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New Routes for Aerobic Biodegradation of Dimethylsulfoniopropionate

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Dimethylsulfoniopropionate (DMSP), an osmolyte in marine plants, is biodegraded by cleavage of dimethyl sulfide (DMS) or by demethylation to 3-methiolpropionate (MMPA) and 3-mercaptopropionate (MPA). Sequential demethylation has been observed only with anoxic slurries of coastal sediments. Bacteria that grew aerobically on MMPA and DMSP were isolated from marine environments and phytoplankton cultures. Enrichments with DMSP selected for bacteria that generated DMS, whereas MMPA enrichments selected organisms that produced methanethiol (CH_3SH) from either DMSP or MMPA. A bacterium isolated on MMPA grew on MMPA and DMSP, but rapid production of CH_3SH from DMSP occurred only with DMSP-grown cells. Low levels of MPA accumulated during growth on MMPA, indicating demethylation as well as demethiolation of MMPA. The alternative routes for DMSP biodegradation via MMPA probably impact on net DMS fluxes to the marine atmosphere.

Dimethylsulfoniopropionate (DMSP) is present at high concentrations in some marine phytoplankton and macroalgae and the marsh grass *Spartina alterniflora*, where it fulfills an osmotic function (9, 14, 23, 27, 28). DMSP has attracted attention as a precursor of dimethyl sulfide (DMS), which may account for over 90% of natural sulfur emissions from marine regions and about 50% of the global biogenic sulfur entering the atmosphere (1, 15). DMS influences climate because it is oxidized in the troposphere to sulfuric and methanesulfonic acids, which, as strong acids, attract water and promote cloud formation (7). In anoxic marine sediments, in addition to cleavage with DMS production, DMSP is demethylated to 3-methiolpropionate (MMPA) and then to 3-mercaptopropionate (MPA) (19) (Fig. 1). This alternative demethylation pathway for degradation probably affects the net production of DMS from coastal sediments. Alternative routes for DMSP metabolism undoubtedly operate in other marine environments. In support of this idea, we report the isolation of aerobic marine bacteria that demethylated DMSP and MMPA and demethiolated MMPA with the liberation of methanethiol (CH_3SH).

MATERIALS AND METHODS

Isolation and growth of bacteria. Bacteria were enriched and isolated on a medium containing the major salts of seawater at about 50% strength and 0.05 M Tris buffer, pH 8.0 (25). Organic sulfur compounds were added as the sole sources of carbon and energy at concentrations of 1 to 5 mM. Enrichments were established in either 125-ml Erlenmeyer flasks or 18-ml screw-cap tubes containing 50 and 9 ml of liquid medium, respectively. The enrichments were incubated in the dark without shaking at room temperature (about 22°C). Pure cultures were obtained by streaking the bacteria onto medium solidified with 1.5% (wt/vol) Bacto Agar (Difco, Detroit, Mich.). Bacteria that grew aerobically on MMPA, DMSP, or both were initially isolated from phytoplankton cultures and coastal sediments near Miami, Fla. Most research was carried out with the isolates from these sources. On a subsequent cruise to the Caribbean Sea, enrichments were also carried out with *Trichodesmium* colonies that had been washed in sterile seawater. Stations in the Caribbean Sea were located in the region of 16 to 20°N and 62 to 73°W.

Growth in liquid cultures was monitored by measurements with a Klett-Summerson colorimeter. Cells were harvested at 5°C by centrifugation, washed, and resuspended either in a medium lacking substrate or in a mixture of 0.05 M Tris (pH 8.0), 0.2 M NaCl, 0.05 M $MgSO_4$, and 0.01 M KCl.

Analytical methods. Respirometry was carried out in a 5-ml incubated chamber (30°C) with a Clark-type oxygen electrode (3). Cells were incubated in 0.05 M Tris (pH 8.0)–0.2 M NaCl–0.05 M $MgSO_4$–0.01 M KCl at 30°C. Substrates (10 μM) were added after endogenous rates (no substrate) had been determined. In experiments to measure the production of volatile sulfur compounds, cell suspensions (1 ml containing 1 to 2 mg of protein) were incubated in 14-ml vials sealed with butyl rubber serum caps. Substrates (1 μmol) were added at zero time, and 100-μl samples of the gas phase (air) were removed for analysis by gas chromatography. Volatile sulfur compounds were assayed by gas chromatography with flame ionization detection on a column (1.4 m by 3 mm [inner diameter]) of 40/60 Carbopak B HT 100 at 100°C with a carrier gas (N_2) flow of 66 ml/min (4). Dissolved thiols were determined after derivatization to isoindoles by high-performance liquid chromatography with fluorescence detection (21, 22). Protein was measured by a biuret method (13).

Chemicals. Chemicals were usually obtained from Sigma (St. Louis, Mo.) or Aldrich (Milwaukee, Wis.). DMSP was purchased from Research Plus (Bayonne, N.J.) and was also synthesized (6). MMPA was obtained from its methyl ester by alkaline hydrolysis.

RESULTS

The isolates used in the research are described in Table 1. Organisms that grew on either DMSP (strain MD 14-50) or

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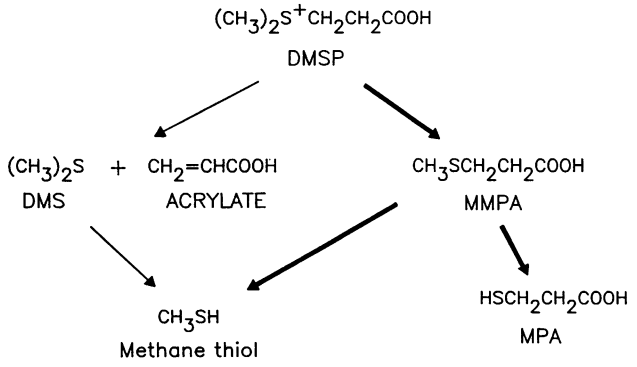

FIG. 1. Aerobic microbial transformations of DMSP in marine environments. Thick lines show transformations proposed in this article.

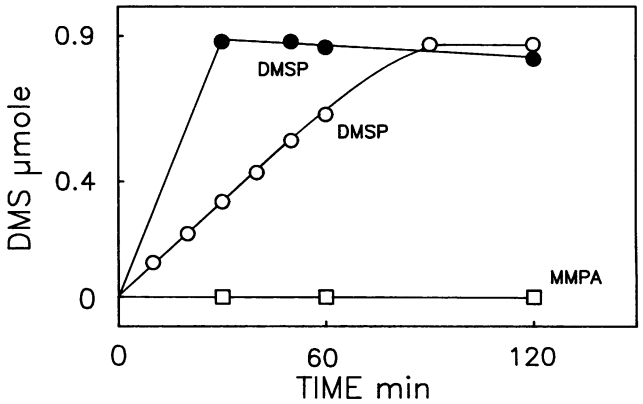

FIG. 2. Production of DMS by strain MD 14-50 grown on DMSP. One milliliter of cell suspension (1.87 mg of protein) was incubated with shaking in a 14-ml vial. One micromole of substrates was added at zero time. DMS was produced from DMSP (●), DMSP (cell suspension, 0.19 mg of protein) (○), and MMPA (□). CH_3SH was not detected in any system.

MMPA (strains DG-AC and DG-8A) or both compounds (strain DG-C1) were investigated. Bacteria that used DMSP were obtained at the 6 stations in the Caribbean Sea at which enrichments were established, and those that used MMPA were obtained at 19 stations. Enrichments and isolates on DMSP generated DMS, whereas those on MMPA produced CH_3SH.

Cells of strain MD 14-50 grown on DMSP showed rapid production of DMS from DMSP but not from MMPA; CH_3SH was not formed from either DMSP or MMPA (Fig. 2). In contrast to strain MD 14-50, the other three organisms (Table 1) generated CH_3SH but not DMS from DMSP or MMPA. An example with strain DG-C1 grown on DMSP is shown in Fig. 3; in this experiment, CH_3SH was rapidly produced from DMSP or MMPA, but DMS was undetectable. CH_3SH did not arise via cleavage of DMS from DMSP and subsequent demethylation, because strain DG-C1 did not grow on or metabolize DMS. DMS was neither consumed nor converted to CH_3SH by cells of strain DG-C1 grown on DMSP, even though CH_3SH was produced from DMSP (Fig. 4). Also, strain DG-C1 was unable to grow on other C_1 compounds (methylamine, trimethylamine, methanol, and formate) or thiosulfate.

Cells of strain DG-C1, when grown on MMPA rather than DMSP, rapidly produced CH_3SH from MMPA but only slowly attacked DMSP, as judged by CH_3SH production (Table 2). More evidence for separate enzymatic steps in the transformation of DMSP and MMPA was provided by strain DG-8A, which did not grow on DMSP; cells grown on MMPA accordingly produced CH_3SH from MMPA but not from DMSP, even upon prolonged incubation. All three strains which grew on MMPA produced CH_3SH at millimolar levels from MMPA during growth as well as in cell suspension experiments. In addition to CH_3SH formation, MMPA was demethylated to MPA. Cultures growing on MMPA accumulated MPA to micromolar levels. MPA was oxidized by cells grown on MMPA but not by propionate-grown cells, as shown for strain DG-AC in Table 3. CH_3SH was not detected as a product of MPA metabolism by cell suspensions of strain DG-C1 grown on DMSP or strain DG-AC grown on MMPA.

DISCUSSION

DMSP was metabolized with either DMS or CH_3SH production; the latter route probably involved demethylation to MMPA and subsequent demethiolation. Strain MD 14-50, which was isolated on DMSP, cleaved DMS from DMSP, presumably by the action of a DMSP lyase similar to enzymes reported to be present in bacteria and algae (16, 26):

$$(CH_3)_2S^+CH_2CH_2COO^- = (CH_3)_2S + CH_2{=}CHCOO^- + H^+$$

Strain MD 14-50 did not metabolize MMPA, in contrast to the organisms isolated on MMPA. Enrichment with DMSP selected for organisms that cleaved DMS from DMSP, whereas MMPA secured bacteria that used other pathways for DMSP degradation. Bacteria isolated on MMPA did not produce DMS from DMSP and thus presumably lacked a DMSP lyase, and CH_3SH was the volatile sulfur compound produced from DMSP and MMPA. Separate enzymes were needed to metabolize DMSP and MMPA because organisms which grew only on MMPA or on both MMPA and DMSP were isolated. Cells grown on MMPA were induced only for MMPA metabolism, whereas those grown on DMSP immediately used both DMSP and MMPA, indicating an initial biochemical rather than chemical (28) demethylation of DMSP. The fate of the methyl group was not determined and is intriguing, since neither DMS nor other C_1 compounds supported the growth of strain DG-C1. Metabolism of the

TABLE 1. Characteristics of marine bacteria isolated on DMSP or MMPA

Organism	Source	Isolation substrate	Growth substrate(s)
MD 14-50	*Trichodesmium* colony	DMSP	DMSP, propionate, acrylate
DG-AC	Cyanobacterial culture	MMPA	MMPA, propionate
DG-8A	*Thalassia* sediment	MMPA	MMPA, propionate, acrylate
DG-C1	*Emiliania huxleyi*	MMPA	DMSP, MMPA, propionate, acrylate

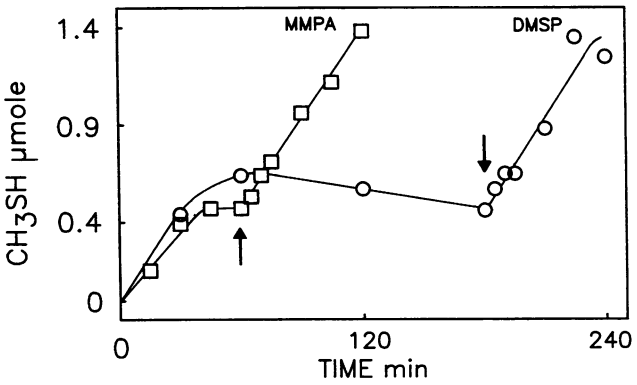

FIG. 3. Production of CH_3SH by strain DG-C1 grown on DMSP. One milliliter of cell suspension (1.58 mg of protein) was incubated with shaking in a 14-ml vial. One micromole of substrate was added at zero time; a further 2 μmol of MMPA was added at 60 min, and 1 μmol of DMSP was added at 180 min (arrows). CH_3SH was produced from DMSP (○) and MMPA (□). DMS was not detected in any system.

methyl group may occur with oxidation, carboxylation, or transmethylation to a suitable terminal acceptor such as halide ions (28) or sulfide ions (10, 12). It appears that bacteria are specialized for the metabolism of either methylated sulfides (15) or DMSP and MMPA but not both suites of compounds.

MMPA was metabolized by demethiolation, to generate CH_3SH, and demethylation, to yield MPA. CH_3SH formation proceeded neither via MPA nor via DMS. Strain DG-C1 did not grow on DMS, and cells grown on DMSP produced CH_3SH from DMSP but not from DMS or MPA. Aerobic demethiolation of MMPA was rapid and may have occurred by either an elimination mechanism or reductive cleavage to yield either acrylate or propionate, respectively. Regardless of the mechanism, demethiolation of MMPA dominated over demethylation, in contrast to anoxic transformations in sediment slurries, in which demethylation of MMPA apparently prevailed (19). The relationship between demethylation

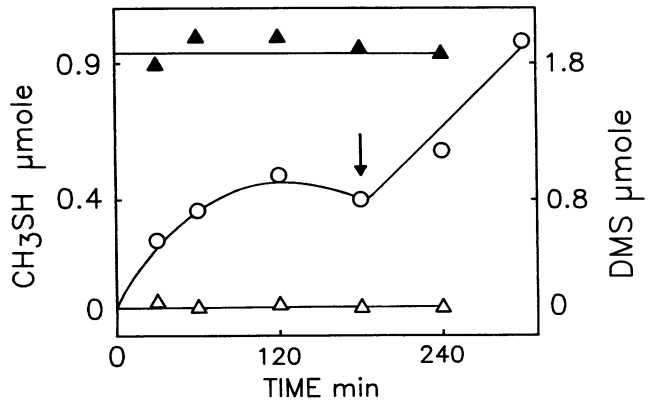

FIG. 4. Metabolism of DMSP but not DMS by strain DG-C1 grown on DMSP. One milliliter of cell suspension (1.02 mg of protein) was incubated with shaking in a 14-ml vial. Two micromoles of DMS or 1 μmole of DMSP was added at zero time; a further 1 μmol of DMSP was added at 180 min (arrow). Symbols: ▲, DMS as substrate; △, CH_3SH produced from DMS; ○, CH_3SH produced from DMSP.

TABLE 2. CH_3SH production from DMSP and MMPA by strain DG-C1 grown on MMPA[a]

Time (h)	CH_3SH produced (μmol)[a] from:	
	DMSP	MMPA
0.5	0.01	0.50
1.0	0.02	0.70
2.0	0.06	0.70
19	0.52	0.43

[a] 1.58 mg of cell protein.

and demethiolation of MMPA needs to be examined with a range of MMPA concentrations because substrate levels (millimolar in the current study as opposed to micromolar previously [19]) may favor demethiolation. Furthermore, demethiolation in slurries may have been obscured by the demethylation of DMS which yielded CH_3SH (19).

The ecology and interactions of the microbial processes which influence the net flow from DMSP to DMS entering the atmosphere are complex but poorly understood. Kiene and Bates (17) recently established that DMS oxidation by bacteria significantly competes with its flux to the atmosphere from surface oceanic waters. The demethylation and demethiolation pathways for DMSP catabolism probably decrease the formation of DMS and its net release to the atmosphere and generate MMPA, CH_3SH, and MPA. The products of the demethylation-demethiolation pathway for DMSP metabolism, CH_3SH and MPA, may be metabolized to H_2S and contribute, together with carbonyl sulfide hydrolysis (11), to the low levels of H_2S in surface ocean waters (8, 20). MPA, as noted previously (18), could also be assimilated by microorganisms and even remethylated to DMSP for use as an osmolyte. CH_3SH may also be oxidized, chemically and biochemically, to dimethyl disulfide and methanesulfonic acid. The only known fate for dimethyl disulfide is microbial reduction to CH_3SH and oxidation to H_2S and then to sulfate (24), a process analogous to the cycling of DMS to dimethyl sulfoxide and back again by photochemical (5) and biochemical (29) oxidations and microbiological reduction (30). The microbial degradation of methanesulfonic acid has been reported (2), but, with the exception of the DMS-to-dimethyl sulfoxide conversion (29), transformations of methylated sulfur compounds by pure cultures of marine microbes have been neglected.

The ease with which bacteria growing aerobically on MMPA were isolated suggests the significance of MMPA in the biodegradation of DMSP in marine environments. One aspect of understanding DMS release to the marine atmosphere will be evaluating how environmental factors affect

TABLE 3. Compounds oxidized by strain DG-AC grown on MMPA or propionate[a]

Substrate	Net oxygen uptake (nmol/min/mg of protein) by DG-AC grown on:	
	MMPA	Propionate
MMPA	68	4
Propionate	17	50
MPA	15	2
Acrylate	23	40
Acetate	58	32

[a] DMSP was not oxidized by either batch of cells.

the flow of DMSP through the DMS-producing and demethylating-demethiolating routes of microbial degradation.

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