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# FORMATION AND EMISSION OF MONOHALOMETHANES FROM MARINE ALGAE

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**Key Word Index**—methyl bromide; methyl iodine; marine macroalgae; marine microalgae; *S*-adenosyl-L-methionine (SAM); halide ion methyltransferase.

**Abstract**—Methyl bromide (5.9 ng g wet algae$^{-1}$ hr$^{-1}$) and methyl iodide (6.9 ng g wet algae$^{-1}$ hr$^{-1}$) were produced by the microalga *Pavlova gyrans*, and methyl iodide (21.4 ng g wet algae hr$^{-1}$ for *Papenfusiella kuromo* and 4 ng g wet algae hr$^{-1}$ for *Sargassum horneri*) and a trace amount of methyl bromide by macroalgae. Methyl halides were synthesized from *S*-adenosyl-L-methionine (SAM) in cell-free extracts of *P. gyrans*, *P. kuromo* and *S. horneri*. This mechanism corresponded to the emission of methyl halides from the three algae *in vivo*. We have studied the optimal pH, and halide ion and methyl donor specificities of the novel enzyme from *P. gyrans*. 

## INTRODUCTION

The marine environment is considered the major source of several volatile halomethanes, including $CH_3Cl$, $CH_3Br$, $CH_3I$, $CHBr_3$, $CH_2Br_2$ and $CHClBr_2$ [1–10]. The emission mechanism regarding di- and trihalomethanes, such as $CHBr_3$ and $CH_2Br_2$, involves the bromoperoxidase reaction in marine macroalgae such as *Corallina* [11, 12] and *Ascophyllum* [13, 14]. The enzyme produces $CHBr_3$ by its reaction with some ketoacids, halide ions and hydrogen peroxide [9]. However, little is known about the marine monohalomethane formation mechanism except for that of $CH_3Cl$. Methyl chloride is produced by marine microalgae [10], and also by reactions involving chloride methyltransferase, which has been found in marine macroalgae [15], a white rot fungus [16] and a terrestrial succulent plant [15]. Other sources of monohalomethanes are anthropogenic sources, such as synthetic methyl bromide as a fumigant [17], biomass burning [18], water chlorination, and natural methyl bromide and methyl iodide from the marine environment [2, 4, 7, 8, 10, 19, 20]. Although methyl bromide and methyl iodide have been detected in the ocean atmosphere and water at high concentrations [2, 19–21], and the direct formation of methyl bromide and methyl iodide has been reported from marine macroalgae [4, 7], information on them is limited to coastal macroalgae and does little to explain $CH_3Br$ emissions from the pelagic ocean [22] and their emission mechanism. We detected methyl halides from a marine phytoplankton, *Pavlova gyrans*, and the marine macroalgae, *Papenfusiella kuromo* and *Sargassum horneri*, and measured the emission rates of methyl halides. We partially purified and characterized a novel halide ion methyltransferase from *P. gyrans* that catalyses the methylation of bromide and iodide ions with *S*-adenosyl-L-methionine (SAM). In this article, we discuss the oceanic source of methyl halides from marine phytoplankton and their formation mechanism at the enzyme level.

## RESULTS AND DISCUSSION

### *Methyl halide-producing marine macro- and microalgae and their methyl halide production rates*

*In vivo* formation of monohalomethanes was measured for 44 free-living macroalgae and 14 artificially cultured microalgae. Among them, we observed the emission of methyl bromide and methyl iodide from a marine microalga, *Pavlova gyrans* (Haptophyceae, Pavlovales), and macroalgae, *Papenfusiella kuromo* (Phaeophyceae, Chordariales) and *Sargassum horneri* (Phaeophyceae, Fucales) (Table 1). Although the artificial culture of microalgae and their collection by centrifugation might have stressed the cells and thereby affected methyl halide emission, this was the first report for the methyl bromide emission from an identified marine microalga. We established that the

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Table 1. Emission of monohalomethanes from marine macro- and microalgae

| Algal species * | Methyl halide production (ng g$^{-1}$ wet algae hr$^{-1}$) $CH_3Cl$ | $CH_3Br$ | $CH_3I$ |
|---|---|---|---|
| *Microalgae* | | | |
| *Pavlova gyrans* CS-213 (non-axenic) | N.D.† | 5.9 | 6.9 |
| *Stichococcus* sp. CS-92 (non-axenic) | N.D. | N.D. | trace |
| *Nannochloris atomus* CS-183 (axenic) | N.D. | N.D. | trace |
| *Macroalgae* | | | |
| *Papenfusiella kuromo* | N.D. | trace | 21.4 |
| *Sargassum horneri* | N.D. | trace | 4.0 |
| *Ecklonia kurome* | N.D. | N.D. | trace |
| *Laminaria angustata* | N.D. | trace | trace |

* Monohalomethanes were not detected from the following macro- and microalgae under the conditions described in Experimental: macroalgae: *Caulerpa okamurae*, *Chaetomorpha moniligera*, *Codium fragile*, *Enteromorpha intestinalis*, *Pseudochlorodesmis furcelate*, *Ulva pertusa* (Chlorophyceae), *Amphiroa zonata*, *Chondria crassicaulis*, *Chondrus occellatus*, *Corallina pilulifera*, *Gelidium amansii*, *Gigartina tenella*, *Gymnogongrus flabelliformis*, *Grateloupia divaricata*, *Grateloupia okamurae*, *Lithothamnium cystocarpioideum*, *Lomentaria catenata*, *Neorhodomela aculeata*, *Odonthalia corymbifera*, *Pachymeniopsis elliptica*, *Pachymeniopsis lanceolata*, *Pterocladia capillacea*, *Schizymenia dubyi* (Rhodophyceae), *Colpomenia sinuosa*, *Dictypoteris prolifera*, *Dictyota dicotoma*, *Dictyota linearis*, *Fucus distichus*, *Gratleria multifida*, *Hydroclathrus clathraths*, *Padina arborescens*, *Padina crassa*, *Pelvetia wrightii*, *Sargassum hemiphyllum*, *Sargassum thunbergii*, *Sargassum ringgoldianum*, *Sargassum fulvellum*, *Sargassum patens*, *Undaria pinnatifida* (Phaeophyceae); microalgae: *Chlamydomonas parkeae* NIES-440 (NIES-collection, Tuskuba, Japan) (Chlorophyceae), *Oscillatoria amphibia* NIES-361 (Cyanophyceae), *Amphora* sp. CS-10 (CSIRO Culture Collection, Tasmania, Australia) (Bacillariophyceae), *Cricosphaera roscoffensis* NIES-8, *Hymenomonas caterae* CCAP-961 (Culture Collection of Algae and Protozoa, Cumbria, U.K.), *H. pringsheimii* CCAP-944 (Haptophyceae), *Chattonella marina* NIES-8 (Raphydophyceae), *Tetraselmis chuii*, CS-26 (Prasinophyceae), *Chroomonas salina* CS-174 (Cryptophyceae), *Nannochloropsis salina* CS-190 (Eustigmatophyceae), *Porphyridium purpureum* CS-25 (Rhodophyceae).

† N.D. not detected.

coexisting bacteria in the microalgal cultures were not the producers of methyl halides (see Experimental), but the contribution of tightly surface-bound or symbiotic bacteria to methyl halides synthesis was not completely eliminated. In addition, trace amounts (around the detection limit of GC-ECD) of methyl iodide were generated by *Ecklonia kurome* (Phaeophyceae, Laminariales), *Stichococcus* sp. (Charophyceae, Klebsormidiales) and *Nannochloris atomus* (Eustigmatophyceae, Eustigmatales); and those of methyl bromide by *Laminaria angustata* (Phaeophyceae, Laminariales). The macroalgae *Ulva*, *Corallina*, *Pterocladia*, *Dictyota* [7] and *Fucus* [8] have been reported to produce methyl iodide. However, we could not confirm this (Table 1), probably due to the detection limits of our analysis system, since we measured the methyl halides in a headspace vial and focused on the high methyl halide-producing algae, while other workers have adopted a purge and cryogenic concentrating system [7, 8]. The other reasons were due to different species and environmentally induced physiological differences such as age, temperature, nutrition, light and tide movement, which probably affected methyl halide production. Yamada *et al.* [24] have shown that the bromoperoxidase activity in the coralline algae is rather different among the species, and Itoh and Shinya [9] have reported that there is a dramatic change in the bromoform emission from *Corallina* throughout the year, depending on the varying bromoperoxidase activity.

The initial production rates of monohalomethanes, which were calculated from the data in Fig. 1, from *P. gyrans* were 5.9 ng g wet algae$^{-1}$ hr$^{-1}$ for methyl bromide and 6.9 ng g wet algae$^{-1}$ hr$^{-1}$ for methyl iodide, and those of methyl iodide were 21.4 ng g wet algae$^{-1}$ hr$^{-1}$ from *P. kuromo* and 4.0 ng g wet algae$^{-1}$ hr$^{-1}$ from *S. horneri*. Wuosmaa and Hager [15] have reported that methyl chloride is produced by the marine algae *Endocladia muricata* (a red alga), *Mesembryanthemum crystallium* (a terrestrial succulent plant) and *Phellinus promaceus* (a white rot fungus), and their evolution rates are between 11.8 and 50.6 pg g cells$^{-1}$ hr$^{-1}$. On the other hand, Schall *et al.* [8] have reported that methyl iodide is emitted by an ice alga, *Fucus distichus*, at a rate of around 20–50 pg g wet algae$^{-1}$ hr$^{-1}$, and Manley and Dastoor [21] have shown the evolution of methyl halides from *Macrocystis pyrifera* (giant kelp) at a rate of 0.7–0.8 ng g wet

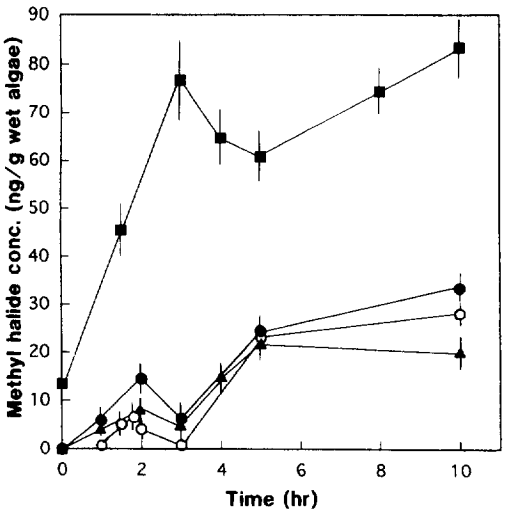


Fig. 1. Time courses of the methyl halide production from *P. gyrans*, *P. kuromo* and *S. horneri*. Methyl bromide (○) and methyl iodide (●) from *P. gyrans* were analysed using GC-MS (SIM), and methyl iodide (■) from *P. kuromo* and (▲) from *S. horneri* using GC (ECD) as described in the Experimental. *P. gyrans* cells were obtained from a 20-l culture ($5 \times 10^7$ cells ml$^{-1}$) to ensure the culture phase. Data points represent results from three samples and the mean value. The bars indicate the variations in the three measurements.

algae$^{-1}$ hr$^{-1}$ for $CH_3Cl$, 0.13–0.17 ng g wet algae$^{-1}$ hr$^{-1}$ for $CH_3Br$ and 0.6–0.7 ng g wet algae$^{-1}$ hr$^{-1}$ for $CH_3I$. Compared with their data, the methyl bromide and methyl iodide emission rates from *P. gyrans*, *P. kuromo* and *S. horneri* were one or two orders of magnitude higher (Table 1). These data show that methyl halides are released from various marine organisms, especially macro- and microalgae, but their emission levels vary considerably. From our data, the high methyl halide-producing marine algae are a minority of the marine algae.

*Profiles of methyl halide production*

Figure 1 indicates the emission profiles of methyl halides from *P. gyrans*, *P. kuromo* and *S. horneri*. Methyl bromide and methyl iodide levels in the vials under dark conditions increased linearly between 1 and 3 hr, but then the emission rapidly dropped, followed by gradual increases. The same profiles were evident for three algae tested. This profile was not seen in the control run of authentic methyl bromide or methyl iodide in seawater without algae. The reason for such declines in the amount of methyl halides produced from algae is unclear. Zafiriou [25] has proposed that atmospheric methyl chloride is produced through the intermediate of biogenic methyl iodide. In this hypothesis, methyl iodide directly produced by marine algae undergoes replacement with chloride ions in seawater to generate methyl chloride. This hypothesis indicates that the nucleophile reactivity towards methyl iodide in seawater is: $Cl^- > H_2O > Br^-$ (relative reactivity 9.4:1:0.12 [25], and methyl bromide formation is lower than the hydrolysis of methyl iodide with $H_2O$. We could not detect the conversion of methyl iodide into methyl chloride or methyl bromide under our experimental conditions (20 in natural seawater for about 10 hr in the dark). The half-life of methyl iodide in seawater at 19.2 is estimated to be 20 days [25]. Therefore, the rapid decay of the formed methyl iodide and methyl bromide could not be explained by this theory. We speculated that they might be eliminated by re-absorption and degradation by the algae in a closed system or by an unknown degrading effect of the epiphytic bacteria on the algae. However, we isolated 20 bacteria from *P. gyrans* culture broth and *P. kuromo* and *S. horneri* thalli surfaces and confirmed that they could not metabolize methyl halides using a suspension of their intact cells (Itoh *et al.*, unpublished data). Therefore, the latter speculation is unlikely.

The production rates from the algae were calculated from the initial emission of methyl halides, as shown in Table 1. The profiles were not significantly influenced by light (200 $\mu$E m$^{-2}$ s$^{-1}$) (data not shown), therefore, methyl halide emission was independent of photosynthesis in such a short time. Methyl iodide emission was observed at 0 time incubation in *P. kuromo*, indicating that the methyl iodide produced was reserved in the tissue or cells of *P. kuromo*.

*Formation mechanism of methyl halides*

We detected SAM : halide ion methyltransferase activity in the cell-free extracts of *P. gyrans*, *P. kuromo* and *S. horneri*. Methyl iodide was produced with SAM and iodide ions, but not with other methyl donor compounds such as DMSP, *S*-methyl methionine (SMM) and L-methionine and iodide ions. Therefore, these enzymes were a SAM : halide ion methyltransferase. Figure 2 shows the relationship between the activities of the enzyme in the freshly prepared cell-free extracts and the methyl halide production rates of three algae *in vivo* in Fig. 1. Although the data of *P. gyrans* was the sum of the rates of methyl bromide and methyl iodide, there was an almost linear relationship between them. These results showed that methyl halide production from algae was catalysed by the reaction of SAM : halide ion methyltransferase *in vivo*, and its production rate depends on the enzyme's activity in the algae. Therefore, methyl halides are most probably produced by such enzymes from a marine environment. This formation mechanism is completely different from that of tri- and dihalomethanes by bromoperoxidase [9, 26]. Taking into account the data of Wuosmaa and Hager [15] and those of *P. gyrans*, *P. kuromo* and *S. horneri*, there are different types of SAM : halide ion methyltransferase in marine macro- and microalgae, although most occur in small amounts in algae and are rather unstable *in vitro*. The enzymes may have a role in regulating the concentrations of halide ions in the cells. However, con-

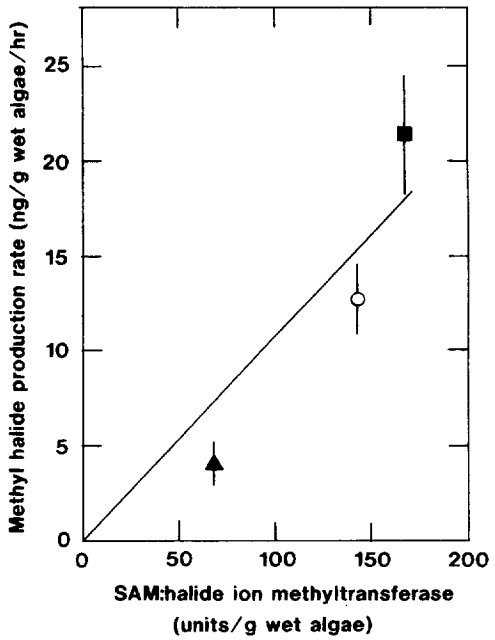


Fig. 2. Relationship between the methyl halide production rate *in vivo* and SAM: halide ion methyltransferase activity in algae. The production rate from *P. gyrans* (○) was the sum of methyl iodide and methyl bromide. The others were those of methyl iodide (■) from *P. kuromo* and (▲) from *S. horneri*. One unit of the enzyme activity was defined as the amount that produced 1 pmol of methyl iodide $min^{-1}$ as described in the Experimental.

sidering that SAM synthesis from L-methionine is an ATP (energy)-consuming reaction [27], methyl halide production by algae seems to have other important physiological functions in marine algae such as an allelopathic chemical.

*Some properties of SAM : halide ion methyltransferase in* P. gyrans

The enzymes from *P. kuromo* and *S. horneri* were unstable and lost their activities almost completely upon storage for a few days at 4 or −20 even if dithioerythritol, EDTA, protease inhibitor or glycerol were added to the extracts. The extract of *P. kuromo* was too viscous with contaminating polysaccharides to purify it. In addition, the enzymes from *P. kuromo* and *S. horneri* were specific for iodide ions, and activity toward bromide ions was not detected *in vitro*. Therefore, we focused on the enzyme of *P. gyrans* because its activity was more stable than those of other sources, and it catalysed not only iodination but also bromination of SAM *in vitro*. The supernatant of the disrupted cells was used as the crude enzyme. The enzyme was characterized using this preparation and iodide ions as the halide, unless otherwise indicated. The control run generated a small amount of methyl iodide from iodide ions with SAM. Therefore, we subtracted it from the measured values. To reveal the effect of pH on the enzyme, its activity was measured in 50 mM potassium phosphate buffer (pH 5.5–7.5) and 50 mM Tris–HCl buffer (pH 7.5–9) at 30°, as described in the Experimental using gas chromatography with an electron capture detector. The high enzyme activity was between pH 7.0 and 7.5, and it exhibited about 60% of maximum activity at pH 6 and 8. The optimal pH was similar to the chloride methyltransferase isolated from the marine red algae, *Endocladia muricata* [15]. The Michaelis constant $K_m$ for iodide ions calculated from the Lineweaver–Burk plot was 63 mM, and that for SAM was 24 μM (Fig. 3). However, the enzyme reaction was inhibited at an iodide ion concentration of over 25 mM as shown in Fig. 3. The relatively high $K_m$ value towards iodide ions of the enzyme suggested their accumulation in *P. gyrans*, considering the low concentration of iodide ion in seawater ($I^-$ 60 μg $l^{-1}$ of seawater).

The halide ions and methyl donor specificities of the enzyme were measured by head space-GC-mass spectrometry analysis. The results are shown in Table 2. The enzyme from *P. gyrans* was specific for bromide and iodide ions, but inactive toward chloride ions.

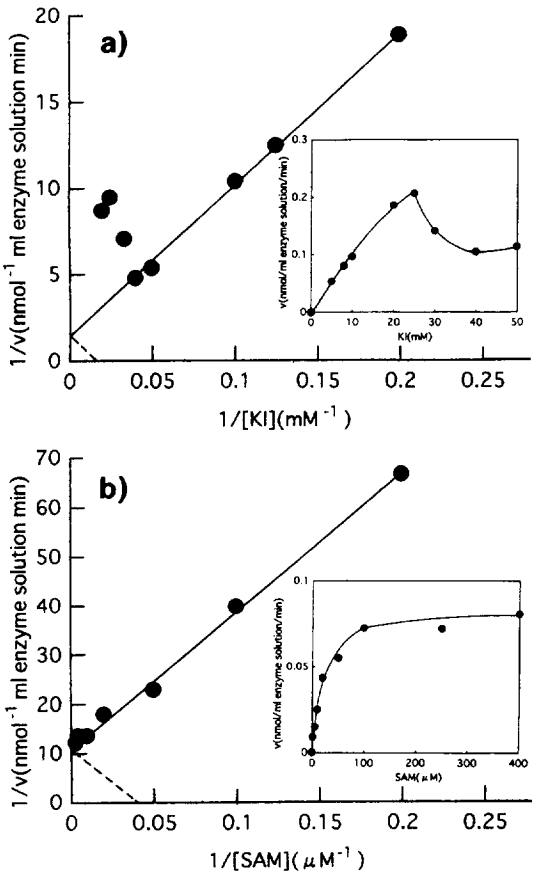


Fig. 3. Lineweaver–Burk plots for the reaction of SAM: halide-ion methyltransferase from *P. gyrans*. The reaction proceeded at pH 7.0 (50 mM MES buffer), as described in Fig. 2, with (a) varying amounts of KI as indicated at 2 μmol SAM; and (b) varying amounts of SAM at 0.2 mmol KI, in a total volume of 10 ml. Methyl iodide levels were analysed by GC (ECD).

Table 2. Substrate specificity of methyl halide transferase from *P. gyrans* CS-213. Headspace gas was analysed as described in the Experimental section by GC-MS (SIM)

| Halide | Methyl donor | Production rate of methyl halide (pmol $min^{-1}$ ml-enzyme$^{-1}$) |
|---|---|---|
| $Cl^-$ | SAM | 0 |
| Br | SAM | 1.8 |
| $I^-$ | SAM | 19.5 |
| $I^-$ | DMSP | 0 |
| $I^-$ | SMM | 0 |
| $I^-$ | L-methionine | 0 |

This differed from the *E. muricata* enzyme [15]. Because the trans-methylation reaction was considered to be a nucleophilic substitution of halide ions with SAM, other likely methyl donors reported in the marine organisms for the reaction were tested. However, SAM served as a substrate for the enzyme, whereas DMSP [23], SMM and L-methionine could not serve as a methyl donor. Therefore, this enzyme was defined as a novel SAM : halide ion methyltransferase.

*Oceanic methyl halides emission*

The monohalomethanes produced by marine microalgae are probably important in the global cycling of gaseous organohalogen species, especially bromine and iodine, because of their large biomass ($5.9 \times 10^{14}$ g carbon) [28]. From the viewpoint of stratospheric ozone depletion, methyl bromide is the most destructive compound because it has a high ozone depletion potential (ODP of 0.7), as a result of its bromine content and its relatively long half-life in the air (about 2.0 years) [20] compared with bromoform and dibromomethane. Khalil *et al.* [20] have estimated that the ocean flux of methyl bromide is $3.5 \times 10^{10}$ g $year^{-1}$, and Singh *et al.* [2] considered it to be $30 \times 10^{10}$ g $year^{-1}$, whose values were calculated from the average concentration of methyl bromide in air and seawater using the model of Liss and Slater [29]. On the other hand, Lobert *et al.* [30] lately reported that there was no supersaturation of methyl bromide in seawater (−16%) and that the oceans may be a net sink, not a source of methyl bromide. This large discrepancy is mainly due to the degree of supersaturation of methyl bromide in seawater. Although our results cannot provide information on whether the ocean is the net source of, or sink for, methyl bromide, the results showed marine microalgae to be one of the producers of methyl bromide and its source from the pelagic ocean. From the biomass of phytoplankton, we could roughly calculate the prevalence of methyl bromide-producing phytoplankton in seawater. Assuming that the flux from the sea surface of methyl bromide is about $3.5 \times 10^{10}$ g $year^{-1}$ [20], the emission rate of methyl bromide from its producing phytoplankton is $5.9 \times 10^{-9}$ g g wet $algae^{-1}$ $hr^{-1}$ (about $51 \times 10^{-6}$ g g wet $algae^{-1}$ $year^{-1}$ from our data) and the biomass is $7.9 \times 10^{15}$ g wet wt algae, which is based on the fact that the dry wt of algae is 25% of the wet wt and that 30% of the dry wt is carbon ([28], our unpublished data), the emission rate is calculated based on the following equation: rate (g $year^{-1}$ = $7.9 \times 10^{15}$ g wet algae $\times 51 \times 10^{-6}$ g g wet $algae^{-1}$ $year^{-1}$ × the prevalence of methyl bromide-producing algae in the total algae (%). Calculating the equation, the prevalence of methyl bromide-producing microalgae in seawater is 8.8%. This value appears to be somewhat high, but is conceivable. Although we have not yet obtained sufficient information concerning the distribution of methyl bromide-producing microalgae in pelagic ocean, our data suggest that marine microalgae are the main oceanic source of methyl bromide.

## EXPERIMENTAL

*In vivo* formation of monohalomethanes was measured in 44 macroalgae and 14 microalgae. Macroalgae living in the intertidal and benthic areas were collected from the beach on the Sea of Japan (Mikuni, Fukui Prefecture, Uozu, Toyama Prefecture), and the Tsugaru Channel (Eyama, Hokkaido) between May 1993 and July 1994 by SCUBA diving and were brought to our laboratory, within 12 hr, in seawater. We used the algal samples as free of visible epiphytes as possible. If necessary, we removed the visible epiphytes from the algae by brushing. After washing with the autoclaved seawater, the macroalgae were dried between paper towels for 2 min. Whole algal thalli were used when possible. Some tissue disks cut from the middle of mature blades were used with thalli too large for analytical vials. The macroalgal sample or tissue disk (1.5–3 g wet wt) was transferred into 50 mol of autoclaved seawater in a vial (20 ml headspace), which was then sealed with silicone rubber and an aluminium cap and incubated at 20 in the dark for between 0 and 10 hr. Thereafter, the vial was heated at 50 for 15 min to ensure the transfer of volatile halomethanes in the tissues or the cells to the gas phase, which was withdrawn (100 $\mu$l) and analysed using a GC or GC-MS. The heat treatment did not affect the concn levels of control methyl halides in soln.

Marine microalgae, which were supplied from the NIES-collection (Tsukuba, Japan), CSIRO Culture Collection (Tasmania, Australia) and CCAP (Culture Collection of Algae and Protozoa, Cumbria, U.K.), were maintained by the methods of the suppliers. They were cultivated in a seawater medium supplemented with some nutrition for 10–20 days. Cultivation conditions of each microalgal strain were as follows: (a) marine enrichment basal medium (Sigma) [31] in seawater (2.5 l) in a 3-l conical flask at 25 with sterile air-bubbling (*ca* 25 ml $min^{-1}$) with a filter (0.2 $\mu$m) at 200 $\mu$E $m^{-2}$ $s^{-1}$ (12 hr light and 12 hr dark) for 10–

20 days (*Chlamydomonas*, *Stichococcus*, *Nannochloris atomus*, *Oscillatoria*, *Cricosphaera*, *Hymenomonas* and *Pavlova*); (b) marine enrichment basal medium (Sigma) ([32] *f*/2) in seawater at 25° for 20 days under the above conditions (*Porphyridium*, *Tetraselmis*, *Chroomonas*, *Nannochloris salina*); at 20° for 10 days under the above conditions (*Amphora*); and at 25° for 20 days of static cultivation under the same conditions (*Chattonella*). The level of bacteria in the cultures for non-axenic algal samples was monitored by using a Nikon Optiphoto-II phase contrast microscope with a hematometer for bacteria (0.0025 $mm^2 \times 0.02$ mm) or by counting the living cells grown on the agar plate for 5 days at 20° consisting of 0.02% (w/v) yeast extract, 0.02% $(NH_4)_2SO_4$, 0.025% $KH_2PO_4$, 0.005% EDTA, 0.0005% $FeSO_4 \cdot 7H_2O$ and 1.5% Bacto agar in seawater (pH 7.5). In all cases, the total bacterial count was between $1 \times 10^7$ and $1 \times 10^8$ cells $ml^{-1}$ and that of living cells was less than $5 \times 10^6$ cells $ml^{-1}$. The contaminating bacterial biomass estimated was less than 5% of the microalgal biomass for *Pavlova* and *Stichococcus*, therefore it was thought to be negligible. Especially for the methyl halide-producing algae, we confirmed that the contaminating bacteria were not the producers of methyl halides by using the cultures of them in medium (a) or (b), and in the medium used for the living-cells count without agar, as described above, in the dark. For large scale culture of *P. gyrans*, we used 20-l medium in a polycarbonate container for 10 days cultivation with sterile air-bubbling (*ca* 100 ml $min^{-1}$) in the same manner described above. The intact cultured microalgae cells were collected by centrifugation (9600 *g*, 15 min) and washed once with the autoclaved seawater. The cells (1–2 g wet wt) were transferred into 25 ml of autoclaved seawater in a vial (10 ml headspace) and incubated at 20° in the dark for 0–10 hr. Thereafter, the headspace gas was analysed in the same manner described above.

Monohalomethanes were analysed by a Yamako G-1880 GC with an ECD (Tokyo, Japan) or Shimadzu QP-5000 GC-MS (quadruple polar type) (Kyoto, Japan). Throughout the analysis, autoclaved seawater (105°, for 20 min) was prepd before use to exclude the effect of contaminating volatile compounds in natural seawater. The detection limits of the GC (ECD) equipped with a packed column (3.2 mm × 2 mm) of Poropak Q (column temp. 150°, injection and detection temp. of 250°, flow rate 25 ml $min^{-1}$ of $N_2$) for $CH_3Cl$ and $CH_3Br$ or a packed column (3.2 mm × 4 m) of Chromosorb W (60–80 mesh) coated with silicone DC-550 (20%) (column temp. 80°, injection and detection temp. of 200°, flow rate 20 ml $min^{-1}$ of $N_2$) for $CH_3I$, were 20 ng $ml^{-1}$ (0.02 ppm) for $CH_3Cl$, 2 ng $ml^{-1}$ for $CH_3Br$ (0.002 ppm) and 10 pg $ml^{-1}$ ($10 \times 10^{-6}$ ppm) for $CH_3I$, and those of GC-MS [SIM: selective ion monitoring, DB-624 capillary column (0.3 mm × 30 m, 3 μm film), injection temp. 100°, column temp. 40°, flow rate 20 ml $min^{-1}$ of He, split ratio 5, ionization 70 eV at 200°] were 0.5 ng $ml^{-1}$ (0.0005 ppm) for $CH_3Cl$ and 0.1 ng $ml^{-1}$ for $CH_3Br$ and $CH_3I$. Unfortunately, unknown compounds, due to air or water, hindered the accurate measurements of $CH_3Cl$. In both analyses, calibration curves were made using the standards prepd from dilution. Authentic soln of $CH_3I$ was first dissolved into MeOH; then it was diluted with autoclaved $H_2O$ to the specified concn using a sealed headspace vial and a gas-tight syringe. For $CH_3Cl$ and $CH_3Br$, authentic gaseous samples ($100 \pm 2$ ppm in He) were supplied from Sumitomo Seika Co. Ltd, Osaka, Japan, and they were diluted with He gas and autoclaved $H_2O$ using a sealed headspace vial and a gas-tight syringe. The methyl halide production was confirmed by ion-scanning mode mass-spectrometry.

The SAM : halide ion methyltransferase was obtained from the cells of *P. gyrans* ($2–5 \times 10^7$ cells $ml^{-1}$, *ca* 0.5 g wet wt l culture $broth^{-1}$). *P. gyrans* cells collected from the culture broth (1 g wet wt) were disrupted in a cold mortar with aluminium oxide and extracted with 5-ml of 50 mM 2-(*N*-morpholino)ethane sulfonate (MES) buffer (pH 7.0) containing 10% glycerol. After centrifugation (9600 *g*, 20 min), the supernatant was dialysed against the buffer and was used as the enzyme. For macroalgal samples, 1 g wet wt of the thalli was disrupted with sea sand instead of aluminum oxide. The crude enzyme (0.2–0.4 ml) was incubated in a 10-ml reaction mixture in a vial with a 5-ml headspace consisting of 500 mmol MES buffer (pH 7.0), 200 mmol potassium halide and 2 mmol for SAM. The mixture was incubated for 10–60 min at 30° in the dark. Headspace gas was analysed as described above.

Dimethylsulfoniopropionate (DMSP) was synthesized from dimethylsulfide and bromopropionic acid by the methods of ref. [33].

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