µg/ml: sodium arsenite, sodium arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Two milliliters of raw sewage was added to each of the bottles, which were incubated at room temperature in a hood. When growth appeared in the mixtures, a 2 ml aliquot was transferred to fresh medium containing 500 µg/ml of each arsenic compound. When growth appeared, another transfer was made in media containing the appropriate arsenic compound at a concentration of 1000 µg/ml, and finally the process was repeated in media with arsenic compounds at concentrations of 2000 µg/ml. If a garlic odor was detected in the cultures, we then attempted to isolate the responsible organisms by streaking the culture on isolation medium agar plates containing the appropriate arsenic compound.

Determination of Trimethylarsine Production by Pure Cultures-Fifty milliliter volumes of sterilized media in dilution bottles were inoculated with pure cultures, and the bottles were incubated at room

temperature in a hood. As soon as growth was evident and a garlic odor was detected, a serum stopper was placed on the dilution bottle to allow sufficient accumulation of volatile compound to detect by gas chromatography. A typical assay was performed by removing a 1 ml aliquot with a gas-tight syringe from the headspace above the culture and injecting the material into the gas chromatograph.

RESULTS AND DISCUSSION

After approximately a one-month incubation period, garlic odors were detected in enrichment cultures containing DMA at pH 4, 5, and 7, in those containing MMA at pH 5, and in those containing sodium arsenate at pH 4. To date we have been successful in isolating three different species of fungi which are capable of producing trimethylarsine when growing in presence of MMA at pH 5. They have been tentatively identified as (1) Candida humicola (Dazewska) Diddens & Lodder; (2) Gliocladium roseum Bain; and (3) a species of Penicillium. Each of these organisms was grown on three different media and in presence of four different arsenic sources to determine the extent of their ability to produce trimethylarsine gas.

The production of trimethylarsine gas in growing cultures of C. humicola is given in table 1. As is evident, this organism is able to produce TMA from all of the arsenic sources tested when growing at pH 5. There was no detectable production of TMA in cultures growing at pH 6 or 7 in solutions containing arsenate or those growing at pH 7 with MMA.

TABLE 1

Compound	nmole TMA in headspace		
	pH 5	pH 6	pH 7
DMA	87	41	2
MMA	9	6	0
$AsO_4^{\equiv}$	6	0	0
AsO_2^-	8	6	11

Culture age: 1 week, time after capping: 3 days.

The ability of G. roseum to produce TMA when growing in various media is presented in table 2. This organism appeared to produce larger amounts of TMA when growing in the presence of MMA than DMA at all pH ranges. However, no attempt was made to determine the amount of gas produced per unit of culture growth. No TMA was de-

tected by gas chromatography nor was a garlic odor detected when this organism was grown in the presence of sodium arsenate or sodium arsenite at concentrations of 100, 500, or 1000 µg/ml.

TABLE 2

Compound	nmole TMA in headspace		
	pH 5	pH 6	pH 7
DMA	10	52	253
MMA	2970	3700	2970
$AsO_4^{\equiv}$	0	0	0
AsO_2^-	0	0	0

Culture age: 1 week, time after capping: 6 days.

The unidentified species of Penicillium produced TMA gas as shown in table 3. As with the Gliocladium species, this organism produced detectable TMA while growing in the presence of MMA and DMA but not in the presence of arsenate or arsenite at concentrations of either 100, 500, or 1000 µg/ml.

TABLE 3

Compound	nmole TMA in headspace		
	pH 5	pH 6	pH 7
DMA	22	14	6
MMA	429	62	254
$AsO_4^{\equiv}$	0	0	0
AsO_2^-	0	0	0

Culture age: 4 days, time after capping: 3 days.

In conclusion, we utilized a simple gas chromatographic assay to determine the ability of three sewage microorganisms to produce

trimethylarsine gas when cultured in the presence of different arsenic sources. At least two of these organisms (the Candida and the Gliocladium) were previously not known to have this ability. These data suggest that acid conditions in sewage might be conducive to TMA production from several arsenic sources by some fungi.

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