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Dimethyl Sulfoxide as an Electron Acceptor for Anaerobic Growth

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Abstract. The isolation from lake mud of a bacterium which can use dimethyl sulfoxide (DMSO) as an electron acceptor for growth is described. The isolate, called strain DL-1, was a small, gram negative, nonmotile spiral. The sole product of DMSO reduction was dimethyl sulfide (DMS). Other electron acceptors used by the isolate included sulfite, thiosulfate, elemental sulfur, methionine sulfoxide, tetramethylene sulfoxide, nitrate, and oxygen (microaerophilically). Sulfate was not reduced and could not even be assimilated. Lactate or succinate could serve as electron donors, with acetate as the main product. Hydrogen could be used as an electron donor if acetate was present in the medium as a carbon source. The organism has a *c*-type cytochrome, and most likely uses electron transport phosphorylation during DMSO reduction. Cultures of *Desulfovibrio* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* were tested for growth using DMSO as an electron acceptor, and only the *Proteus* strain grew. Both *Proteus* and strain DL-1 are versatile at coupling reductions with energy generation. There is a marked resemblance between strain DL-1 and the recently described sulfur-reducing spirillum of Wolfe and Pfennig.

Key words: Dimethyl sulfoxide reduction – Electron acceptors.

Dimethyl sulfoxide (DMSO) is a waste product of the paper industry, and has received attention in recent years because of its potential use in medicine as a solvent which can transport drugs across lipid membranes (Leake, 1967). It has been suggested that DMSO plays a role in the global sulfur cycle as an atmospheric oxidation product of dimethyl sulfide (DMS) (Lovelock et al., 1972).

Sulfoxides are generally formed via oxidation of sulfides by peroxides (Snow et al., 1976). Methionine sulfoxide and biotin sulfoxide are natural metabolites which are reduced by *Escherichia coli* (Cleary and Dykhuizen, 1974; Sourkes and Trano, 1953) and *Saccharomyces* (Black et al., 1960), as well as by other organisms.

Ando et al. (1957) found that *Escherichia coli* reduced DMSO to DMS, while strains of *Salmonella* and *Shigella* did not, and proposed DMSO reduction as a diagnostic reaction. Distefano and Borgstedt (1964) found that DMS could be detected in the breath of cats, after administration of DMSO. DMSO reduction has also been found in other animals including cows (Tiews et al., 1975), and plants (Smale et al., 1975).

In preliminary studies on sulfur metabolism in Lake Mendota (Wisconsin) sediments, we found that DMSO was rapidly reduced to DMS. Since it was likely that this process was biological, we tried enrichments patterned after the lactate-sulfate media used for enriching sulfate-reducing bacteria. This report describes the properties of an isolate from such an enrichment, and presents results on dissimilatory DMSO reduction by laboratory cultures of bacteria.

Abbreviations. DMS = dimethylsulfide; DMSO = dimethyl sulfoxide; O.D. = optical density

MATERIALS AND METHODS

Organisms Used. Strain DL-1 was isolated from lake sediments as described in the results section. *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were obtained from the University of Wisconsin, Department of Bacteriology culture collection. *Desulfovibrio* sp. was isolated from sewage digestor sludge by the authors.

Growth Media and Conditions. The basal medium used for enrichment and growth of strain DL-1 contained 0.2 g NH_4Cl, 0.11 g Na_2HPO_4, 0.1 g disodium ethylenediaminetetraacetic acid, 0.1 g $MgSO_4 \cdot 2\,H_2O$, 1 ml of a trace metal solution (Tuovinen and Kelly, 1973), and 0.1 g yeast extract (Difco Laboratories, Inc.,

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Detroit, MI) per liter distilled water. The final pH was adjusted to 7.0. The medium was bubbled vigorously for 10 min with prepurified nitrogen gas (scrubbed by hot copper filings), and was dispensed into anaerobic culture tubes, which were gassed with nitrogen and were sealed with neoprene stoppers. The tubes were autoclaved in a tube press at 121° C for 20 min. One molar solutions of sodium lactate and DMSO, sterilized by passage through 0.45 μm membrane filters (Gelman Instrument Co., Ann Arbor, MI), were added via sterile syringes through the stoppers. The final concentration was 10 mM. Since the volume of these solutions added was only 1% of the total volume, oxygen contamination was minimal and did not affect the growth of this microaerophile. Other electron donors and acceptors were added in a similar fashion. Cultures were incubated at 25° C. Growth was measured as optical density at 400 nm using a Spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, NY).

E. coli, *P. aeruginosa*, and *P. vulgaris* were grown in the same basal medium, except yeast extract was omitted, and 10 mM lactate was the electron donor. For growth of *Desulfovibrio* sp., 0.1% sodium thioglycollate was added to the medium before autoclaving, and sulfate salts were replaced with chloride salts.

Chemical Methods. Dimethyl sulfide in the headspace over cultures was determined using a Packard 419 gas chromatograph (Packard Inst., Co., Inc., Downers Grove, IL) fitted with a flame photometric detector (FPD). The FPD is a sensitive and selective detector which will detect nanogram quantities of volatile sulfur compounds in the presence of a 10000-fold excess of carbon compounds (Banwart and Bremner, 1974). The column used was that of Stevens et al. (1971) and was purchased from Supelco, Inc., Bellefont, PA. The gas flows and operating conditions were those described by Banwart and Bremner (1974).

Acetate and other short chain fatty acids were measured using a Packard 407 gas chromatograph fitted with a flame ionization detector. A 1 m, 3 mm O.D. teflon column was used, packed with Carbopack B/3% Carbowax 20 M, 0.5% H_3PO_4 (Supelco, Inc.).

Methionine was determined by the color reaction with sodium nitroprusside, as described by Greenstein and Winitz (1961). This reaction is specific for the methiol group and does not detect methionine sulfoxide.

Cytochrome Spectrum Analysis. Cells were grown in stoppered 1 l bottles, and were harvested and washed three times in 50 mM phosphate buffer, pH 7.0. The final pellet was suspended in 5 ml phosphate buffer and the cells were broken by passage through a French pressure cell at a pressure of 8–10000 psi and at 4° C. The resulting preparation was centrifuged at 27000 × g for 20 min, and the supernatant was carefully drawn off using a Pasteur pipet. These extracts were scanned with a Beckman DB-G spectrophotometer (Beckman Inst., Inc., Fullerton, CA) calibrated with a holmium oxide standard. Cytochromes were reduced by addition of sodium dithionite to a concentration of 1 mg/ml.

Chemicals Used. DMSO was purchased from Fisher Scientific Co., Fair Lawn, N.J. L-methionine-*dl*-sulfoxide and dimethyl sulfone were purchased from Sigma Chemical Co., St. Louis, MO. The methionine sulfoxide was shown to be free from methionine by thin layer chromatography. Tetramethylene sulfoxide and diphenyl sulfoxide were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. All other chemicals were of reagent grade.

RESULTS

Isolation of Strain DL-1 from Lake Mud

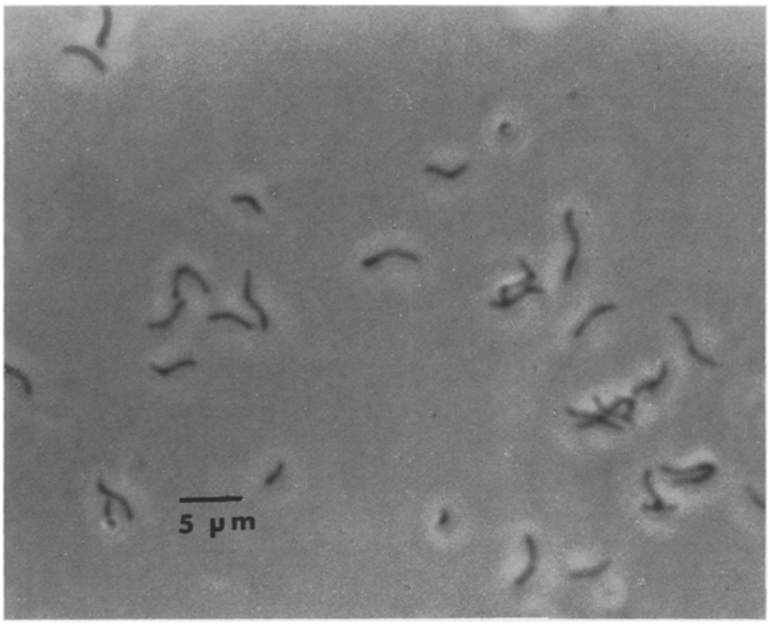
When a small amount of lake mud was incubated at 25° C in DMSO plus lactate medium, turbidity developed within 2 days. This enrichment was transferred once to liquid medium, and then streaked out onto agar plates of the same medium. The plates were incubated in GasPak anaerobic jars (Becton, Dickinson and Co., Cockeysville, MD) for 5 days. The large white colonies which resulted were picked and restreaked. The colonies formed were all of one type and were transferred to liquid medium. As shown in Figure 1, strain DL-1 is a small (0.5 μm diameter, 2 μm long), spiral, which occasionally may form longer spirals, depending on the growth conditions. It is gram negative, and motility has never been observed.

DMSO Dependent Growth of Strain DL-1

Figure 2 shows that growth of the culture is dependent on the presence in the medium of both DMSO and lactate. Doubling time for the culture was 4–5 h. An analysis of the products found after growth of the culture is presented in Table 1. No H_2S or any other volatile product of DMSO reduction other than DMS was detected. The major product formed from lactate was acetate. Oxidation of lactate to acetate produces four electrons while DMSO reduction to DMS requires two electrons. Since the concentrations of both DMSO and lactate were 10 μmole/ml, only half of the lactate would be oxidized to acetate, while all the DMSO would be reduced. Thus, the concentrations of these products conform well to the stoichiometry predicted by the equation: $^1/_2$ lactate + DMSO $\rightarrow$ $^1/_2$ acetate + DMS + $^1/_2$ HCO_3^- + $^1/_2$ H^+. The small amounts of propionate and butyrate formed are most likely secondary fermentation products, or were formed from the small amount of yeast extract in the medium.

Nutrition of Strain DL-1

As seen in Table 2, strain DL-1 is very versatile in the electron acceptors it can use for growth. Although it could not reduce sulfate or tetrathionate, other forms of inorganic sulfur were reduced to hydrogen sulfide. The DMSO analogues, methionine sulfoxide and tetramethylene sulfoxide, were readily reduced, but the insoluble diphenyl sulfoxide was not. When a methionine analysis was performed after growth of a culture using methionine sulfoxide as an electron acceptor, 92% of the methionine sulfoxide added was recovered as methionine. No methyl mercaptan or other volatile sulfur compounds were found. Dimethyl sulfone, more oxidized by two electrons than DMSO, was not reduced. Strain DL-1 grew when oxygen was supplied at 0.02 atm but not at 0.2 atm (normal atmospheric pressure). Nitrate was also used as an electron acceptor for growth.



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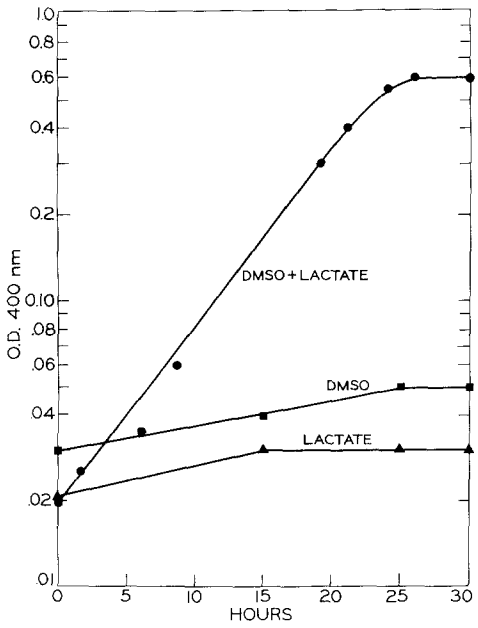

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Fig. 1. Phase micrograph of strain DL-1

Fig. 2. Dependence of strain DL-1 on both DMSO and lactate (both 10 μmole/ml for growth

Table 1. Metabolic products of strain DL-1 after growth on 10 μmole/ml lactate plus 10 μmole/ml DMSO

Product	Final concentration μmole/ml
Acetate	5.5
Proprionate	0.1
Butyrate	0.2
Dimethyl sulfide	10.2

Table 2. Growth of strain DL-1 using various electron donors and acceptors. All concentrations are 10 μmole/ml and incubations were done under a nitrogen atmosphere unless otherwise noted

Electron donor	Electron acceptor	Growth
Lactate	DMSO	+
Lactate	$SO_4^=$	−
Lactate	$SO_3^=$	+
Lactate	$S_2O_3^=$	+
Lactate	$S_4O_6^=$	−
Lactate	S°	+
Lactate	dimethyl sulfone	−
Lactate	methionine sulfoxide	+
Lactate	tetramethylene sulfoxide	+
Lactate	diphenyl sulfoxide	−
Lactate	NO_3^-	+
Lactate	O_2 (0.2 atm)	−
Lactate	O_2 (0.02 atm)	+
Succinate	DMSO	+
Acetate	DMSO	−
Glucose	none	−
Glucose	DMSO	−
Ethanol	DMSO	−
H_2 (1 atm)	DMSO	−
H_2 + Acetate	DMSO	+

Electron donors were not surveyed extensively. Succinate was the only other organic electron donor besides lactate which supported growth, and acetate was the major product. Strain DL-1 could not grow using acetate as a donor, which is consistent with acetate builtup during growth on lactate and succinate. Acetate could, however, be used as a carbon source when hydrogen was supplied as the electron donor. The yield when using this combination was about one third that when lactate was the electron donor, even though all the DMSO in the medium was reduced to DMS. Glucose was neither fermented nor oxidized.

The Role of Yeast Extract in the Medium

It had been found that the 100 mg/l yeast extract in the medium was essential for growth of the organism when using DMSO as the electron acceptor. However, when thiosulfate was used as the electron acceptor, yeast extract was not necessary for growth. When small amounts of thiosulfate, sulfide, or methionine were added to medium without yeast extract, the culture grew. Therefore, it appears that DL-1 is not able to use either the sulfate in the medium, or DMSO and its reduction product DMS, as sulfur sources.

Table 3. Growth of microorganisms using 10 μmole/ml lactate and various electron

Organism	Electron acceptor					
	none	O_2	NO_3^-	$S_2O_3^=$	DMSO	$SO_4^=$
Desulfovibrio sp.	−	ND	ND	+	−	+
Escherichia coli	−	+++	+	−	−	ND[a]
Pseudomonas aeruginosa	−	+++	++	−	−	ND
Proteus vulgaris	−	+++	++	−[b]	+[c]	ND

[a] Not determined
[b] Hydrogen sulfide produced
[c] DMS produced

Cytochromes of Strain DL-1

The dithionite-reduced spectrum of a crude extract of strain DL-1 showed α, β, and γ absorption peaks at 552, 523, and 416 nm respectively, and the oxidized γ peak was at 407 nm. This is typical of a *c*-type cytochrome. No desulfoviridin, a pigment found in many strains of *Desulfovibrio* (Postgate, 1959), was detected, either by absorbance at 630 nm or by the fluorescence test described by Postgate (1959). That DL-1 makes a cytochrome during growth using DMSO as an electron acceptor suggests that it may be generating energy via electron transport. Further evidence for electron transport phosphorylation is that growth is inhibited by 0.2% sodium azide and 0.5 mM 2,4-dinitrophenol.

Growth of Laboratory Cultures Using DMSO

Since it is possible that DMSO is an analogue of an intermediate in sulfate reduction, the ability of a *Desulfovibrio* sp. isolate to grow using DMSO as an electron acceptor was tested. Also tested were cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*, all of which were shown to substantially reduce DMSO to DMS when grown aerobically on complex medium.

As shown in Table 3, DMSO could not serve as an electron acceptor for *Desulfovibrio* sp., while sulfate and thiosulfate could. DMSO added to medium containing a small amount of sulfate neither enhanced nor inhibited growth. A small amount of the DMSO in the medium was reduced to DMS but this may have been due to chemical reduction by hydrogen sulfide. *E. coli* and *P. aeruginosa* grew using oxygen and nitrate as acceptors but not DMSO or thiosulfate. *P. vulgaris* did grow using DMSO as an electron acceptor, producing DMS, and could also use oxygen and nitrate. Although *P. vulgaris* did not grow in medium containing thiosulfate, sulfide was produced, and it is possible that sulfide toxicity prevented growth, as suggested by Oltmann et al. (1975).

DISCUSSION

Strain DL-1 can grow reducing DMSO to DMS, and oxidizing lactate to acetate. Because of its spiral shape, it was originally thought to be a sulfate reducer, but it is unable to reduce sulfate, even for assimilation, does not contain desulfoviridin, and is a facultative aerobe.

Strain DL-1 is morphologically and physiologically similar to spirillum 5175, recently described by Wolfe and Pfennig (1977), which could grow using sulfite, thiosulfate, and elemental sulfur as electron acceptors. Spirillum 5175 is a microaerophile, can grow using hydrogen as an electron donor and acetate as a carbon source, and has a cytochrome spectrum identical with that of strain DL-1. Strain DL-1 is not motile, while spirillum 5175 is, but motility may easily be lost. There is also some physiological similarity between strain DL-1 and the facultatively anaerobic marine bacteria described by Tuttle and Jannasch (1973), which reduced sulfite, thiosulfate and nitrate.

Although we know of no one who has specifically enriched for a DMSO reducer in the past, S. C. Rittenberg (1941) tested *Desulfovibrio* to see whether it could grow by reducing various sulfoxides, sulfones and sulfonic acids (although not DMSO). He found no growth, and we have verified this for DMSO. Recently, Yen and Marrs (1976) described dissimilatory DMSO reduction by *Rhodopseudomonas capsulata*. Growth only occurred when a fermentable electron donor, such as glucose, was used, and they concluded that electron transport phosphorylation did not occur in that organism. All attempts to grow strain DL-1 phototrophically have failed.

The free energy of DMSO reduction using lactate as the electron donor is −30 kcal/mole per two electrons (calculated using $\Delta G'$ formation for DMSO = −23.7 kcal/mol, and for DMS = +3.4 kcal/mol). This is roughly half of that obtained using oxygen as an electron acceptor and three times the energy derived from sulfate reduction. The presence of a cytochrome, and the growth inhibition by azide and dinitrophenol, are indicative of electron transport phosphorylation.

Growth using hydrogen as an electron donor is further evidence for electron transport phosphorylation, since it is difficult to imagine substrate level phosphorylation involving hydrogen. However, it should be mentioned that the ATP requirements of the organism, when growing on lactate and perhaps on hydrogen and acetate, may be met by substrate level phosphorylation from acetyl phosphate.

It is unlikely that DMSO is present in large amounts in the lake sediments strain-DL-1 was isolated from. That methionine and tetramethylene sulfoxide were also reduced suggests that strain DL-1 may have a general sulfoxide reductase system to reduce sulfoxides formed naturally via the peroxidation of sulfides. Since strain DL-1 does not grow at normal oxygen tensions, it is likely to be sensitive to oxygen radicals. Strain DL-1 appears to be versatile at coupling reductions with energy production. It is interesting that *Proteus*, a genus which is also versatile at coupling energy production with reduction (Oltmann et al., 1975), can grow reducing DMSO. Since *Desulfovibrio* can grow reducing sulfite and thiosulfate, and *E. coli* and *P. aeruginosa* have nitrate reductase, it appears that these enzymes are not directly responsible for DMSO reduction for growth. A related phenomenon is the ability of *Salmonella typhimurium* to grow using trimethylamine oxide as an electron acceptor (Kim and Chang, 1974). A mutant which could not reduce nitrate was shown not to be able to grow on this substrate. However, Yamamoto and Ishimoto (1977) were able to obtain anaerobic growths of *E. coli* with trimethylamine oxide as electron acceptor on formate, provided peptone was also added. Conceivably, some nutritional factor also prevents anaerobic growth of *E. coli* on DMSO.

Strain DL-1 cannot use sulfate as a sulfur source, but can use more reduced forms of sulfur such as thiosulfate. This implies that it lacks the enzyme system which reduces sulfate to sulfite via 3′-phosphoadenylyl sulfate. This phenomenon has been reported for the facultative autotroph, *Thiobacillus intermedius* (Smith and Rittenberg, 1974), and in certain photosynthetic bacteria (Kelly, 1971). In lake sediments, reduced sulfur compounds are plentiful so that this would probably not be a disability to the organism.

Lovelock et al. (1972) have postulated that dimethyl sulfide plays an important role in the atmospheric transfer of sulfur, and that DMSO may be an important atmospheric oxidation product of DMS. If any of this DMSO were to reach the sediments it would rapidly be reduced to DMS by a variety of organisms. In other studies (S. H. Zinder and T. D. Brock, to be published), we have found that ^{14}C labelled DMS is also rapidly metabolized to methane, carbon dioxide and an inorganic sulfur component, most likely sulfide.

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