Effects of Alternative Selenium and Sulfur Sources on Dimethylselenide Production by Two Fungi Isolated from Natural Systems

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The considerable interest of recent years in toxicological problems in natural systems has resulted in reports of organisms isolated from nature that possess the ability to methylate selenium under laboratory conditions (FLEMING and ALEXANDER, 1972, BARKES and FLEMING, 1974). While many ubiquitous fungi mediate this potentially intoxicating reaction, there are few reports of such methylation in natural systems. The study to be reported was an effort to evaluate factors restrictive to methylation of selenium. As selenium and sulfur are similar chemically and both present in soil (STROCK, 1935), an appraisal was made of the effects of different sulfur and selenium compounds on growth and dimethylselenide (DMSe) production by two fungi isolated from natural systems.

MATERIALS AND METHODS

Organisms - Penicillium strain F, isolated from sewage (FLEMING and ALEXANDER, 1972), and Fusarium strain A, obtained from soil (BARKES and FLEMING, 1974), were used in this study as they yielded results representative of the widest variations in response to experimental combinations of sulfur and selenium compounds in preliminary studies with 12 different fungal isolates.

Chemicals - All chemicals used were of reagent grade. Production of DMSe was evaluated by comparison with a DMSe standard obtained from Alfa Chemicals.

Medium - Effects of selenium and sulfur compounds on growth and DMSe production were characterized in a minimal medium that contained 5.8g maleic acid, 6.02g tris(hydroxymethyl)aminomethane(tris), 0.5g NH_4NO_3, 0.11g $CaCl_2$, 0.1g K_2HPO_4, 20mg $MgCl_2 \cdot 6H_2O$, and 1mg $FeCl_2 \cdot 4H_2O$ per liter of distilled water. Sufficient NaOH was added to adjust the pH to 7. Glucose was added to 0.1% concentration and Na_2SeO_3 and Na_2SeO_4 added at concentrations to 1000µg/ml of medium. Sulfur compounds employed were $Na_2S_2O_3$, Na_2SO_3, and Na_2SO_4. Sulfur compounds were added to give 10^{-3} or 10^{-2} molar sulfur concentration. Fifty milliliter volumes of

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medium were inoculated and incubated under stationary conditions at room temperature in a ventilated hood. Cultivation was in 100ml dilution bottles (165ml total volume) with small mouth openings to facilitate trapping of DMSe for subsequent identification and measurement.

Estimation of Growth - Media were inoculated with 0.25 ml aliquots of a spore suspension with an optical density of 0.1 to 0.3 (525nm). Spores were harvested from growth on Saboraud's Dextrose agar (Difco) and suspended in 0.85% NaCl. Growth was quantitated by total culture filtration through pre-weighed membrane filters of 0.45μM pore size (Millipore Corp.), followed by drying at 70C to constant weight prior to final weighing. Growth studies utilized three replicate cultures.

Estimation of DMSe Production - Following incubation for two days, culture vessels, inoculated as for growth studies, were sealed with serum stoppers to permit accumulation of DMSe for gas-liquid chromatographic (GLC) evaluation. A 0.5ml gas sample was taken from the headspace of each culture vessel and injected into the chromatograph with a gas-tight syringe. GLC procedures utilized a Hewlett-Packard Model 402 chromatograph with flame ionization detectors. Glass columns (183cm with a 3.2mm internal diameter) were used with a packing of either 3% XE60 80/100 mesh WHP or 3% QF-1 80/100 mesh WHP (Applied Sciences Laboratories, State College, Penna.). Injector and detector temperatures were maintained at 110C and 120C, respectively, while the column was at 70C. The carrier was helium. The production of DMSe was qualitatively and quantitatively surveyed by peak area comparisons with known amounts of DMSe standard. The total DMSe in the headspace of each culture vessel was calculated from that present in the 0.5ml sample, and reported below as the average from three replicate cultures.

RESULTS AND DISCUSSION

Preliminary studies with the experimental organisms, as well as 10 other fungal isolates, indicated that each was more severely inhibited by 1000μg/ml Na_2SeO_4 than by the same level of Na_2SeO_3. The two fungi selected for additional studies were chosen because they represented the total range of the inhibitory effects of high selenate concentrations. As shown in tables 1 and 2, growth of Penicillium strain F was severely inhibited, while the effect on Fusarium strain A was not as great. By comparison, the same selenite concentrations inhibited growth of these organisms by a

maximum of 30%. Though the relationships between sulfur sources, selenate, selenite, and DMSe production were of greatest interest, attempts to eliminate selenate toxicity for these organisms were continued.

TABLE 1

Effect of sulfur source on growth of Penicillium strain F with selenate[a]

µg/ml Na_2SeO_4	Sulfur (M)	Total mg of mycelium		
		$S_2O_3^{-2}$	SO_3^{-2}	SO_4^{-2}
0	10^{-3}	17.15	17.44	16.59
	10^{-2}	16.90	19.30	16.95
100	10^{-3}	0.41	0.18	1.23
1000	10^{-3}	0.15	0.09	0.14
	10^{-2}	0.67	0.08	1.88

[a]Culture age: 7 days.

TABLE 2

Effect of sulfur source on growth of Fusarium strain A with selenate[a]

µg/ml Na_2SeO_4	Sulfur (M)	Total mg of mycelium		
		$S_2O_3^{-2}$	SO_3^{-2}	SO_4^{-2}
0	10^{-3}	15.11	15.17	16.96
	10^{-2}	22.71	16.45	18.32
100	10^{-3}	14.28	9.13	12.17
1000	10^{-3}	10.28	0	2.30
	10^{-2}	17.59	6.26	12.55

[a]Culture age: 7 days.

Efforts to ameliorate the inhibitory effects of selenate for the Penicillium isolate were unsuccessful, and only the addition of high concentrations of sulfate or thiosulfate to the Fusarium cultures resulted in substantial growth in the presence of 1000µg/ml of selenate. The values for growth are average values obtained from three separate trials. Little variation occurred between trials, with differences of less than 10% being typical.

Of greatest interest is the observation that high

sulfite concentrations provided little protection from selenate toxicity, while thiosulfate and sulfate were particularly effective. The bases for the protective effects have yet to be characterized, but it is probable that the combination of selenium and sulfur compounds present in any natural system will markedly influence the ability of such organisms to grow. It has been proposed (WEISSMAN and TRELEASE, 1955) that sulfate is antagonistic to selenium transport in the fungal genus Aspergillus, and it may also be possible that thiosulfate plays a significant role in the pathway for sulfate assimilation in these isolates. Unfortunately, the intermediary sulfur metabolism of the fungi has not been thoroughly resolved.

Tables 3 and 4 indicate the abilities of these two fungi to produce DMSe in the presence of different combinations of selenium and sulfur compounds. Again, the values represent the averages of three trials with each set of cultivation conditions. Variations between trials were generally less than 10%, with an extreme of 20%.

TABLE 3

Effect of sulfur source on DMSe production by Penicillium strain F[a]

Selenium source	Sulfur (M)	Total µg DMSe in headspace		
		$S_2O_3^{-2}$	SO_3^{-2}	SO_4^{-2}
100µg/ml SeO_3^{-2}	10^{-3}	10.9	3.2	3.4
	10^{-2}	2.0	4.9	4.1
1000µg/ml SeO_3^{-2}	10^{-3}	12.7	5.8	6.5
	10^{-2}	2.2	5.0	6.8
1000µg/ml SeO_4^{-2}	10^{-3}	0[b]	0	0
	10^{-2}	0.6	0	0

[a]Culture age: 4 days; Time after capping: 2 days.

[b]No DMSe detected.

Penicillium strain F produced little DMSe from selenate regardless of type or level of sulfur supplementation, a result expected in view of the lack of growth in the presence of selenate. Most notable, however, is the observation that high sulfur concentration will reduce the amount of DMSe produced from selenite.

The data relating to Fusarium strain A demonstrate a similar phenomenon, and may be of more value as this organism grows well in the presence of either selenite or selenate. A high concentration of sulfite or sulfate almost completely eliminated DMSe production from selenite. While the data for combinations

of selenate and alternative sulfur compounds are not as striking, they also show suppression of DMSe production.

TABLE 4

Effect of sulfur source on DMSe production by *Fusarium* strain A[a]

Selenium source	Sulfur (M)	Total µg DMSe in headspace		
		$S_2O_3^{-2}$	SO_3^{-2}	SO_4^{-2}
100µg/ml SeO_3^{-2}	10^{-3}	4.2	0[b]	0
	10^{-2}	3.5	0	0.5
1000µg/ml SeO_3^{-2}	10^{-3}	4.6	1.6	1.4
	10^{-2}	3.2	2.0	1.8
1000µg/ml SeO_4^{-2}	10^{-3}	6.1	0	5.3
	10^{-2}	3.2	4.6	1.2

[a]Culture age: 7 days; Time after capping: 5 days.

[b]No DMSe detected.

Lowered DMSe production in response to sulfur supplementation may only reflect competition for transport mechanisms. Diminished DMSe yields, like improved growth, could result from competitive inhibition of transport of selenium compounds into the fungal cells.

Biochemical mechanisms that satisfactorily explain the observed results have yet to be fully defined and characterized, but it is clear that the ability of fungi to grow and methylate selenium in any natural system will be determined, at least in part, by the particular combination of selenium and sulfur sources present in the system. The data relating to selenate are considered to be of special significance as it has been suggested (LAKIN, 1972) that selenium is present predominantly in the selenate form in seleniferous soils. The apparent greater toxicity of selenate for the organisms studied, as well as the limited amounts of DMSe produced from selenate, could be major contributing factors to the lack of reports of selenium volatilization in natural systems.

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