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Demethylation of Dimethylsulfoniopropionate to 3-Mercaptopropionate by an Aerobic Marine Bacterium

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A bacterium, strain BIS-6, that grew aerobically on dimethylsulfoniopropionate (DMSP) was isolated from an intertidal mud sample. Strain BIS-6 quantitatively demethylated DMSP and 3-methiolpropionate to 3-mercaptopropionate. Strain BIS-6 was a versatile methylotroph growing on the osmolytes DMSP and glycine betaine and their methylated degradation products (dimethyl glycine, sarcosine, methylamines, and dimethyl sulfide).

Dimethylsulfoniopropionate (DMSP) is an organic sulfur compound which occurs at high concentrations in some marine plants and cyanobacteria (4, 7, 16, 26, 28). When DMSP is released into the environment, it is degraded by bacteria with either an initial cleavage to yield dimethyl sulfide (DMS) and acrylate or demethylation to 3-methiolpropionate (MMPA) (Fig. 1) (21). MMPA is further metabolized with demethylation to 3-mercaptopropionate (MPA) or by conversion to methanethiol (Fig. 1) (21). Isolates of bacteria that aerobically or anaerobically cleave DMS from DMSP have been described previously (8, 12, 27). The demethylation of DMSP to MMPA was shown with pure cultures from marine habitats, both an aerobe (strain DG-C1) (22) and an anaerobe (*Desulfobacterium* sp. strain PM4) (23). The second demethylation of MMPA to MPA was observed with anoxic slurries of coastal marine sediments (11), where MPA was a major organic sulfur compound in the pore waters (10). Strain DG-C1 mainly converted MMPA to CH_3SH, although traces of MPA were detected in culture media during growth on DMSP or MMPA. We now describe an aerobic marine bacterium, strain BIS-6, that quantitatively demethylates DMSP and MMPA to MPA, without the formation of CH_3SH. MPA accumulates from DMSP and MMPA, indicating methylotrophic growth on these compounds. Indeed, the isolate is a versatile methylotroph, growing on a range of methylated compounds that are common in marine environments (9).

Medium. The medium contained (in grams per liter) NaCl (25.0), NH_4Cl (0.2), $CaCl_2 \cdot 2H_2O$ (0.225), KCl (0.2), $MgCl_2 \cdot 6H_2O$ (0.2), KH_2PO_4 (0.02), and Na_2CO_3 (2.0) and was supplemented with $FeSO_4 \cdot 7H_2O$ (1 mg/liter), the trace element solution (1 ml/liter) of Widdel and Pfennig (29), and either yeast extract (0.1 g/liter) or the vitamin solution (1 ml/liter) of Pfennig (15). The pH was adjusted to 7.5 after autoclaving with sterile HCl or NaOH. The solid medium contained 1.5% (wt/vol) Bacto Agar (Difco Laboratories, Detroit, Mich.).

Cell suspension experiments. Cultures were grown at 25°C without shaking in 100-ml batches in 250-ml Erlenmeyer flasks. Growth substrates were used at 5, 10, or 20 mM concentrations. Growth was monitored by measurements with a Klett-Summerson colorimeter and by determining cell protein. Cells in the late-exponential phase were harvested by centrifugation (10 min at 10,000 × *g* at 5°C) and washed twice in medium lacking the growth substrate. Cell suspensions were used to test substrate oxidation. Oxygen uptake was measured in a 5-ml chamber incubated at 30°C by using a Clark-type electrode (1). Substrates (50, 100, or 200 nmol) were added after endogenous rates had been determined for 1 to 2 min.

Analytical methods. DMS and CH_3SH were determined by gas chromatography with flame ionization detection (model GC-14A; Shimadzu Corp., Kyoto, Japan) and a column of 40/60 Carbopak BH T 100 (Supelco Inc., Bellefonte, Pa.) at 110°C with a carrier gas (N_2) flow rate of 60 ml/min (2). The retention time for DMS was 1.3 min. DMS was calibrated by headspace analysis in the 14-ml vials of solutions diluted in water or methanol or alkaline decomposition of DMSP in the vials (26). CH_3SH production was calibrated by the reduction of dimethyl disulfide with tributylphosphine. DMSP concentrations in media were determined by alkaline hydrolysis (5, 28). Dissolved thiols (MPA and CH_3SH) were derivatized, by reaction with *o*-phthalaldehyde and 2-aminoethanol, to isoindoles that were separated and quantified by reverse-phase high-performance liquid chromatography with fluorometric detection (11, 14). Protein was measured colorimetrically with bicinchoninic acid (19).

Chemicals. Chemicals were purchased mainly from the Sigma Chemical Co., St. Louis, Mo., or Aldrich Chemical Co., Milwaukee, Wis. DMSP was synthesized (2) and purchased from Research Plus, Inc. MMPA was obtained by alkaline hydrolysis of its methyl ester [methyl(3-methylthio)propionate; Aldrich].

Bacteria that demethylated DMSP and/or MMPA to MPA were readily detected and common in most-probable-number dilutions on DMSP or MMPA from the upper waters of the Caribbean Sea (24) and surface muds of coastal environments (25). Strain BIS-6 was isolated from a 10^6 dilution, in medium mixed with an equal volume of sterile seawater (25), of surface mud from a sediment in Bear Cut of Biscayne Bay, Fla., with 0.6 mM DMSP as the sole source of carbon and energy. The liquid enrichment was streaked onto separate solid media

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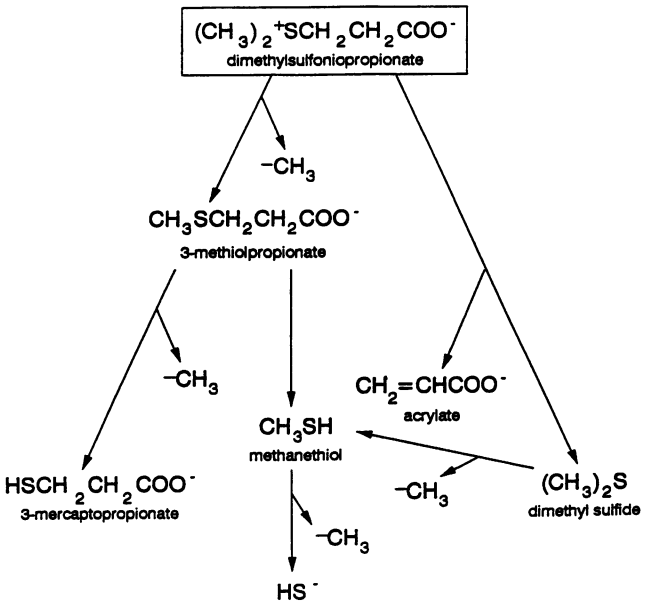

FIG. 1. Routes for the degradation of DMSP by marine bacteria.

containing the following growth substrates: DMSP, MMPA, MPA, and glycine betaine. Colonies developed on the methylated substrates but not on MPA. The medium initially lacked organic growth factors, and colony development was slow upon subculture onto identical media unless 0.01% yeast extract was added. Yeast extract was subsequently replaced by a defined vitamin mixture (15). The isolate was a short (about 0.5- by 1.0-μm) nonmotile rod that grew aerobically on a variety of *N*-methyl and *S*-methyl compounds.

The organism grew well on DMSP, MMPA, DMS, glycine betaine, trimethylamine, dimethylamine, and methanol (Table 1). Growth yields were approximately proportional to the number of methyl groups in the substrate (Table 1). Dimethyl disulfide, *N,N'*-dimethylglycine, and *N*-methylglycine (sarcosine) were also good growth substrates, but methylamine and *S*-methyl cysteine were less effective. Of nonmethylated compounds, acetate and propionate supported good growth (similar to that with MMPA) but butyrate was less effective. Strain BIS-6 did not grow on MPA, glycine, acrylate, or diethyl sulfide.

MPA was accumulated during growth on DMSP or MMPA in amounts that were equivalent to the initial substrate concentration (Table 2). Neither DMS (headspace analysis) nor CH_3SH (headspace and medium analysis) were detected during growth on DMSP.

TABLE 1. Growth parameters for strain BIS-6 on some methylated compounds

Substrate	μ (h^{-1})	Yield (mg of protein/mmol of substrate)
Glycine betaine	0.23	23.5
Trimethylamine	0.18	20.4
DMSP	0.14	12.3
DMS	0.08[a]	10.8
Dimethylamine	0.09[a]	14.5
MMPA	0.10	8.2
CH_3OH	ND[b]	9.7

[a] Determined from measurements of A_{550}; all other results were based on direct protein determinations.
[b] ND, not determined.

TABLE 2. MPA production by strain BIS-6 during growth on DMSP or MMPA

Growth substrate	Initial substrate concn (mM)	MPA produced (mM)
DMSP	3.06	2.82
	5.12	4.51
MMPA	3.00	2.37
	3.00	2.64

Cells of strain BIS-6 grown on either glycine betaine or trimethylamine oxidized both compounds at similar rates. Furthermore, cells grown on trimethylamine immediately oxidized a wide variety of methylated sulfur and nitrogen compounds (Table 3).

Strain BIS-6 is a versatile methylotroph capable of utilizing several methylotrophic substrates that are common in marine environments (9). These substrates abound because of the roles of methylated nitrogen and sulfur compounds as osmolytes in marine organisms. DMSP, glycine betaine, trimethylamine *N*-oxide, and their degradation products (methyl sulfides and methylamines) are the principal compounds that are available in marine waters. Strain BIS-6 demethylated glycine betaine to glycine, as observed for a recently isolated marine bacterium, strain MD 14-50, and previously for other aerobes (5). The ability of strain BIS-6 to use methylamines for growth, unlike the oceanic strain MD 14-50, may reflect its proximity to anoxic zones in its intertidal habitat, where methylamines are generated from the fermentation of methylated glycines (6, 13). The indication that strain BIS-6 can immediately use a variety of methylated compounds (Table 3) deserves further investigation. Even though the metabolism of methylated substrates often requires the induction of specific enzymes, the situation is not always simple; *Hyphomicrobium* sp. strain EG, for example, is inducible for methylamine metabolism but constitutive for DMS metabolism (20). Constitutive enzymes might be advantageous in an environment where a multiplicity of methylated compounds at low concentrations exists. Strain BIS-6 adds to the variety of marine methylotrophic bacteria previously described (17, 18). It further identifies the sea as a habitat that is suitable for the methylotrophic mode of life (9).

An interesting metabolic and ecological feature of strain BIS-6 is its ability to demethylate DMSP and its degradation product DMS. In natural environments, strain BIS-6 would

TABLE 3. Oxidation of methylated compounds by cells of strain BIS-6 grown on trimethylamine (20 mM)

Substrate	Oxygen uptake[a] (nmol/min/mg of protein)
Trimethylamine	27
Glycine betaine	34
Dimethylamine	19
Methylamine	14
DMSP	18
MMPA	12
DMS	16
Acetate	20

[a] Endogenous (no substrate) rates subtracted. Endogenous rates were 1 to 2 nmol of O_2 per min per mg of protein. Glycine, MPA, and diethyl sulfide did not stimulate uptake of the endogenous rate.

benefit from DMSP release by plants and cyanobacteria and the activities of bacteria that cleave DMSP. Strain BIS-6 potentially contributes to DMSP degradation at two points in the multiple routes that operate for its destruction. Organisms like strain BIS-6 are probably common in marine habitats that contain DMSP (24, 25).

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