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Metabolism of methyl bromide and dimethyl sulfide by marine bacteria isolated from coastal and open waters†

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ABSTRACT: Marine methylotrophic bacteria that consumed methyl bromide (MeBr) and/or dimethyl sulfide (DMS) were isolated: 4 strains from Long Island Sound, USA (LIS) and 1 from the tropical North Atlantic (TNA). Substrates used for enrichment were DMS, trimethylamine (TMA) or TMA plus MeBr. Attempts to obtain isolates on MeBr as the sole source of carbon and energy were unsuccessful. Three of the isolates used MeBr at rates that were higher than chemical loss, and one of these, isolated from coastal LIS, could use this C_1-substrate for biomass production but only when other carbon sources were available simultaneously. Microbial consumption stopped when the MeBr concentration was higher than 0.5 mM and, similarly, when the DMS concentration was higher than 5 mM. The presence of DMS greatly enhanced MeBr consumption in cell suspensions. Inhibitor studies showed that DMS and MeBr were used by the same metabolic pathway in 2 LIS isolates, one of which displayed hydroxypyruvate reductase activity, indicative of the serine pathway of carbon assimilation. A third isolate obtained from the TNA that used MeBr did not use DMS, whereas 2 additional LIS strains isolated on DMS did not use MeBr. Therefore, MeBr utilization is not a common trait of DMS-degrading bacteria. MeBr concentrations in the marine environment are in the pM range and are the highest in coastal waters. This study indicates that growth on MeBr alone is unlikely to support bacterial growth and that removal of MeBr may be a cometabolic phenomenon. However, regardless of growth, consumption by marine bacteria may limit the flux of MeBr to the atmosphere.

KEY WORDS: Methyl bromide · Dimethyl sulfide · Methylotrophy · Trimethylamine

INTRODUCTION

Methyl bromide (MeBr) is an atmospheric trace gas of both natural and anthropogenic origin. It is believed to be the principal source of stratospheric bromine, a highly effective catalyst for the destruction of ozone. MeBr may account for as much as 10 to 15% of the depletion in stratospheric ozone (Albritton & Watson 1992). Currently in the atmosphere at a volume mixing ratio of approximately 10 parts per trillion (Butler & Rodriguez 1996), atmospheric levels are believed to be increasing at a rate of 3% yr^{-1} (Khalil et al. 1993). Although bromine is present at approximately 400 times lower concentrations than chlorine in the stratosphere, on a per atom basis, bromine is 40 to 100 times more effective than chlorine in removing ozone from the stratosphere. The use of MeBr as an agricultural and industrial fumigant has led to its inclusion in the 'Montreal Protocol on Substances that Deplete the Ozone Layer' (UNEP 1992). There remains considerable uncertainty, however, in the factors controlling its atmospheric budget and lifetime (Butler 1995).

The oceanic lifetime is an important parameter in global models seeking to predict the atmospheric response to proposed changes in MeBr emissions from anthropogenic sources. Oceanic loss due to chemical degradation is well defined; however, microbial consumption of MeBr in the marine water column may also affect the oceanic lifetime. While biological consumption of MeBr in coastal water samples has been

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demonstrated (King & Saltzman 1997, Goodwin et al. 1998), the organisms and metabolic pathways responsible have not been identified.

MeBr is one of many one-carbon (C_1) compounds found in the marine environment. The biological production of MeBr and other low-molecular weight halocarbons has been associated with marine macroalgae (Fenical 1981, Wever et al. 1991, Nightingale et al. 1995), as well as phytoplankton (Moore & Webb 1996, Sæmundsdóttir & Matrai 1998, Baker et al. 1999). Sources of C_1-compounds significant in the marine environment include the methylated substrates glycine betaine (GB), dimethylsulfoniopropionate (DMSP) and trimethylamine-*N*-oxide, all common osmolytes in marine organisms. Degradation of these compounds results in C_1-substrates (methylamines, dimethyl sulfide [DMS] and methanethiol) which may be utilized by marine methylotrophs (Kiene 1993, Sieburth et al. 1993). C_1-utilizing bacteria (methylotrophs) represent an important functional group in oceanic waters (Hanson 1980, Visscher et al. 1992, Sieburth et al. 1993). Enzymes that catalyze degradation of some of these C_1-compounds may also accept MeBr as a substrate (Visscher et al. 1996, Goodwin et al. 1998). Other marine bacteria capable of MeBr degradation include ammonium oxidizers (Rasche et al. 1990). However, methanotrophs, which oxidize MeBr in soils (Oremland et al. 1994b) and fresh water samples (Goodwin et al. 1998), were not involved in MeBr oxidation in coastal water samples (Goodwin et al. 1998). Moreover, most cell-specific rates reported compete poorly with chemical and physical loss rates, especially when reasonable population densities are considered (Rasche et al. 1990, Goodwin et al. 1998).

Although standing seawater concentrations of MeBr (~10 pM) may not be sufficient to support bacterial growth and metabolism, we hypothesize that co-metabolic uptake of MeBr with other C_1-substrates, such as DMS or trimethylamine (TMA), is likely. DMS is ubiquitous in marine surface waters and bacterial consumption of DMS has been demonstrated in a variety of marine environments (Visscher et al. 1991, Kiene 1993, Visscher & Taylor 1993). For this present study, DMS metabolism was used as a model for potential pathways of MeBr degradation. In addition, trimethylamine (TMA) enrichments were carried out, because DMS enrichments are often unsuccessful due to toxicity of this volatile organosulfur compound (de Zwart et al. 1996). This work presents data from several strains of marine methylotrophic bacteria, isolated from 2 different geographic locations, some of which consume MeBr. Our results suggest that microbial pathways exist for the metabolism of MeBr in some marine methylotrophs and that in some organisms, similar pathways may exist for MeBr and DMS degradation.

MATERIALS AND METHODS

Sample collection. Seawater samples from Long Island Sound, USA (LIS) were collected in glass bottles and used as inoculum for enrichments immediately upon collection. Additional enrichments were carried out with *Fucus vesiculosus* thallus material: *F. vesiculosus* was collected, blotted dry and placed in 100 ml bottles that contained filtered (0.45 µm) and autoclaved seawater. After shaking (1 h, 50 rpm), the seawater fraction was then used for enrichments upon serial dilution to 10^{-6}. Additional samples were collected from the tropical North Atlantic (TNA) (15° 43.3' N, 48° 5.3' W; collected at 85 m depth; courtesy of Dr K. Rolfhus). These samples were shipped and stored on ice until processed within 1 wk.

Medium. A carbonate-buffered mineral medium (Visscher et al. 1991) consisted of the following (in g l^{-1}): NaCl (20.0), NH_4Cl (0.2), $CaCl_2 \cdot 2H_2O$ (0.225), KCl (0.2), $MgCl_2 \cdot 6H_2O$ (0.2), anhydrous KH_2PO_4 (0.02), and anhydrous Na_2CO_3 (2.0). The medium was supplemented with a trace element solution (1 ml l^{-1}; Widdel & Pfennig 1981) and 1 ml l^{-1} of the vitamin solution of either Pfennig (1978) or Kanagawa et al. (1982), and the carbon source as described. Carbonate, carbon sources, vitamins and trace elements were added after autoclaving the mineral medium.

Isolation and cultivation. Isolations of methylotrophs were carried out using one of the following carbon sources: TMA (0.5 or 5 mM), DMS (0.5 mM; replenished upon depletion), and MeBr (0.3 mM) in combination with TMA (0.5 mM; both replenished upon depletion). Enrichments on MeBr (10 µM to 0.5 mM) alone were not successful. The enrichment medium consisted of a 1:1 (v/v) mixture of filtered and autoclaved seawater and mineral medium. Incubations were carried out in the dark at 30°C, either statically or on a shaking incubator (50 rpm). Liquid cultures were transferred at least 3 times before being plated to check for purity. Plates contained 1.5% (w/v) agar and 5 mM of a carbon source (TMA, GB, methanol [MeOH]), or mineral medium with MeBr or DMS in the headspace. The MeBr and DMS plates were kept in sealed containers with air. As MeBr- and DMS-consuming organisms were often difficult to grow on agar plates, serial dilution to extinction was performed to confirm purity.

Routine cultivation was carried out in flasks with cotton plugs, using GB (16 mM) or MeOH (25 mM) as a carbon source. Growth on MeBr and DMS was accomplished in 72 ml crimp-sealed serum bottles containing 20 ml medium and 52 ml headspace. MeBr was added to final concentrations of 75 µM, alone or with MeOH (3 mM). MeBr was replenished upon depletion (every 1 to 3 d). Concentrations of MeBr higher than 250 µM

retarded growth, while 500 µM completely inhibited MeBr consumption. DMS was added at a 3 mM concentration, because higher concentrations supported very slow or no growth. Controls for chemical losses of MeBr consisted of uninoculated medium with the substrate. The average chemical loss rate was approximately 0.48 µM MeBr h^{-1} (standard deviation = 0.12, n = 28), except when TMA was present. Chemical MeBr loss rates in the presence of TMA were concentration dependent, and ranged from 1.25 µM MeBr h^{-1} (standard deviation = 0.10, n = 3) in the presence of 0.5 mM TMA to 2.81 µM MeBr h^{-1} (standard deviation = 0.14, n = 4) when 50 mM TMA was added.

Isolates were tested for their ability to grow aerobically on various C_1-substrates, sugars, organic acids and as ammonium oxidizers. Growth was monitored by measuring optical density at 580 nm, by assessing cell densities obtained from acridine orange direct counts (Hobbie et al. 1977) using an Olympus Vanox epifluorescence microscope and, when appropriate, by measuring MeBr or DMS decrease in the headspace using a gas chromatograph (GC; see below).

Cell suspension experiments. Cells were harvested by centrifugation (15 min at 10000 × *g* at 5°C), washed twice and resuspended in medium lacking growth substrate. An aliquot of the suspension was removed for cell counts, then 2 ml was dispensed into 14 ml serum bottles which were crimp-sealed with butyl rubber stoppers. Substrates were added via sterile syringe. Treatments with MeBr alone and MeBr plus a co-substrate (GB, MeOH, TMA or DMS) were tested. MeBr was added to a final liquid concentration of 85 to 100 µM. Co-substrates were added to a final concentration of 5 to 50 mM. Inhibitors were used to identify potential metabolic pathways of MeBr consumption: chloroform (0.5 mM) as a transmethylation inhibitor and methyl butyl ether (0.5 mM) as an inhibitor of monooxygenases (Visscher & Taylor 1993). Chemical loss was measured in bottles containing medium plus substrate(s) only. Substrate uptake was measured by headspace analysis using a GC (see below).

Hydroxypyruvate reductase activity. Hydroxypyruvate reductase (EC 1.1.1.81) activity, which is indicative for the serine cycle, was determined spectrophotometrically according to Krema & Lidstrom (1990). Cell-free extracts were prepared from cultures of all isolates grown on MeOH or GB. *Methylobacterium extorquens* AM1 (kindly provided by Dr Mary Lidstrom) was used as a positive control. Protein content of cell-free extracts was determined according to Smith et al. (1985).

Analytical methods. MeBr was determined by headspace analysis on a Shimadzu GC-14A gas chromatograph equipped with a column of 5% Krytox 143 AC Graphpac GB (Alltech Assoc., Inc., Deerfield, IL, USA) and ^{63}Ni-electron capture detection. DMS was determined by headspace analysis on a Shimadzu GC-14A GC equipped with a column of 40/60 mesh Carbopak B-HT 100 (Supelco Inc., Bellefonte, PA, USA), a cryogenic purge-and-trap system and either flame ionization or flame photometric detection.

Chemicals. Standard chemicals used were of analytical quality and purchased from Sigma Chemical Co., St. Louis, MO, USA, or Aldrich Chemical Co., Milwaukee, WI, USA. DMSP was purchased from Research Plus, Inc., Bayonne, NJ, USA. MeBr was purchased from Scott Specialty Gases, Plumsteadville, PA, USA.

RESULTS

Pure cultures of methylotrophic bacteria were obtained on TMA (strain FV, isolated from *Fucus vesiculosus*, and strain TNA), DMS (strain LIS-1 and strain LIS-2), and MeBr plus TMA (strain LIS-3) (Table 1). Organic carbon sources consumed that supported growth included: methylated amines, MeOH, GB, and dimethylsulfoxide as well as other simple, non-methylated organic compounds including acetate, glycine and glucose

Table 1. Isolation procedure and key metabolic characteristics of the 5 marine methylotrophic strains isolated in this study. S = serial dilution, D = direct isolation, TMA = trimethylamine, DMS = dimethyl sulfide, MeBr = methyl bromide, C = cometabolism during growth, G = growth, U = uptake in cell suspension only, NU = not used, MBE = methyl butyl ether, $CHCl_3$ = chloroform, ($CHCl_3$) = slight inhibition upon chloroform addition

	Strain FV	Strain LIS-1	Strain LIS-2	Strain LIS-3	Strain TNA
Isolation method	S, *Fucus vesiculosis* thallus	D, coastal LIS	D, coastal LIS	D, coastal LIS	D, TNA
Substrate used for isolation	TMA (5 mM)	DMS (0.5 mM)	DMS (0.5 mM)	MeBr (0.3 mM) + TMA (0.5 mM)	TMA (5mM)
Use of MeBr	C	NU	NU	G	U
Use of DMS	G	G	G	G	NU
C_1 inhibitor	MBE	MBE	$CHCl_3$	$CHCl_3$	($CHCl_3$)
Hydroxypyruvate reductase activity	+	+	–	–	–

Table 2. Growth of the marine methylotrophic isolates using various substrates. DMSP = dimethylsulfoniopropionate, DMS = dimethyl sulfide, DMSO = dimethylsulfoxide, MeBr = methyl bromide, GB = glycine betaine, TMA = trimethyl amine, DMA = dimethyl amine, MeOH = methanol, $CHOO^-$ = formate, CH_2O = formaldehyde, +++ = $OD_{580nm} \geq 0.4$, ++ = $0.4 > OD_{580nm} \geq 0.2$, + = $0.2 > OD_{580nm} \geq 0.1$, +/– = $0.1 > OD_{580nm} \geq 0.06$, – = $OD_{580nm} < 0.06$, ND = not determined

Substrate	Strain FV	Strain LIS-1	Strain LIS-2	Strain LIS-3	Strain TNA
DMSP	+	++	++	–	+/–
DMS	+	+	+	+	–
DMSO	+	–	–	–	ND
MeBr	–	–	–	+	–
GB	++	++	+++	++	++
TMA	++	–	–	+	++
DMA	+	–	–	+/–	+/–
MeOH	+	++	++	+	+
$CHOO^-$	–	–	+/–	–	+
CH_2O	+/–	+/–	–	–	–
Acetate	++	++	+++	++	++
Glucose	++	+++	+++	+++	++
Acrylate	–	–	–	–	++
Glycine	++	+/–	+/–	+/–	++
CH_4	–	–	–	–	–
NH_4^+	–	–	–	–	–

(Table 2). DMS consumption occurred in all 4 LIS isolates. Strains LIS-1 and LIS-2, isolated on DMS, did not grow on methylated amines. Strains TNA and FV grew well on glycine, while the other strains used this substrate only marginally. None of the isolates oxidized methane or ammonia (Table 2). Use of MeBr alone was not successful and concentrations higher than 0.5 mM inhibited growth. It appears from these results that all strains are facultative, non-methanotrophic methylotrophs.

Cell suspension experiments

Pure cultures of all isolates were tested for their MeBr-degrading capability in cell suspension. Strains LIS-1 and LIS-2 did not degrade MeBr. In contrast, consumption occurred at much faster rates than chemical loss in strains FV, LIS-3 and TNA (Table 3). In these strains, the rates of MeBr consumption were influenced by the presence of additional substrates (Table 3). The addition of GB inhibited MeBr degradation rates in all strains. Increasing GB concentrations had a greater inhibitory effect: at a 50 mM GB addition, 100, 82 and 73% inhibition was observed for FV, LIS-3 and TNA, respectively. Similarly, TMA additions to cell suspensions of these 3 strains inhibited MeBr consumption, especially at a concentration of 50 mM (89, 69 and 50% inhibition in strain FV, LIS-3 and TNA, respectively). DMS addition tripled the MeBr consumption rate in strain FV and doubled it in strain LIS-3. DMS-degrading strains (FV, LIS-1, LIS-2 and LIS-3) consumed DMS in cell suspension at rates that were approximately 2 to 3 orders of magnitude higher than those for MeBr degradation (Table 4).

Chloroform inhibited DMS consumption in strains LIS-2 and LIS-3 and MeBr consumption in strain LIS-3. In contrast, methyl butyl ethyl (MBE) had no effect. In cell suspensions of these strains, use of MeBr was inhibited by 52% (Table 3) and consumption of DMS by 82 to 97% (Table 4). The metabolic pathway of both DMS and MeBr consumption in strain LIS-3 is therefore likely to involve transmethylation reactions (Visscher & Taylor 1993). In contrast, DMS degradation in strains FV and LIS-1 was inhibited by MBE only, the addition of which resulted in a reduced rate of substrate oxidation of 81 and 94%, respectively (Table 4). These data indicate that these strains use a monooxygenase for DMS metabolism (Visscher & Taylor 1993). MBE also inhibited MeBr metabolism in strain FV (Table 3), indicating that this isolate uses the same pathway for DMS and MeBr metabolism. Consumption of MeBr plus MeOH was slightly inhibited (by 18%) in strain TNA upon $CHCl_3$ addition, while the presence of MBE did not affect MeBr degradation (Table 3).

Table 3. Rates of MeBr degradation in cell suspensions of FV, LIS-3 and TNA, and effects of additional substrates and inhibitors, $CHCl_3$ and MBE. All rates are corrected for the chemical loss that applied for the specific incubation condition. Chemical loss rates averaged 567 nmol MeBr l^{-1} h^{-1} (standard deviation = 118, n = 24). Data represent average of 1 to 8 experiments ± standard deviation. ND = not determined

Substrate	Strain FV	Strain LIS-3	Strain TNA
	(nmol MeBr consumed h^{-1} 10^{-9} cells)		
MeBr (92 ± 7 µM)	2.39 ± 1.00	1.78 ± 0.56	1.04 ± 0.51
MeBr + 0.5 mM TMA	2.50 ± 0.86	ND	ND
MeBr + 5 mM TMA	1.98 ± 0.39	1.03 ± 0.20	ND
MeBr + 50 mM TMA	0.26 ± 0.37	0.56 ± 0.11	ND
MeBr + 0.5 mM GB	2.26 ± 0.33	1.80 ± 0.25	1.27 ± 0.20
MeBr + 5 mM GB	1.02 ± 0.21	1.99	0.74 ± 0.16
MeBr + 50 mM GB	0 ± 0	0.49 ± 0.15	0.18 ± 0.13
MeBr + 0.5 mM DMS	3.78 ± 0.42	2.13 ± 0.42	–[a]
MeBr + 5 mM DMS	9.76 ± 1.36	4.00 ± 0.25	–[a]
MeBr + 0.5 mM $CHCl_3$	2.48 ± 0.71	0.85 ± 0.15	1.23 ± 0.16
MeBr + 0.5 mM MBE	0.64 ± 0.18	1.71 ± 0.61	0.85 ± 0.13

[a]Strain TNA does not utilize DMS (see Table 2)

Table 4. Rates of DMS degradation in cell suspensions of FV, LIS-1, LIS-2 and LIS-3. Averages of 1 to 3 experiments ± standard deviation

Substrate	Strain FV	Strain LIS-1	Strain LIS-2	Strain LIS-3
	(nmol DMS 10^6 cell^{-1} h^{-1})			
DMS	0.48 ± 0.08	2.45 ± 0.62	4.16 ± 0.11	0.78 ± 0.15
DMS + 50 mM GB	0.08	0.45	0.52 ± 0.18	0.22 ± 0.09
DMS + 0.5 mM $CHCl_3$	0.52 ± 0.04	2.39 ± 0.40	0.75 ± 0.23	0.02 ± 0.03
DMS + 0.5 mM MBE	0.09 ± 0.06	0.15 ± 0.16	4.03 ± 0.21	0.74 ± 0.11

Apparent MeBr uptake rates in cell suspensions of LIS-3 changed with increasing concentrations of MeBr: from 25 to 150 µM, consumption increased with increasing MeBr, but uptake rates decreased at 250 µM and no consumption occurred at 500 µM MeBr. Rates observed in the concentration range of 25 to 150 µM were used in an Eadie-Hofstee plot, and apparent kinetic parameters v_{max} (1.58 µM h^{-1}) and K_S (12.9 µM) were estimated (Fig. 1).

Growth experiments on MeBr and DMS

Experiments were performed with strains FV, LIS-3 and TNA to determine if MeBr or DMS uptake resulted in growth. Under conditions applied (50 to 250 µM MeBr, with and without addition of MeOH), MeBr was not notably consumed in strain TNA and did not support an increase in cell density. As mentioned above, this isolate degraded MeBr, but not DMS, in cell suspension.

Growing cultures of strain FV only consumed MeBr when a second substrate (DMS or MeOH) was present and MeBr was partially consumed (Fig. 2). When the culture was supplemented with additional MeOH, MeBr consumption did not resume. However, when cells were harvested and resuspended in fresh medium, MeBr consumption proceeded (Fig. 2), indicating possible formation of inhibitory metabolic products. MeBr consumption by strain FV did not support production of biomass; however, growth on DMS resulted in increase in cell numbers (Fig. 3). The addition of MeOH slightly stimulated DMS consumption rates in this strain. The cell yield was the same for MeOH and DMS (1.25×10^6 cells µg^{-1} C consumed).

Complete degradation occurred in cultures of strain LIS-3 inoculated with 75 or 150 µM MeBr. This consumption resulted in a modest increase in cell numbers (Fig. 4). Furthermore, the cell density in the MeBr plus MeOH added cultures was greater than in the cultures on MeOH alone. The presence of MeOH did not inhibit MeBr consumption (Fig. 4), indicating that this strain utilizes MeBr directly, rather than MeOH formed through chemical MeBr hydrolysis. Growth on MeOH and 250 µM MeBr resulted in a longer lag phase, but similar doubling time (data not shown). The cell yield of strain LIS-3 on MeBr was 1.92×10^6 cells µg^{-1} C (standard deviation = 1.07×10^6, n = 4).

Strain LIS-3 grew on DMS as the sole source of carbon and energy. DMS consumption doubled when MeOH was added. The cell yield of this strain was 2.60×10^6 cells µg^{-1} C (standard deviation = 0.13×10^6, n = 4) when growing on MeOH, and 1.46×10^6 cells µg^{-1} C (standard deviation = 0.20×10^6, n = 4) when growing on DMS. In both FV and LIS-3, total consumption of DMS only occurred in cultures amended with 3 mM DMS or less. Higher levels of DMS slowed

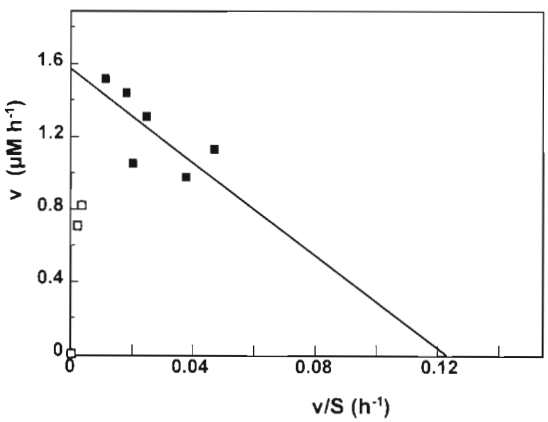

Fig. 1. Eadie-Hofstee single-reciprocal plot of methyl bromide (MeBr) consumption dynamics. (■) Data of rate-limiting MeBr concentrations; (□) inhibitory concentrations which were not included in the plot. $R^2 = 0.50$. The slope is defined as $-K_S$

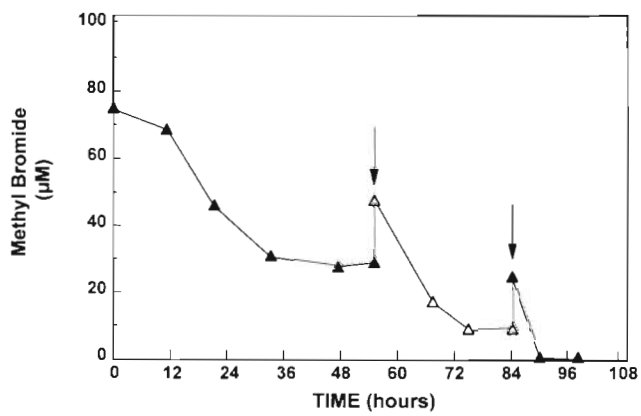

Fig. 2. Consumption of MeBr in a culture of strain FV, growing on 3 mM MeOH plus MeBr. Arrows indicate time of cell harvest and resuspension in fresh medium

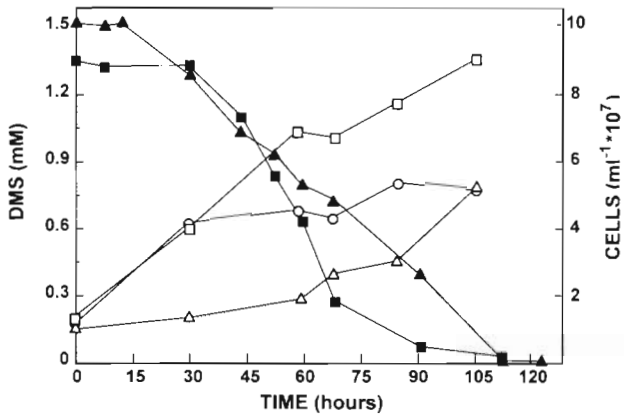

Fig. 3. Growth of strain FV on dimethyl sulfide (DMS). Closed symbols represent DMS concentrations, open symbols designate cell densities. Cells were grown on DMS alone (▲, △), DMS plus 3 mM MeOH (■, □), or MeOH alone (○). Data represent single-bottle experiments that were repeated 3 times

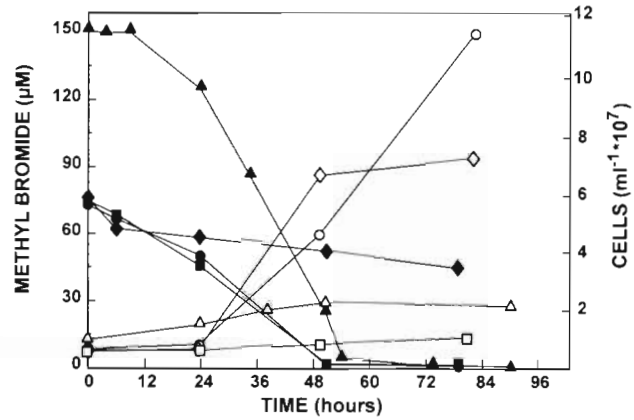

Fig. 4. MeBr utilization by strain LIS-3. Closed symbols indicate MeBr concentrations: chemical loss (♦); 150 µM MeBr (▲); 75 µM MeBr (■); 75 µM MeBr plus MeOH (●). Open symbols represent cell densities: 150 µM MeBr (△); 75 µM MeBr (□); 75 µM MeBr plus MeOH (○); MeOH (◊). Data represent single-bottle experiments that were repeated twice

growth while concentrations >5 mM proved fully inhibitory.

Hydroxypyruvate reductase

None of the isolates showed hydroxypyruvate reductase activity when grown on GB. However, strains FV and LIS-1 grown on MeOH tested positive (Table 1) and rates were 0.23 and 0.09 µM NADH oxidized min^{-1} mg^{-1} protein, respectively. Strains TNA, LIS-2 and LIS-3 grown on MeOH did not have hydroxypyruvate reductase activity (Table 1).

DISCUSSION

MeBr was degraded by 3 out of 5 marine bacterial cultures isolated in this study; 2 originated from coastal LIS and 1 from the TNA. Cell suspensions of these facultative methylotrophic bacteria consumed MeBr faster than chemical destruction. DMS was consumed by 4 strains, all of which were isolated from LIS. This indicates that some, but not all, DMS degraders can use MeBr as a substrate (Table 1). Previous studies indicated the same: The marine methylotrophic strain BIS-6, isolated from Biscayne Bay, Florida, on DMSP, used a variety of methylated substrates, including DMS and MeBr (Visscher & Taylor 1994, Visscher et al. 1996) and MeBr consumption required the presence of DMS or TMA. In contrast, Connell et al. (1997) did not find stimulation of MeBr consumption when DMS was added in water samples of Mono Lake, California.

Methyl halides, including MeBr, are C_1-substrates (Kelly et al. 1993) and so methylotrophs are likely candidates for MeBr degradation. However, MeBr consumption was not found in all of our isolates and supported biomass production in only 1 strain, and is clearly not a common trait of methylotrophs. This is not surprising, since oceanic concentrations are in the pM range (Butler 1995, Moore & Webb 1996, Baker et al. 1999), a concentration that cannot sustain significant bacterial growth by itself.

MeBr-consuming bacteria are most likely to be found in coastal waters since these may contain supersaturated MeBr concentrations (Singh et al. 1983, Manley & Dastoor 1987, Baker et al. 1999). Bacteria in nearshore waters could possibly benefit from high MeBr production rates associated with phytoplankton blooms (Moore & Webb 1996, Baker et al. 1999) or macrophyte populations (Manley & Dastoor 1987). However, experiments with MeBr-producing, unialgal phytoplankton cultures revealed concentrations that peaked at only 0.6 to 2.5 nM MeBr after a 30 to 35 d incubation period (Moore et al. 1996), which makes it unlikely that these primary producers support bacteria that use MeBr as their sole energy and carbon source. In an extensive survey of 19 phytoplankton species by Sæmundsdóttir & Matrai (1998), MeBr was predominantly produced during the stationary phase. The highest production rates occurred in *Phaeocystis* sp. and *Hemiselmis* sp. cultures and were 317 and 98 fmol CH_3Br µg^{-1} chl *a* d^{-1}, respectively. Crashing blooms may be a source of MeBr, and assuming 10 µg chl *a* l^{-1} for a dense *Phaeocystis* bloom (Baker et al. 1999), approximately 3.2 pmol l^{-1} d^{-1} or 38 pg C d^{-1} is pro-

duced. Assuming no chemical loss, a marine bacterial carbon content of 20 fg C $cell^{-1}$ (Cho & Azam 1988) and a bacterial growth efficiency of 20% (Ducklow 1999), this amount of MeBr can sustain a production of only 380 bacterial cells l^{-1} d^{-1}. This extrapolation indicates that it is unlikely that natural MeBr production reported in the literature sustains any significant bacterial production. An earlier study found that *Phaeocystis* sp. produced MeBr during the exponential phase (Scarratt & Moore 1996), but the production rate reported was expressed per nitrogen assimilated, which does not allow for a simple conversion to *in situ* production. Clearly, production patterns by phytoplankton and associated *in situ* consumption by bacteria need further attention.

Brown seaweeds are known producers of methyl halides (Manley & Dastoor 1987, Nightingale et al. 1995, Itoh et al. 1997). In a laboratory experiment, *Macrocystis* sp. thallus produced 4.3 ng MeBr g^{-1} wet tissue d^{-1} (Manley & Dastoor 1987). Extrapolating this to the field assuming a biomass of 76 to 98 kg m^{-2} and an average water depth of 8 to 10 m (Jackson 1987), a kelp bed could produce up to 4.5 nmol l^{-1} d^{-1}, and may therefore be one of the few ecosystems in which MeBr alone is sufficient to sustain bacterial biomass production. Assuming the same bacterial carbon content of 20 fg C $cell^{-1}$ (Cho & Azam 1988) and also the same bacterial growth efficiency of 20% (Ducklow 1999), this amount of MeBr can sustain a production of 0.8×10^6 cells l^{-1} d^{-1}. This production could sustain a small fraction of the total bacterial population (approximately 10^9 cells l^{-1}; Ducklow 1999). Alternatively, our study offers a scenario in which bacteria consume MeBr in combination with other substrates and so keep the concentration in the water column low enough to prevent loss due to ventilation. MeBr consumption did not support biomass production but was consumed rapidly in strain FV, isolated from a 10^6 dilution obtained from *Fucus* sp. thallus material. This demonstrates that a significant part of the bacteria associated with this macrophyte was able to consume MeBr, and may restrict the potential flux to atmosphere. A similar scenario was proposed by Goodwin et al. (1997) for microbial consumption of dibromomethane. Recently, Goodwin et al. (1998) reported growth of a coastal marine enrichment culture on MeBr as the sole source of carbon and energy. Similar to our findings, these authors hypothesize that *in situ* MeBr is consumed as a secondary substrate, while other C_1-compounds are primarily supporting growth in this enrichment culture.

TMA and DMS are common C_1-substrates in the marine environment (Sieburth & Keller 1991, Kiene 1993) and arise from compatible solutes such as GB and DMSP (King 1983, Kiene 1993). These compounds are involved in osmoregulation and may be produced in relatively large amounts in estuarine environments (Reed & Stewart 1985, Kirst 1996) such as LIS. MeBr and DMSP were positively correlated in Eastern North Atlantic waters and coastal water off the British Isles (Baker et al. 1999). Moreover, these authors reported high concentrations of MeBr associated with *Phaeocystis* sp. blooms, an organism known to contain DMSP (Keller et al. 1989) and a producer of DMS (Stefels & van Boekel 1993) as well as MeBr (Scarratt & Moore 1996). Interestingly, MeBr consumption in this study increased in the presence of DMS in the 2 coastal isolates, FV and LIS-3, similar to observations for strain BIS-6 (Visscher et al. 1996). This observation suggested a role of DMS in MeBr metabolism and led to inhibitor studies with MBE and $CHCl_3$, similar to previous investigations of DMS metabolism (Visscher & Taylor 1993). In the current study, MeBr and DMS consumption rates in both FV and LIS-3 were greatly reduced by the same inhibitor (Tables 3 & 4). This suggests that DMS and MeBr are metabolized by the same pathway in these isolates: strain FV oxidizes MeBr and DMS via a monooxygenase reaction and strain LIS-3 uses a transmethylation pathway (Fig. 5). GB and TMA inhibited MeBr degradation in strains FV, LIS-3 and TNA (Table 3). Kiene & Gerard (1995) reported a similar effect of GB on DMSP use, and Wolfe & Kiene (1993) found that GB inhibited DMS consumption. In the strains isolated in this study, GB and TMA seemed to function as competitive substrates for MeBr consumption, while DMS greatly stimulated MeBr degradation (Table 3). Strains LIS-1 and LIS-2, both isolated on DMS, did not grow on TMA but did very well on its precursor GB. Since growth on glycine was slow in these strains and supported much less biomass (Table 2), these strains may only partially demethylate GB. Other studies using $^{14}CH_3Br$ found that TMA stimulated bacterial MeBr degradation (Visscher et al. 1996, Connell et al. 1997, Goodwin et al. 1998), and it is clear that other metabolic pathways than that of DMS could be important in MeBr degradation in the marine environment.

The lack of biomass production in the presence of MeBr in strains FV and TNA points to cometabolism of MeBr in the presence of DMS or MeOH. A similar cometabolic feature was suggested for the marine methylotroph BIS-6 (Visscher et al. 1996), and TMA-stimulated MeBr degradation in Mono Lake (Connell et al. 1997). Marine nitrifiers were also shown to use MeBr cometabolically (Rasche et al. 1990). These observations are in agreement with the low standing concentration of MeBr *in situ*, which is unlikely to support biomass production. A specialized enzyme system that accepts only MeBr is unlikely to exist from a bioenergetic point of view. Since phytoplankton are a source for other methylated substrates like GB, DMSP and

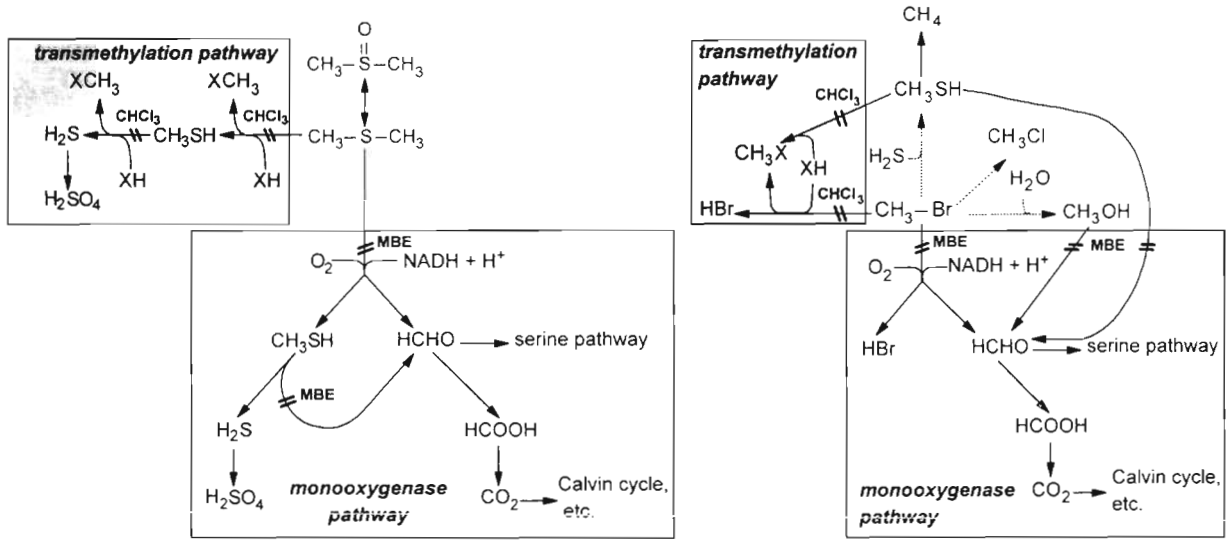

Fig. 5. Proposed metabolic pathways of MeBr (right-hand side) and DMS (left-hand side) consumption. Solid lines depict biological reactions, dashed lines indicate chemical hydrolysis and substitution reactions of MeBr. Monooxygenase pathway (light boxes) is inhibited by methyl butyl ether (MBE) and involves O_2 and NADH. The methyltransferase pathway (dark boxes) is blocked by $CHCl_3$ and involves a methyl acceptor, XH, such as cobamide

DMS (Keller et al. 1989, Sieburth & Keller 1991, Kiene 1993), microorganisms that are adapted to growth on these substrates are ubiquitous (Visscher et al. 1992, Kiene 1993) and may also metabolize MeBr.

Strains LIS-1 and LIS-2, isolated on DMS, did not degrade MeBr. DMS consumption in these strains involved a monooxygenase and transmethylation pathway, respectively (Table 1, Fig. 5), both of which have been found in marine thiobacilli (Visscher & Taylor 1993). Interestingly, strains FV and LIS-1 are both inhibited by MBE, and strains LIS-2 and LIS-3 are both inhibited by $CHCl_3$ (Tables 1, 3 & 4). However, only FV and LIS-3 consume MeBr, which suggests that these strains may have different monooxygenases and transmethylases, respectively, for DMS use than strains LIS-1 and LIS-2 possess.

None of our isolates oxidized CH_4 or NH_4^+ (Table 2), although methanotrophs and nitrifiers are likely MeBr consumers (Rasche et al. 1990, Oremland et al. 1994b) and both are ubiquitous in the coastal marine environment (Sieburth et al. 1993, Voytek & Ward 1995). Based on methyl fluoride inhibition experiments, Goodwin et al. (1998) concluded that in seawater samples off the coast of California these organisms were not responsible for MeBr oxidation. Marine thiobacilli, present in high population densities, consume DMS in the marine environment (Visscher et al. 1991, Visscher & Taylor 1993) and may consume DMS as well. Similarly, hyphomicrobia use monooxygenases to degrade DMS (Kelly et al. 1993) and methyl halides (Leisinger et al. 1993) and are common in the marine environment (Poindexter 1992).

Chemical loss of MeBr was rapid, especially in experiments were methylated amines were added. TMA may accept the methyl group of MeBr to produce tetramethyl ammonium ion [$(CH_3)_4N^+$; G. W. Luther III pers. comm.]. Although MeBr consumption in live cell suspensions was significantly higher than chemical loss, *in situ* use of MeBr could be a combination of chemical and biological reactions. In the marine environment, chemical decomposition of MeBr yields primarily methyl chloride (MeCl) and MeOH (King & Salzman 1997), and microbial use of both of these products has been widely demonstrated (Hanson 1980, Kelly et al. 1993). Hence, MeCl and MeOH consumption by marine methylotrophs could possibly enhance chemical destruction of MeBr through removal of halide-exchange and hydrolysis products, respectively. A similar mechanism was reported by Oremland et al. (1994a) where MeBr reacted with H_2S in anoxic sediments to form methane thiol, which was rapidly consumed by sulfate-reducing bacteria and methanogens.

King & Salzman (1997) demonstrated in a size-fraction experiment that biological sinks for MeBr exist in the marine environment. In their experiment, most of the activity was associated with the 0.2 to 1.2 µm size fraction and they attributed consumption to bacteria. Several studies (Visscher et al. 1996, Goodwin et al. 1998) including the present report have shown that marine bacteria can consume MeBr at rates that are higher than chemical degradation. Some of the observations are of ecological significance: both strains BIS-6 and FV were obtained from 10^6-fold diluted samples.

Although MeBr is unlikely to support significant biomass production, consumption is expected, predominantly in the coastal environment. This microbial consumption may thus decrease the MeBr flux resulting from nearshore production, but is unlikely to have a significant impact in the open ocean. However, further work is needed to evaluate *in situ* rates of microbial MeBr consumption as well as investigate pathways other than C_1-metabolism.

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