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# Distinct bacterial community and diversity shifts after phytoplankton-derived dissolved organic matter addition in a coastal environment

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ABSTRACT

Dissolved organic matter derived from phytoplankton (DOMP) can affect the bacterial growth and community composition, with concomitant changes in DOM characteristics, of aquatic ecosystems. Here, we examined the chemical and fluorescent characteristics of DOMPs derived from the diatom *Thalassiosira weissflogii* and the dinoflagellate *Heterocapsa triquetra*, and the community and diversity responses of free-living bacteria to these DOMPs. The DOMP quality was evaluated based on amino acid composition and excitation-emission matrix (EEM) analyses. In addition, EEM analysis was used to examine compositional changes in DOMPs. The total hydrolysable and free amino acid (THAA and FAA, respectively) compositions of each DOMP differed between phytoplankton strains. In addition, THAA and FAA compositions in diatom DOMPs were slightly different between the exponential and stationary growth phases. Terminal restriction fragment-length polymorphism (T-RFLP) analysis with 16S rRNA genes revealed that the community shifts varied significantly with different DOMP additions. Furthermore, the specific amino acids in the diatom and dinoflagellate DOMPs potentially affected the bacterial community shifts. Similarity percentage analysis with 16S rRNA gene deep-sequencing revealed that distinct DOMP additions caused the community and diversity shifts with the growth of some specific bacterial lineages. *Alteromonas* (class Gammaproteobacteria) and Bacteroidetes lineages were strongly associated with the diatom DOMPs, whereas the proportion of Rhodobacteraceae sequences (class Alphaproteobacteria) among the total sequences increased in response to the addition of the dinoflagellate DOMP. EEM analysis revealed that the fluorescent DOM (FDOM) compositions of the diatom and dinoflagellate DOMPs changed in association with shifts in the bacterial community. Especially, a decrease in the fluorescence intensities of DOM was observed from the dinoflagellate treatment with the growth of Rhodobacteraceae lineage. These results indicate that there was a strong linkage between FDOM dynamics and Rhodobacteraceae lineage in the coastal water. This study suggests that DOMPs from different phytoplankton constitute a primary factor that alters the dominant bacterial groups with compositional changes in FDOM in coastal environments.

## 1. Introduction

Dissolved organic matter (DOM), which represents one of the largest reduced carbon pools in the ocean, is involved in global biogeochemical processes (Hansell et al., 2009). In general, a large portion of the labile DOM is released via the exudation of photosynthetic products from phytoplankton cells and their lysis which can be caused by zooplankton predation (Møller et al., 2003) and viral lysis (Middelboe and Lyck, 2002). DOM derived from phytoplankton is hereafter referred to as DOMP. Recent studies have revealed that there was a positive correlation between the percent extracellular carbon release and the specific cell lysis rates of phytoplankton in the natural environments (Agustí and Duarte, 2013; Lasternas and Agustí, 2014). These data suggest that the phytoplankton lysate and exudate are important sources affecting the production and community composition of bacteria in aquatic environments.

Some early studies have shown that the chemical characteristics of DOMP (such as amino acids, monosaccharides, and organic sulfur compounds) differ among phytoplankton lineages or their growth phases (e.g., Brown, 1991; Geider and La Roche, 2002; Wetz et al., 2008). Thus, the shifts in the composition of phytoplankton communities and their growth phases should effectively change the DOM

*Abbreviations:* DOMP, dissolved organic matter derived from phytoplankton; EEM, excitation-emission matrix; FDOM, fluorescent dissolved organic matter

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characteristics in the ocean. In addition, marine bacteria play a key role in shaping the characteristics of DOM, not only as consumers but also as producers (Amon and Benner, 1996; Azam, 1998; Kirchman, 1993; Ogawa et al., 2001; Jiao et al., 2010). Many previous reports have demonstrated that DOMP enhanced specific bacterial growth and led to shifts in the bacterial community and diversity in the ocean (e.g., Cole, 1982; Azam and Ammerman, 1984; Kirchman, 1994; Riemann et al., 2000; Pinhassi et al., 2004; West et al., 2008; Tada et al., 2011; Teeling et al., 2012; Landa et al., 2013, 2014, 2015; Tada and Suzuki, 2016). Other studies have demonstrated that the growth of marine bacteria can contribute to the transformation of DOMP characteristics, such as amount of fluorescent DOM (FDOM) and composition of amino acids (Romera-Castillo et al., 2011; Sarmento et al., 2013). Thus, the characteristics of DOMPs derived from taxonomically and physiologically distinct phytoplankton cells would strongly link the shifts of bacterial community composition and diversity, which could change the DOMP characteristics. However, information concerning the direct relationships among DOMP characteristics, related shifts in bacterial community and diversity, and changes induced in DOM remain limited.

Two components of DOM, dissolved total hydrolysable and free amino acids (THAA and FAA, respectively) should be important as carbon and nitrogen sources for bacterial growth in the ocean (Kujawinski, 2011). Many previous studies have shown that phytoplankton are primary producers of these amino acids in aquatic environments (e.g., Hellebust, 1965; Admiraal et al., 1986; Myklestad et al., 1989; Brown, 1991). For example, the proportion of THAA in phytoplankton cultures ranged from 12% to 35% of dry weight (Brown, 1991), and their composition varied not only with phytoplankton species but also with physiological conditions such as growth phases (Admiraal et al., 1986). Marine phytoplankton excrete both THAAs and FAAs as the labile DOM, which is the major energy source for bacterial growth (Kirchman, 1994; Fuhrman, 1987). In addition, recent studies have revealed that the composition of THAA and FAA could be one of the important factors determining the composition and diversity of the bacterial community in natural environments (Landa et al., 2013; Sarmento et al., 2013; Canelhas et al., 2016; Tada and Suzuki, 2016). Thus, information regarding the concentration and composition of THAA and FAA in DOMPs derived from taxonomically and physiologically (exponential or stationary growth phases) distinct phytoplankton cells would help to better understand the interactions among phytoplankton communities, DOM characteristics, and the diversity of bacterial communities in the ocean.

It has shown that changes in DOM produced by phytoplankton during heterotrophic bacterial degradation could be qualitatively evaluated by determining the fluorescent DOM (FDOM) composition (Romera-Castillo et al., 2011). For example, humic-like and protein-like fluorophores in the FDOM are considered to be recalcitrant and labile to bacterial degradation, respectively (Chen and Bada, 1992; Yamashita and Tanoue, 2003b; Nieto-Cid et al., 2006). In addition, different phytoplankton lineages have reportedly produce FDOM with distinct compositions, and the production rates vary with the growth phases of the phytoplankton (Romera-Castillo et al., 2010). Thus, quantitative and qualitative analyses of DOMPs and their compositional changes during bacterial degradation through EEM fluorescence spectroscopy should facilitate a better understanding of the dynamics of DOM via interactions between phytoplankton and bacteria in the ocean.

The goal of this study was to examine the THAA and FAA compositions of DOMP from phytoplankton cells in distinct lineages and growth phases, and the community and diversity changes of free-living bacteria in response to the addition of DOMP to coastal seawater, using T-RFLP and deep-sequencing analyses of 16S rRNA genes. Moreover, we analyzed EEMs to evaluate qualitative changes in DOMPs caused by the diversity of the bacterial community.

## 2. Materials and methods

### 2.1. Culturing phytoplankton strains and preparation of the DOMPs

The diatom *Thalassiosira weissflogii* (CCMP1336) and the dinoflagellate *Heterocapsa triquetra* (CCMP449) were grown axenically in f/2 medium (Guillard, 1975) at 10 °C with a 12-h light and 12-h dark cycle (see Fig. S1 in the Supporting information). The diatom cells were harvested during the exponential and stationary phases, whereas those of dinoflagellates were collected during the exponential phase by centrifugation at 11,000 rpm for 10 min at 4 °C. The pellets were washed with Milli-Q water three times to remove residual nutrients from the culture medium. The cells were lysed using a bead-beater (3200 rpm, 60 s × 2) with 0.5 g pre-combusted (450 °C for 4 h) glass beads (0.5-mm diameter, Bio Spec Products, OK) and Milli-Q water at < 4 °C (Tada and Suzuki, 2016). Crushed cellular debris was removed using an acid-washed, Milli-Q water-rinsed plastic syringe with a polyvinylidene fluoride (PVDF) filter (0.2-μm pore size, Millipore, MA). These DOMPs were dispensed into pre-combusted (450 °C for 4 h) glass bottles and stored at − 30 °C until further analysis.

### 2.2. Preparation of the seawater inoculum and medium for the incubation experiments

The experimental procedure was described in Fig. S2. For the incubation experiment, we used modified Aquil medium (artificial seawater without EDTA and vitamin solution as carbon sources) (Morel et al., 1979) as a low DOC growth medium. Before use, the water temperature and salinity of the artificial seawater were adjusted to those of natural seawater (23 °C and 32.4 psu, respectively). The nutrient concentrations in the artificial seawater were adjusted to the value determined for the same month and location of the previous year (0.28 μM $NO_3$, 0.7 μM $NH_4$, 0.2 μM $PO_4$) (Takao et al., unpublished data). The artificial seawater was sterilized with a 0.2-μm pore size PVDF filter (Millipack 20, Millipore).

The natural seawater for the incubation experiments was collected from the surface (0 m) of Oshoro Bay, southwestern Hokkaido, Japan (43°13′N, 140°52′W) at 28 Jul 2015. Approximately 20 l of seawater were collected in acid-cleaned (1 M HCl) polycarbonate bottles (Nalgene, Rotherwas, UK) (defined as natural seawater). For the preparation of inoculum seawater (defined as filtered seawater), the natural seawater was pre-filtered through 0.8-μm pore size polycarbonate membrane filters (111109, Whatman, Buckinghamshire, UK) by gravity filtration to remove bacterivorous protozoa.

For the incubation experiment, an inoculum (100 ml) of the filtered seawater was diluted with the artificial seawater (900 ml) in 1000-ml polycarbonate bottles (washed with 1 M HCl and Milli-Q water before use). The diluted seawater samples were amended with different DOMPs, each with a carbon concentration of 10 μM C (final). The treatment without DOMP addition was defined as “Control”. These samples were then incubated for 3 days at the in situ temperature (23 °C). All incubations were performed in the dark in duplicate. Incubation bottles were mixed once a day before subsampling. During incubation, we collected the samples for bacterial count every day. Samples for FDOM analysis were collected at Day 0 and Day 3 after DOMP addition (see Table S1 in the Supporting information). Bacterial community structure samples were collected after incubation (Day 3). To analyze the bacterial community composition of natural and filtered seawater, these seawater samples were filtered through 0.2-μm pore size Supor membrane filters (25-mm diameter, Pall Corporation) and stored at − 80 °C until DNA extraction.

### 2.3. Dissolved organic carbon, macronutrients and amino acid analyses of the DOMPs

Dissolved organic carbon (DOC) in the DOMPs was analyzed with a