Annual monitoring of DMS-producing bacteria in Tokyo Bay, Japan, in relation to DMSP

Takushi Niki1•, Masayuki Kunugi2, Kunio Kohata2, Akira Otsuki1

1Department of Marine Science and Technology, Tokyo University of Fisheries, 4-5-7 Konnan, Minato-ku, Tokyo 108, Japan
2National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305, Japan

ABSTRACT: The population of dimethylsulfide (DMS)-producing bacteria together with the DMS and dimethylsulfoniopropionate (DMSP) concentrations were monitored from July 1995 to August 1996 in Tokyo Bay, Japan. The concentration of DMS varied widely in the range from 2 to 130 nM, and the total, dissolved and particulate DMSP concentrations varied from 5 to 220 nM, from below the detection limit to 75 nM, and from 5 to 200 nM, respectively. The observed particulate DMSP concentration was well explained by the DMSP attributable to Dinophyceae, estimated from the species-specific DMSP per cell contents. The population of DMS-producing bacteria enumerated by the Most Probable Number method was in the range from $1.6 \times 10^3$ to $9.2 \times 10^4$ cells ml$^{-1}$. A positive relationship was found between the population of DMS-producing bacteria and the total DMSP concentration in some months, suggesting an important role of bacteria in DMS production. However, some other data points showed the small population of DMS-producing bacteria in spite of the high concentrations of DMS and total DMSP. These data did not agree with the above relationship and suggest a contribution of non-bacterial DMS production under certain circumstances.

KEY WORDS: Dimethylsulfoniopropionate, Dimethylsulfide, Bacteria, Dinophyceae

INTRODUCTION

Dimethylsulfide (DMS) constitutes the major proportion of volatile organic sulfur compounds in the marine environment (Andreae 1985), and its flux to the atmosphere is considered to be one of the most important biogenic sources of non-sea-salt-sulfate (nss-sulfate) aerosols. These nss-sulfate aerosols are important as cloud-condensation nuclei (CCN), which affect the earth’s albedo and, hence, the global climate. Thus, the biological climate regulation hypothesis proposed by Charlson et al. (1987) is of great interest with respect to the biogeochemical cycling of oceanic DMS.

It is known that DMS is derived from biological transformation of β-dimethylsulfoniopropionate (DMSP). DMSP is produced by certain phytoplankton species, especially members of the Prymnesiophyceae and Dinophyceae (Keller et al. 1989, Belviso et al. 1990, Gibson et al. 1990). However, many previous works have revealed no direct correlation between DMS concentration and phytoplankton biomass in the field (Turner et al. 1989, Belviso et al. 1993, Holligan et al. 1993). Bacterial degradation of dissolved DMSP has been regarded as one of the most important processes in DMS production due to the ubiquity of dissolved DMSP in sea water and the high rate of bacterial consumption of dissolved DMSP (Kiene 1990, 1992, Bates et al. 1994, Kwint & Kramer 1996).

Release of DMS from phytoplankton cells to the dissolved pool is necessary for bacterial DMS production. Some processes, such as senescence of phytoplankton (Stefels & van Boekel 1993, Kwint & Kramer 1995), zooplankton grazing (Leck et al. 1990, Christaki et al. 1996) and viral infection (Braščak et al. 1995), have been proposed as mechanisms of DMS release from phytoplankton cells to sea water. However, information about the relationship between bacterial DMS production activity and DMS production in natural marine systems is quite scarce.

In the present study, not only the DMSP and DMS concentrations but also the population of DMS-produc-
ing bacteria were investigated in Tokyo Bay, Japan, during the period from July 1995 to August 1996. The main aim of this study was to investigate the relationship between the DMS-producing bacteria and the DMSP and DMS occurrence in a marine environment, and to evaluate the role of bacteria in DMS production.

MATERIALS AND METHODS

Sample collection. Surface seawater samples were collected approximately monthly (sampling was not conducted in October and November 1995 and June 1996 due to bad weather) at Stn B (Fig. 1; 35° 33' N, 139° 54' E), located at the head of Tokyo Bay, during survey cruises of the vessels 'Kiyosumi' of Chiba Prefecture and 'Hiyodori' of the Tokyo University of Fisheries. Samples for DMS and DMSP analysis and bacterial enumeration were collected in 2 HCl-rinsed 1 l polyethylene bottles washed thoroughly with seawater from the sampling site before collection. Pigment samples were collected in 2 l polyethylene bottles. No fixation procedure was applied to these samples. Samples for light microscopic phytoplankton enumeration were taken in 1 l and 500 ml polyethylene bottles. Samples taken in the former were fixed with glutaraldehyde (final concentration 1 %) and those taken in the latter were not fixed. All samples were stored in a cool box and prepared for analysis in the laboratory within 8 h of collection.

Analysis. A purge and trap/gas chromatography method was employed for DMS analysis. Between 1 and 10 ml of sample was carefully transferred to the sparging device. The sparging duration was 10 min at 100 ml min⁻¹ nitrogen flow. Stripped DMS was adsorbed to porous polymer (Tenax TA; GL Science Inc., Tokyo) in a straight glass tube at -20°C through a gas-drying condenser (U-shaped empty glass tube chilled at -20°C). No obvious loss of DMS in the condenser was observed. DMS retained in the Tenax tube was injected to the gas chromatograph (GC) system with a TCT (Thermal-desorption Cold Trap injector, CHROMPACK) attached to a Hewlett-Packard 5890A series II GC equipped with a flame photometric detector (FPD). A PorapLOT Q (0.32 mm × 25 m; CHROMPACK) column was used and the carrier gas was helium at 2 ml min⁻¹. The column temperature was linearly programmed to increase from 100 to 200°C at 15°C min⁻¹ and then held at 200°C for 5 min. The detection limit of the whole system was about 0.06 nM DMS when a 10 ml sample was analyzed.

In the present study, 3 DMSP concentrations, total, dissolved and particulate, were determined. Dissolved DMSP (DMSPd) was defined as that in the filtrate after gravity filtration through a Whatman GF/F filter (Keller et al. 1989). Particulate DMSP (DMSPp) was calculated by subtracting DMSPd from total DMSP (DMSPt). Filtrations were performed in the laboratory and did not take longer than 10 min. Between 1 and 10 ml of total water sample (for DMSPt) or GF/F filtrate (for DMSPd) was sparged for 10 min to remove DMS, then 1 to 2 ml of 5 N NaOH was added to convert DMSP to DMS (Dacey & Blough 1987), which was determined by the GC system described above. After the DMSP analysis, further DMS yield in the samples was not detected.

Photosynthetic pigments were analyzed by an HPLC system (Kohata et al. 1991). Sample aliquots of 200 to 500 ml were filtered through GF/F filters. These filters were stored at -20°C until the analysis, which was carried out within 2 mo. Pigments on the filters were extracted in 10 ml of 90% acetone by filter homogenization. The pigment extracts (100 to 400 μl) were injected directly into the HPLC system.

Bacterial enumeration. Two bacterial populations, 'DMS-producing bacteria' and 'DOC utilizers', were enumerated by the Most Probable Number (MPN) method (de Man 1975, Visscher et al. 1992). In the present study, 'DMS-producing bacteria' were defined as
bacteria which produced DMS from DMSP, and 'DOC utilizers' were defined as bacteria which grew in a mixture of some dissolved organic substrates. The MPN media were based on STP media, which was used for bacteria-free check of algal strains (Watanabe & Satake 1991), but the organic composition was modified for each population as noted in Table 1. The soil extract was replaced by a trace element solution (1 ml l⁻¹; Widdel & Pfennig 1981) to remove uncertainty about the medium's chemical composition.

Each duplicated sample was diluted from 10⁻¹ to 10⁻⁶ with GF/F-filtered, autoclaved, aged sea water. Aliquots (1 ml) of diluted subsamples were injected into 10 ml media in 25 ml glass test tubes, and capped with polypropylene screw caps. Controls consisted of each substrate without inoculum. All incubations were carried out at 25°C in the dark. Each tube was vortexed daily to mix media with oxygen.

Growth of DOC utilizers on the mixture of organic substrates was checked by observing the turbidity of the media. Growth of DOC utilizers was terminated within 4 to 6 d. Growth of DMS-producing bacteria on DMSP was checked by observing the DMS production in each tube. DMS production was checked by analyzing for DMS in the headspace gas. Headspace analyses were carried out 7 d after inoculation. Headspace gas (5 ml) was sampled from each tube, and directly injected to TCT to analyze DMS by the GC/FPD system described above. The final size of each bacterial population was determined according to de Man (1975).

### RESULTS

#### DMSP and DMS

The DMSP concentration showed large variation during the study period (Fig. 2a). The highest concentration was 220 nM in July and August 1995, and the lowest concentration was 5 nM in January 1996. The DMSP concentration increased in February 1996, and ranged from 50 to 160 nM during February to August 1996. In contrast, the DMSP₄ concentrations did not exceed 20 nM throughout the study period, except for a very high concentration of 73 nM observed in August 1995 (Fig. 2a). From December 1995 onward, the DMSP₄ concentrations were always lower than 3 nM and even fell below the detection limit in February and April 1996. Calculated DMSP₄ concentration ranged from 5 nM in January 1996 to 200 nM in July 1995. The relative proportion of DMSP₄ in DMSP, ranged from 0 to 35%.

The DMS concentration also varied very widely (Fig. 2a). The highest DMS concentration was 130 nM in July 1995, and a concentration of more than 100 nM was also observed in the next month, August 1995 (110 nM). The lowest concentration was 2 nM in December 1995 and May 1996. In February 1996, the DMS concentration increased to 36 nM and showed a small peak of concentration. However, in spite of the increase in DMSP₄ concentration, the DMS concentration did not show further increases in spring and summer of 1996 (from March to August), having lower concentrations than that observed in February 1996.
Changes in DMS concentration did not correspond to DMSP<sub>p</sub>, DMSP<sub>o</sub> or DMSP<sub>d</sub> concentrations (Fig. 2a).

The water temperature ranged from 7.7 to 30.1°C during the study period, and the salinity ranged from 22.2 to 32.4% (Fig. 2b). The low salinity (below 30%) was observed from July 1995 to September 1995, when the high concentrations of DMSP<sub>p</sub> were observed (18 to 75 nM; Fig. 2a).

**MPN enumeration of bacteria**

The population of DOC utilizers ranged from \(3.5 \times 10^3\) cells ml\(^{-1}\) in January 1996 to \(2.9 \times 10^5\) cells ml\(^{-1}\) in May 1996 (Fig. 3). The population of DOC utilizers showed a small peak in February 1996. The maximum population of DMS-producing bacteria was \(9.2 \times 10^4\) cells ml\(^{-1}\) in July 1995. However, in the next month, August 1995, the population of DMS-producing bacteria decreased drastically to \(3.2 \times 10^3\) cells ml\(^{-1}\), the third lowest value observed. The minimum population was \(1.6 \times 10^3\) cells ml\(^{-1}\) in January 1996 (Fig. 3). Although changes in population of DMS-producing bacteria generally appeared to reflect those in DOC utilizers, the relative proportion of DMS-producing bacteria to DOC utilizers was not constant, varying from 4 to 44% (Fig. 4). The smallest proportions of DMS-producing bacteria were found in August 1995 and February 1996 (4 and 9%, respectively).

**Phytoplankton species**

Bacillariophyceae and Dinophyceae were observed throughout the study period (Fig. 5). Since species identification was done with a light microscope, pico- and small nanophytoplankton could not be precisely identified and were enumerated collectively as ‘microflagellates’ (Fig. 5). In July 1995, this fraction constituted 93% of the total algal cell number. Other identified phytoplankton classes were enumerated collectively as ‘others’. Although this fraction constituted more than one-third of total algal cell number in May 1996, the largest fraction in this month was that of *Heterosigma akashiwo* (Raphidophyceae), which does not produce DMS (S. Takizawa, M. Kunugi, M. Watanabe & T. Higashi pers. comm.). In the other months, the ‘others’ fraction constituted less than 18%, and was mainly comprised of Euglenophyceae and Cryptophyceae.

Although pigment analyses were not conducted in all months, chlorophyll \(a\) (chl \(a\)) concentrations ranged from 4.8 µg l\(^{-1}\) in January 1996 to 43.7 µg l\(^{-1}\) in July 1995. Changes in chl \(a\) concentration were dissimilar to those in DMS, DMSP<sub>p</sub>, DMSP<sub>o</sub> and DMSP<sub>d</sub> concentrations (Fig. 2a).

**DISCUSSION**

**Origin of DMSP production**

The DMSP concentration depends largely on phytoplankton species composition rather than total phytoplankton biomass (Turner et al. 1989, Belviso et al. 1990, Malin et al. 1993). Also, in this study, changes in DMSP concentration were not coupled with those in chl \(a\) (Fig. 2a). The results of phytoplankton enumeration showed that the changes in DMSP<sub>p</sub> concentration were more similar to those in Dinophyceae abundance than those in the other categories or total algal cells (Fig. 5). When the July 1995 data were excluded, due to the ex-
extraordinary abundance of microflagellates at that time, a significant correlation was found between Dinophyceae abundance and observed DMSP concentration ($r = 0.75$, $n = 9$). These results suggested a significant contribution of Dinophyceae to the DMSP pool. However, Dinophyceae constituted only small fraction of total algal cells (see y-axes of Fig. 5). Therefore, the DMSP concentration attributable to the dominant Dinophyceae species ($\text{DMSP}_{\text{Dino}}$) was estimated. The dominant Dinophyceae species in this study were as follows, where the used DMSP per cell content, which were taken from Keller et al. (1989), are shown in parentheses: Gymnodiniales (0.69 pmol cell$^{-1}$), *Prorocentrum* spp. (represented by *P. minimum*, 0.16 pmol cell$^{-1}$), *Protoperoa* spp. (represented by the average value of reported Peridiniales, 0.96 pmol cell$^{-1}$), *Ceratium* spp. (0.01 pmol cell$^{-1}$), *Heterocapsa* spp. (0.37 pmol cell$^{-1}$) and *Scrippsiella* spp. (2.84 pmol cell$^{-1}$). Throughout the study period, these dominant species constituted more than 80% of the total Dinophyceae cells observed at our study site.

The observed DMSP concentration was well explained by the calculated DMSP$_{\text{Dino}}$ (Fig. 6). A higher correlation coefficient was obtained ($r = 0.85$) when the data for July 1995 were excluded for the reason stated above. These results indicate that most of the DMSP pool in the months other than July 1995 was contributed by Dinophyceae. Although DMSP per cell contents of Dinophyceae have been reported to vary over the growth cycle (Matrai & Keller 1994), such differences in DMSP content may be averaged in complex natural phytoplankton communities. The good correlation found in this study suggests the possibility that DMSP content data obtained from culture experiments is applicable to natural environments to estimate the size of DMSP pool from available phytoplankton data.

In July 1995, the DMSP$_{\text{Dino}}$ was calculated to be 28.8 nM, accounting for only 15% of actual DMSP (200 nM). The DMSP attributable to Bacillariophyceae was also calculated (only 3.0 nM), but did not account for the discrepancy between the DMSP$_{\text{Dino}}$ and the observed DMSP. Carotenoid analysis of samples taken at that time revealed a high concentration...
(1.6 μg l⁻¹) of 19'-hexanoyloxyfucoxanthin, a characteristic carotenoid of Prymnesiophyceae. Therefore, a significant part of the DMSP₄ in that month can be attributed to Prymnesiophyceae which were probably included in the 'microflagellates' in this study.

**Importance of DMS-producing bacteria**

Released DMSP from phytoplankton cells is consumed rapidly by bacteria (Kiene 1992). The rates or kinetics parameters of DMSP₄ consumption and DMS production in sea water have been reported in some studies (Kiene & Service 1991, Ledyard & Dacey 1996). DMS-producing bacteria have also been isolated (Ledyard & Dacey 1994, de Souza & Yoch 1995) and enumerated (Visscher et al. 1992). However, information about the seasonal changes in both bacterial activity and population has been quite scarce. In the present study, an annual data set of the population of DMS-producing bacteria was obtained by the MPN method. Due to the selectivity of the media, the MPN method may underestimate the true bacterial population. However, due to its selectivity, this method was considered to be useful for the purpose of observing the seasonal changes in DMS-producing bacteria in this study.

Changes in DMS concentration were not similar to those in the population of DMS-producing bacteria, and no obvious relationship was found between them (Fig. 7b). In the spring of 1996, despite the increase in both the DMSP₄ concentration and the population of DMS-producing bacteria, the DMS concentration started to decrease from its peak in February, and fell to 2 nM in May (Figs. 2a & 3). Produced DMS is removed by various processes such as bacterial consumption (Kiene & Bates 1990, Wolfe & Kiene 1993, Kwint & Kramer 1996), photochemical oxidation (Brimblecombe & Shooter 1986) and escape to the atmosphere. The observed concentration of DMS is the net result of production and removal. The discrepancy between the DMS concentration and the DMSP₄ concentration and DMS-producing bacteria population found in this study may be explained by DMS removal.

The seasonal changes in the population of DMS-producing bacteria appeared not to be directly related to those in DMSP concentrations (see Figs. 2a & 3). However, an interesting relationship appeared in the plots of the population of DMS-producing bacteria versus DMSP₄ concentration (Fig. 7a). A coupled increase of both the DMSP₄ concentration and the population of DMS-producing bacteria was shown by some data points (July 1995, May 1996, etc.). An increase in the population of DMS-producing bacteria should be coupled with an increase in the input (production) of DMSP₄. However, the concentrations of DMSP₄ for these data points were low (Figs. 2 & 7c), possibly due to a rapid turnover of the DMSP₄ by bacteria. Therefore, the above relationship between the DMS-producing bacteria and the DMSP₄ concentration may suggest that a significant part of produced DMS could become available for bacteria. These interpretations indicate an important role of bacterial consumption as a sink for DMS in the environment, and suggest that the bacterial transformation was the most important DMS production process.

However, some other data points, such as August 1995, showed the small population of DMS-producing bacteria in spite of the high DMSP concentration (Fig. 7a). These points appear to be independent of the positive relationship discussed above. The non-DMS-producing population (demethylator) is also included in the total DMSP-consuming population (Visscher et
al. 1992), but was not determined in this study since the DMS analysis was used for checking the bacterial growth on DMSP. The relative proportion of the DMSP-producing bacteria to the total DMSP-consuming population might have decreased in these months. The data for August 1995 and February 1996 showed a high DMS concentration but a small population of DMSP-consuming bacteria (Fig. 7b). In these 2 months, the proportions of DMS-producing bacteria to DOC utilizers were also small (4 and 9%, respectively; Fig. 4). These data appear to disagree with the hypothesis that bacteria are important in DMS production. Some previous studies have reported non-bacterial DMSP-lyase activity (Kadota & Ishida 1968, Stefels & van Boekel 1993). Stefels et al. (1995) showed that DMSP-lyase activity in the early stage of a *Phaeocystis* sp. bloom was mostly attributable to *Phaeocystis* itself. There are also some reports that microzooplankton and detritus are important in DMS production (Belviso et al. 1993, Christaki et al. 1996). In this study, large tintinnids were extraordinary abundant (>100 µm in length, not precisely enumerated but probably more than 10 ind. m⁻³) in the February 1996 sample. The discrepancy between the DMS concentration and population of DMS-producing bacteria may suggest a contribution of non-bacterial DMS production in these months. These results stress the need for further research on DMSP₄ generation and non-bacterial DMS production process(es), such as larger particle-associated DMSP-lyase activity, in natural marine environments.

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**LITERATURE CITED**


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