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Effect of temperature on the production rates of methyl halides in cultures of marine proteobacteria

Masahiro Hirata[a], Morito Ikeda[a], Fumiya Fukuda[a], Minami Abe[a], Hiroshi Sawada[b], Shinya Hashimoto[a,*]

[a] Department of Chemistry, College of Humanities & Sciences, Nihon University, 3-25-40 Sakurajosui, Setagaya-ku, Tokyo 156-8550, Japan
[b] Department of Biosciences, College of Humanities & Sciences, Nihon University, 3-25-40 Sakurajosui, Setagaya-ku, Tokyo 156-8550, Japan

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ABSTRACT

Methyl halides released from the ocean serve as important carriers of halogen into the atmosphere. The transported halogens are released into the atmosphere through photolysis, and catalyze ozone depletion. Marine bacteria are known to be a source of methyl halides in marine environments, but the effects of environmental temperature on the production of methyl halides by bacteria have not been studied. Here, we examined the effect of temperature on methyl halide production in a culture of marine bacteria (HKF-1) belonging to *Erythrobacter*, which was incubated for several days at 15 °C, 20 °C, 25 °C, or 30 °C. We also analyzed the effect of temperature on the production of methyl halides in a culture of the marine γ-proteobacterium *Pseudomonas stutzeri*. The concentrations of methyl halides such as CH_3Cl, CH_3Br, and CH_3I in the gas phase above cultured samples were determined using dynamic headspace gas chromatography–mass spectrometry. Bacterial growth was monitored by measuring the optical density at 600 nm. The production rate of CH_3Cl by marine bacteria (HKF-1) was increased with increasing temperature from 15 °C to 30 °C, and the maximum production rate for CH_3Cl was ~19 pmol $(10^9 \text{ cells})^{-1}\ d^{-1}$ at 30 °C, which was about 26 times higher than that at 15 °C (~0.7 pmol $(10^9 \text{ cells})^{-1}\ d^{-1}$). The production rates of CH_3Cl by *P. stutzeri* were also increased with increasing temperature from 15 °C to 30 °C. The production rates for CH_3Br by bacteria (HKF-1) were increased with increasing temperature from 15 °C to 30 °C, whereas changes in the production rates for CH_3I by these two bacteria were relatively small during the temperature rise from 15 °C to 30 °C. Higher production rates of CH_3Cl, CH_3Br, and CH_3I were observed during the exponential phase than during the stationary phase in the cultures of HKF-1. The ratio of production rates (CH_3Cl:CH_3Br:CH_3I) was 1.0:0.12:0.015 in the cultures of HKF-1, and this ratio was almost constant between 15 °C and 25 °C. These results suggested that water temperature could affect the production of methyl halides derived from bacteria and would be a significant factor for estimating the emissions of methyl halides from marine environments.

1. Introduction

Methyl halides such as methyl chloride (CH_3Cl), methyl bromide (CH_3Br), and methyl iodide (CH_3I) have important roles as carriers of halogen to the atmosphere. These compounds release halogens into the troposphere and the stratosphere via photolysis, and the released halogens catalyze ozone depletion (Read et al., 2008). A large portion of the total methyl halides is considered to be derived from natural sources. For example, biomass burning (Yvon-Lewis et al., 2009), coastal salt marshes (Rhew et al., 2000, 2014), fungi (Harper and Hamilton, 1988), rice paddies (Redeker et al., 2000), and terrestrial plant origins such as terrestrial plants (Yokouchi et al., 2002; Wishkerman et al., 2008) and leaf litter (Derendorp et al., 2011, 2012) have all been reported as sources of methyl halides. The oceans are also considered to be one of the natural sources of methyl halides (e.g., Yvon-Lewis et al., 2009; Xiao et al., 2010). In terms of our knowledge of the global budget of methyl halides, the global sinks of CH_3Cl (or CH_3Br) are larger than the known global sources of CH_3Cl (or CH_3Br) (Schäfer et al., 2007; Yvon-Lewis et al., 2009; Montzka et al., 2011). As for the global budget for CH_3I, the sources and a sink (photolysis) of CH_3I were shown to be balanced; however, there remains substantial uncertainty in the estimation of the oceanic source (Bell et al., 2002). Therefore, it remains necessary to clarify the dynamics of CH_3Cl, CH_3Br and CH_3I in the environment.

As for the chemical sources of methyl halides in the ocean, nucleophilic substitution reactions of CH_3Br and CH_3I with chloride ion in

* Corresponding author.
E-mail address: shinya-h@chs.nihon-u.ac.jp (S. Hashimoto).

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seawater are substantial sources of CH_3Cl (Elliott and Rowland, 1993). CH_3I (Moore and Zafiriou, 1994; Happell and Wallace, 1996; Richter and Wallace, 2004) and CH_3Cl (Moore, 2008) in seawater are also thought to be produced by photochemical reactions. In addition, CH_3I and other iodocarbons are also produced from biogenic marine aggregates (Hughes et al., 2008). As for the biological sources, methyl halides in the ocean are known to be produced by macroalgae: e.g., the giant kelp *Macrocystis* produces CH_3Cl (Manley and Dastoor, 1987), the polar macroalgae *Laminaria saccharina* produces CH_3I (Schall et al., 1994) and tropical macroalgae produce CH_3I (Leedham et al., 2013). As the sources of methyl halides in the open ocean, marine phytoplankton are known to produce methyl halides (e.g., *Emiliania huxleyi*, *Phaeodactylum tricornutum*, *Thalassiosira weissflogii*, and *Phaeocystis* sp.; Scarratt and Moore, 1996, 1998). Marine cyanobacteria *Prochlorococcus* and *Synechococcus* (Brownell et al., 2010) and marine proteobacteria such as *Erythrobacter* and *Pseudomonas* (Fujimori et al., 2012) were also reported to produce CH_3Cl, CH_3Br, and CH_3I. In macroalgae, phytoplankton, plants, bacteria and fungi, the production of CH_3Cl, CH_3Br, and CH_3I have been correlated with methyltransferase activity, i.e., the methylation of halide ions using S-adenosyl-L-methionine as a methyl donor produces methyl halides (Manley, 2002).

There have been a few reports on the effects of temperature on methyl halide production in different environments, including peatland (Khan et al., 2012) and salt marshes (Rhew et al., 2014). In peatland, the production rates of CH_3Cl and CH_3Br increase with increasing soil temperature from 10 °C to 35 °C (Khan et al., 2012), while in the salt marshes, the production rates of CH_3Cl and CH_3Br change seasonally, with higher production rates of CH_3Cl and CH_3Br in summer than in winter (Rhew et al., 2014). Recently, temperature was also reported to affect the production of CH_3Cl in the culture of a marine phytoplankton, *Phaeodactylum tricornutum*: the production rate increased along with increasing temperature from 10 °C to 25 °C, and the maximum rate of CH_3Cl production was observed at 25 °C, which was several times higher than that at 10 °C (Abe et al., 2016). Moreover, emission of CH_3Cl from senescent and dead leaves (Hamilton et al., 2003; Derendorp et al., 2011, 2012) and that of CH_3Br from plants (Wishkerman et al., 2008) have been reported to increase greatly with increasing temperature. These results suggest that environmental changes of temperature could have an effect on the production rates of methyl halides by micro-organisms such as bacteria. To date, the impacts of temperature on the biological production rates of volatile organic compounds have been examined in bacterial culture—e.g., the production rate of isoprene was shown to increase as the incubation temperature of the cultures of *Bacillus* increased from 25 °C to 45 °C (Kuzma et al., 1995). However, as far as we know, there has been no report on the effects of temperature on the production rates of methyl halides in a bacterial culture.

The study presented here describes the effects of temperature on the production rates of methyl halides in the culture of a marine bacterium (HKF-1). The HKF-1 used in this study was previously isolated from brackish water (salinity of about 5‰) in Sanaru Lake (latitude 34° 42.7′N; longitude 137° 41.5′E) (Fujimori et al., 2012); the sampling site lay in an estuary connecting Sanaru Lake to the ocean, where seawater repeatedly flowed in and out with the tide (the salinity of the sampling site ranged from about 5 to 20‰). HKF-1 was isolated in a medium for marine bacteria with a salinity of about 35‰, and the 16S rRNA gene sequence comparisons revealed that strain HKF-1 belonged to the marine α-proteobacteria *Erythrobacter* group (Fujimori et al., 2012). For the present study, HKF-1 was chosen because it had earlier been shown to produce all methyl halides (CH_3Cl, CH_3Br and CH_3I), and to produce higher concentrations of methyl halides than eleven other strains of proteobacteria (including *α*-proteobacteria and γ-proteobacteria) when incubated at 25 °C (Fujimori et al., 2012). To study the effect of temperature on the production of methyl halides in the culture of γ-proteobacteria, we also selected the strain *Pseudomonas stutzeri*, which produced the methyl halides in the previous study (Fujimori et al., 2012).

Marine α-proteobacteria and γ-proteobacteria are widely distributed in the ocean—e.g., *Erythrobacter* sp. strains have been isolated from the upper ocean (Koblížek et al., 2003) and marine strains of *Pseudomonas stutzeri* have been isolated from the water column and sediment in the marine environments (Lalucat et al., 2006). *Erythrobacter* sp. strains make up a significant portion of the aerobic anoxygenic photoheterotrophs in the marine microbial community in the open ocean (Béjà et al., 2002). The relative abundance of aerobic anoxygenic photoheterotrophs such as *Erythrobacter*, which make up about 10% of the total bacteria (Kolber et al., 2001), and their photoheterotrophic metabolism suggest that these bacteria make a distinctive contribution to the carbon cycle in the ocean (Kolber et al., 2001; Koblížek, 2015). *P. stutzeri* strains have a wide variety of metabolic activities, including denitrification (Rius et al., 2001).

In the present study, the bacteria were incubated at 15 °C, 20 °C, 25 °C, and 30 °C, respectively, and then the concentrations of methyl halides in the cultures were measured for about 20 days, and the production rates of methyl halides were calculated for the cultured samples at each temperature.

2. Experimental

2.1. Bacterial strains and growth conditions

The strain HKF-1 was used to study the effect of temperature on the production rates of methyl halides. The effect of temperature on the production of methyl halides in the culture of a strain of *Pseudomonas stutzeri* (JCM 21638) obtained from the Japan Collection of Microorganisms (JCM; Riken BioResource Center, Tsukuba, Japan) was also investigated. The strains used in this study were directly streaked on plates containing 37.4 g of marine broth 2216 (Difco BD Biosciences, Tokyo, Japan) and 15 g agar (Wako Pure Chemical Industries, Tokyo, Japan) dissolved in 1.0 L ultrapure water (Milli-Q water; Merck Millipore, Darmstadt, Germany), and samples were incubated in the dark at 25 °C. After several days of incubation, single colonies were selected with a sterile needle and the bacterial strains were pre-incubated in test tubes without agitation, in the dark at a temperature of 15 °C, 20 °C, 25 °C or 30 °C, respectively. Bacteria samples were acclimated at the same temperature for about one week before the start of the experiment—e.g., bacteria samples were incubated at 15 °C for about one week before the start of the time course experiment that included incubation at 15 °C. These bacteria (HKF-1 and *P. stutzeri*) can grow at temperatures from 10 °C to 30 °C. The cells were then collected by centrifugation (3 min at 12,000 revolutions/min), washed three times with fresh medium, and inoculated in 10 mL of marine broth 2216 (37.4 g of marine broth 2216 dissolved in 1.0 L of ultrapure water) with potassium iodide and potassium iodate (KI and KIO_3; both at final concentrations of 0.2 μmol L^{-1}) in 20-mL glass vials (headspace volume: about 10.3 mL). Following the inoculation with bacteria, the culture was incubated at 15 ± 0.2 °C, 20 ± 0.2 °C, 25 ± 0.2 °C, or 30 ± 0.3 °C, respectively, without agitation in the dark for up to 19 days. Vials containing only the medium (without bacteria) were incubated at 15 °C, 20 °C, 25 °C, or 30 °C, respectively, as a control.

2.2. Time course experiment and production rate of methyl halides in cultured samples

To obtain the time courses of the methyl halide concentrations in the bacterial cultures, CH_3Cl, CH_3Br, and CH_3I concentrations in the vials were measured regularly for up to 19 days as described below (see Section 2.3). The production rates of methyl halides were calculated based on the increase in the methyl halides concentrations in a cultured sample. Bacterial growth was monitored by measuring the optical density at 600 nm (OD_{600}) during the culture period using a WPA CO7500 colorimeter (Biochrom, Ltd., Cambridge, UK). After the