Production of Dimethylselenide Gas from Inorganic Selenium by Eleven Soil Fungi

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The biological conversion of inorganic selenium salts to volatile organic products was first confirmed by CHALLENGER and NORTH (1934) who demonstrated that the fungus Scopulariopsis brevicaulis was capable of producing dimethylselenide (DMSe) from selenite and selenate when growing on bread crumbs. In subsequent studies, this capability was also established for two species of Penicillium (BIRD and CHALLENGER 1939) and strains of Schizophyllum commune (CHALLENGER and CHARLTON, 1947) and Aspergillus niger (CHALLENGER et al., 1954). Recently a strain of Penicillium isolated from sewage was shown to produce DMSe from selenate, selenite, and inorganic selenide (FLEMING and ALEXANDER, 1972).

The results of these studies suggest that the production of volatile selenium in seleniferous soils (ABU-ERREISH et al., 1968) may be due to fungal metabolism. In view of the possible attendant health hazards of selenium volatilization, we have attempted to identify the soil microorganisms responsible and determine the extent of their ability to methylate selenium.

MATERIALS AND METHODS

Chemicals - All chemicals used were reagent grade. The purity of the DMSe standard (Alfa Chemicals, Beverly, Mass.) and the identity of DMSe that was produced in culture was confirmed by gas-liquid chromatography (GLC) on two different columns.

Isolation Media - A minimal isolation medium was employed that contained 5.8g maleic acid, 6.02g tris (hydroxymethyl)aminomethane (tris), 0.50g NH_4NO_3, 0.12g $MgSO_4$, 0.11g $CaCl_2$, 0.10g K_2HPO_4, and 2mg $FeSO_4 \cdot 7H_2O$ per liter. Sodium hydroxide was added to yield a pH of either 5 or 7. Soil medium was prepared by adding 1.2kg of local garden soil, previously dried and passed through a 1.6mm mesh screen, to 1.6L of minimal medium. The mixture was shaken and strained through tissue paper to yield one liter of material free of large particles. All solid media contained 1.5% agar. Sodium selenite and glucose were added to all non-commercial isolation media, routinely at 1000µg/ml and 0.1% respectively. Commercial media used were Littman Oxgall agar (LOA) and Sabouraud's Dextrose agar (SAB), both obtained from Difco. All isolation media were sterilized.

Bulletin of Environmental Contamination & Toxicology.
Vol. 12, No. 3 © 1974 by Springer-Verlag New York Inc.

Isolation of DMSe-Producing Microorganisms - Soil agar plates and liquid minimal medium were inoculated with soil crumbs or soil washings and incubated until growth appeared. Colonies exhibiting a reddish hue, indicative of elemental selenium, were picked and streaked for isolation on soil agar and minimal agar plates. Colonies from plates which emitted the characteristic odor of DMSe were picked and streaked for isolation on similar media. Pure cultures were transferred to SAB plates and maintained. In a variation of this procedure, LOA plates were inoculated with soil washings and then incubated until growth appeared. Fungal isolates were picked at random from these plates and streaked on soil agar plates. Isolation then proceeded as described above. All incubations were at room temperature.

Culture Media - Four culture media were used; minimal medium (pH 7) supplemented with different concentrations of Na_2SeO_3 or Na_2SeO_4; and three natural media; marine water, fresh water, and raw sewage, each supplemented with 500 µg/ml Na_2SeO_3. All culture media were sterilized and supplemented with 0.1% glucose.

Determination of DMSe Production by Pure Cultures - Fifty milliliter volumes of culture media in dilution bottles were inoculated with pure cultures, and the bottles incubated at room temperature in a hood. When the characteristic odor of DMSe was noted, serum stoppers were placed on the dilution bottles to allow sufficient accumulation of the volatile compound to detect by GLC. A typical assay was performed by removing a 0.5ml aliquot from the headspace above the culture with a gas-tight syringe and injecting the gas sample into the gas chromatograph. No attempt was made to evaluate the exact amount of growth present in each culture.

Chromatography - GLC procedures were performed with a Hewlett-Packard Model 402 Chromatograph (Hewlett-Packard, Avondale, Penna.) equipped with a flame ionization detector. Glass columns 183cm in length and with an inner diameter of 3.2mm were employed which contained either 3% XE60 80/100 mesh WHP (used routinely) or 3% QF-1 80/100 mesh WHP (both obtained from Applied Science Laboratories, State College, Penna.). The injector and detector temperatures were maintained at 115C while that of the column was at 72C. The carrier gas was helium.

RESULTS AND DISCUSSION

Eleven distinct fungal strains capable of producing DMSe were isolated and have been tentatively identified as four strains of Penicillium, emcompassing at least three species; three strains of Fusarium, including at least two species; a Fusarium-like organism; two species of Cephalosporium; and a species of Scopulariopsis. Three of the organisms were isolated without passage through low pH or LOA.

The production of DMSe in growing cultures of each organism on minimal medium is shown in table 1. As is evident, each organism is capable of producing the gas from selenite. Only six organisms, however, are capable of producing DMSe from selenate.

TABLE 1

Isolate	µg DMSe in Headspace			
	100 µg/ml Na_2SeO_3	1000µg/ml Na_2SeO_3	100µg/ml Na_2SeO_4	1000µg/ml Na_2SeO_4
Cepahlosporium A	0	4.0	0	0
Cepahlosporium B	1.6	2.0	0	1.2
Fusarium A	4.7	9.3	0	1.8
Fusarium B	17.7	8.1	0	0.6
Fusarium C	4.3	3.7	0	0
Fusarium-like	1.9	2.6	0	0
Penicillium A	24.0	22.7	6.2	0
Penicillium B	32.9	30.1	2.0	0
Penicillium C	11.7	5.8	4.7	0
Penicillium D	6.6	13.2	0	0
Scopulariopsis	3.6	0.6	0	0

Culture age: 1 week; time after capping: 1 day.

Growth medium for Cephalosporium B contained 0.05% yeast extract (Difco)

Five isolates were also examined for their ability to produce DMSe on three natural media. As can be seen in table 2, each of the five fungi produced DMSe on some or all of the natural substrates. Sterile sewage appeared to best support production of the gas. This is probably a simple reflection of the greater nutrient content of sewage compared to fresh and marine waters.

A large number of selenium-reducing bacterial isolates were also screened for the production of DMSe. No isolate was found to possess this characteristic, however.

In conclusion, a GLC assay was employed to evaluate DMSe production by eleven soil fungi in the presence of sodium selenite or sodium selenate. Species of Fusarium and Cephalosporium, not previously reported to have this ability, as well as species of Penicillium and Scopulariopsis were shown to produce DMSe from either or both of the inorganic selenium sources. These data suggest that perhaps a large number of different soil fungi may be capable of mediating the same conversion in the soil ecosystem as well as in a variety of water systems.

TABLE 2

	μg DMSe in Headspace		
Isolate	Sewage	Fresh Water	Marine Water
Cephalosporium A	15.8	1.5	1.1
Fusarium A	22.3	5.9	2.9
Penicillium A	68.8	6.7	2.0
Penicillium B	8.6	1.3	0
Scopulariopsis	4.0	0	0

Culture age: 10 days; time after capping: 1 day minimum - caps applied when growth was evident.

ACKNOWLEDGMENTS

The authors wish to thank Dr. D. Mahoney, Department of Biology, California State University, Los Angeles, for his identification of the different fungal isolates used in this study.

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