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Ecological Study of Bacterial Populations Related to Biogenic Gas Transformation in Marine Environments

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Introduction

Marine bacterioplankton regulate sulfer flux from the ocean to the atmosphere by degrading dimethylsulfoniopropionate (DMSP), a phytoplankton product for use as an osmolyte and also the precursor of a climate-related gas dimethylsulfide (DMS). This process is a key to understanding the feedback mechanism from marine ecosystems to climate systems (Kiene *et al*. 2000). Marine bacteria contribute to both the sink and the source of DMS (Vogt *et al*. 2008). Some bacteria transform DMSP to DMS, and others transform DMSP to other compounds by dimethylation. Recently, several genes encoding enzymes related to these processes have been identified (Strom 2008; Howard *et al*. 2008; Todd *et al*. 2009). However, little is known about the relative contribution of these processes to DMS emission in the ocean. Here, we report two studies on bacterial population dynamics related to DMSP degradation and DMS emission in marine environments. Firstly, in order to determine which phylotypes of bacteria are involved in DMSP degradation, the change of bacterial community structures and their growth response were investigated during incubations of natural seawater, after supplementing DMSP and other organic substrates in the Southern Ocean. Secondly, patterns of DMS emission and bacterial population dynamics are described during mesocosm bloom conditions of diatoms and coccolithophores.

Methods

Southern ocean experiment

Surface seawater was collected off the Lützow-Holm Bay, St. L12 (67.43S, 37.60E), and off Cape Danley, St. I-4 (66.39S, 64.48E), St. II-5 (67.06S, 68.06E), St. 23 (53.55S, 97.63E) during the cruise of R/V Umitaka-maru, from December 2007 to January 2008. After filtering the seawater with GF/F filters to remove phytoplankton, it was stored for a few days to reduce the ambient DMSP concentration, and then used for on-deck incubations supplemented with 100 nM each of DMSP, leucine and glucose. The concentrations of DMSP and DMS, and

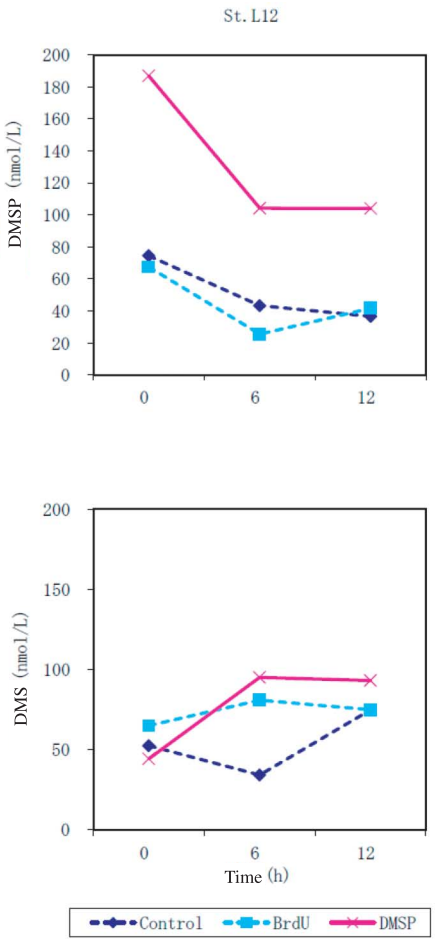

Fig. 1-1. DMS and DMSP concentrations at St. L12.

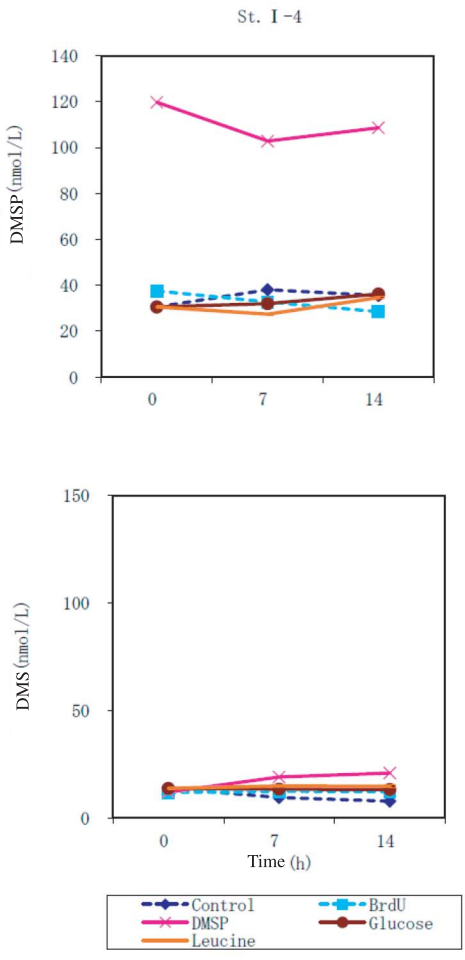

Fig. 1-2. DMS and DMSP concentrations at St. I-4.

bacterial abundance, productivity and community structures were monitored up to 6–22 h. Also, bacterial community structures were compared by means of 16S rRNA gene PCR-DGGE analysis after extracting DNA from bacterial cells collected on membrane filters. The identities of actively-growing bacteria were determined by 16S rRNA gene sequences combined with bromodeoxyuridine (BrdU) labeling techniques (Hamasaki *et al*. 2007).

Mesocosm experiment

Seawater was collected in the Otsuchi Bay in June, 2010. The water was filtered through a 100-μm nylon mesh to remove large zooplankton, and received in four 200-L tanks. A half of the water was further filtered through a 10-μm-pore size filter to remove large phytoplankton. Inorganic nutrients (nitrate, phosphate, silicate) were added to each tank. Coccolithopholid algae, *Gephyrocapsa oceanica*, precultured in a laboratory condition were added to two of the four tanks as a seed population. Phytoplankton abundance, bacterial abundance, and DMSP and DMS concentrations were monitored for 11 days.

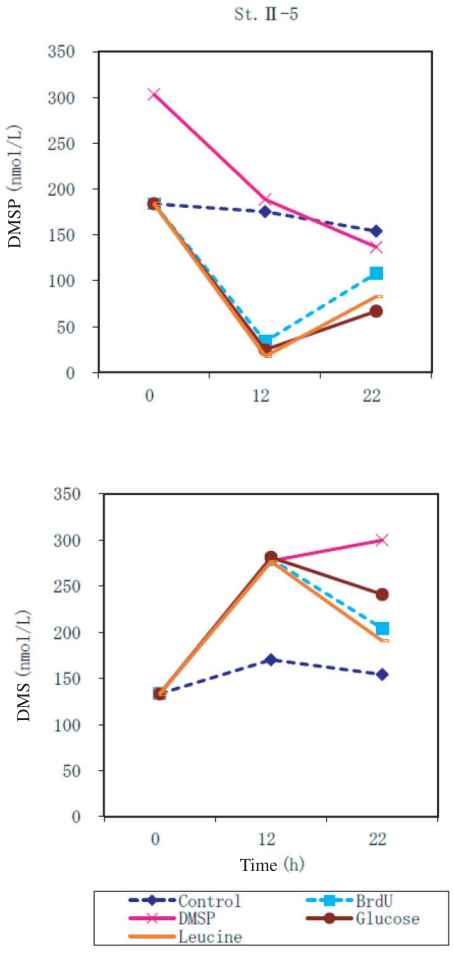

Fig. 1-3. DMS and DMSP concentrations at St. II-5.

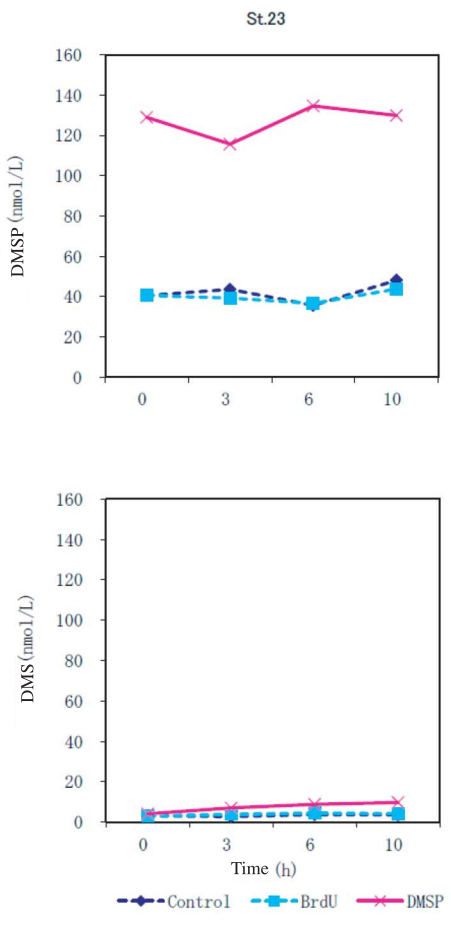

Fig. 1-4. DMS and DMSP concentrations at St. 23.

Results and Discussion

Southern ocean experiment

A decrease of the DMSP concentration was observed in all DMSP amended bottles at all stations (Figs. 1-1, 1-2, 1-3 and 1-4). Also a decrease of the DMSP concentration and subsequent DMS emission was observed in other bottles at high ambient DMSP stations. The ratio of DMS increase/DMSP reduction differed among sampling stations, implying variable activity and community structures of bacteria involving DMSP consumption and DMS emission. A significant reduction of DMS concentration observed in a control bottle, without DMSP addition at St. I-4 might be due to DMS oxidation which can be another possible pathway of its loss process (Fig. 1-2).

Bacterial community structure analysis by PCR-DGGE showed that a change of organic matters input (DMSP, leucine, glucose) resulted in no difference in the bacterial community structures. DGGE banding patterns were compared among

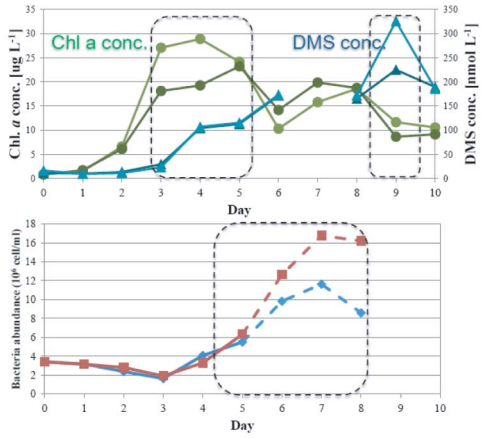

Fig. 2-1a. DMS and chl-a concentration and bacterial abundance during a diatom bloom.

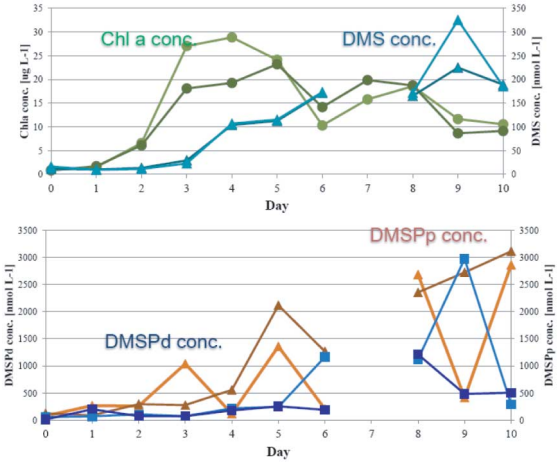

Fig. 2-1b. DMS, DMSP and chl-a concentration during a diatom bloom.

different stations. Also, the bands including BrdU-labeled ones were excised and sequenced to identify the key species during the experiments (Table 1). The dominant band was identified as originating from bacteria closely related to *Polaribacter irgensii*. Bacteria closely related to *Pseudoalteromonas issachenkonii* appeared at St. L12 and I-4. Since significant amount of DMSP is transformed to DMS especially at the St. L12 and II-5, these bacteria are possible major mediators of DMSP consumption and DMS emission. Also, variable patterns of DMS emission, in spite of similar DGGE banding patterns, may be due to the contribution of minor bacteria missed by PCR-DGGE analysis, which should be clarified in future research.

Mesocosm experiment

A rapid increase of chl-a concentration

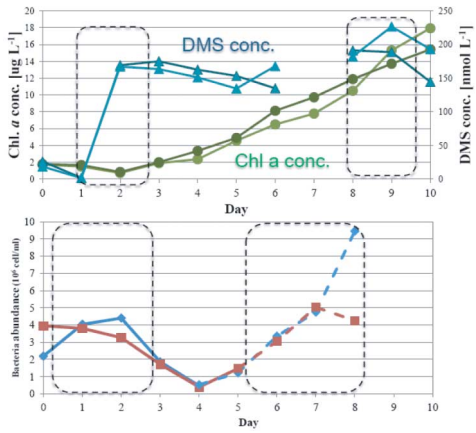

Fig. 2-2a. DMS and chl-a concentration and bacterial abundance during a coccolithophorid bloom.

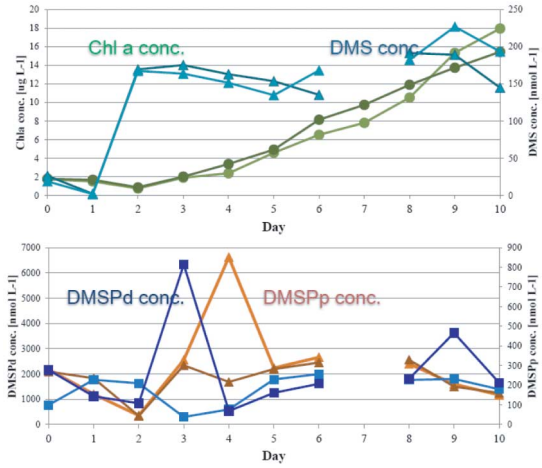

Fig. 2-2b. DMS, DMSP and chl-a concentration during a coccolithopholid bloom.

caused by the growth of diatoms was observed in two nutrient-enriched tanks of natural seawater (Figs. 2-1a and 2-1b). Peaks of chl-a concentration were shown at days 4 and 5 (29 and 24 mg m^{-3}, respectively). Bacterial abundance started to increase after these chl-a peaks and reached a maximum at day 7 (1.6×10^7 and 1.2×10^7 cells m^{-1}, respectively). DMS concentration showed a significant increase after day 3 and peaks at day 9 (340 and 230 nM, respectively). A delayed emission of DMS after the peak of chl-a, and a subsequent increase of bacteria, suggested the importance of bacterial activity for determining the dynamics of DMS.

In the other two tanks, containing a seed population of coccolithopholids, the chl-a concentration started to increase at days 3 and 4, and kept increasing until the end of the experiment at day 10 (Figs. 2-2a and 2-2b). The chl-a concentrations

Table 1. Presence and absence of DGGE bands and its identities at 4 stations in the Southern Ocean.

The most closest species	similarity (%)	L12		I-4		II-5		Surf23
		total	BrdU	total	BrdU	total	BrdU	total
Alphaproteobacteria								
Pelagibacter ubique	99	-	-	+	-	-	-	+
Rhodobacteraceae bacterium	89	-	-	+	+	-	-	-
Roseobacter sp.	95	+	-	-	-	-	-	-
Loktanella vestfoldensis	96	+	-	-	-	-	-	-
Gammaproteobacteria								
Psudoalteromonas issachenkon	100	+	+	+	+	-	-	-
Psudoalteromonas sp.	94	-	-	+	+	-	-	-
Psudoalteromonas haloplanktis	100	-	-	+	+	-	-	-
Psudoalteromonas marina	92	-	-	+	+	-	-	-
Flavobacteria								
Polaribacter irgensii	95	+	+	+	+	+	+	-
Winogradskyella sp.	92	-	-	+	-	-	-	-
Sphingobacteria								
Flexibacteraceae bacterium	86	-	-	+	+	-	-	-

were 16 and 18 mg m^{-3} at the end. Bacterial abundance showed a decrease at first, reached minimum at the day 4 (40.4×10^6 and 0.5×10^6 cells ml^{-1}) and then increased during the latter half of the experiment. DMS concentration quickly increased at day 2, maintained a high concentration for several days, and then increased again at the end. The rapid increase of DMS may be caused by the degradation of DMSP contained in the seed culture of coccolithopholids added at the start of the experiment. DMSP accumulation, after the peak of DMS, corresponded to the decrease of bacterial abundance. Also, the decrease of DMSP and increase of DMS corresponded well to the increase of bacterial abundance. These results imply a close coupling between bacterial activity and DMS emission during the bloom of the phytoplankton.

Acknowledgements

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