Gene 576 (2016) 610–617

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Spatial variation of bacterial community composition at the expiry of spring phytoplankton bloom in Sendai Bay, Japan

Tomoko Sakami [a,*], Tsuyoshi Watanabe [a,b], Shigeho Kakehi [a], Yukiko Taniuchi [c], Akira Kuwata [a]

[a] Tohoku National Fisheries Research Institute, Fisheries Research Agency, 3-27-5 Shinhama, Shiogama, Miyagi 985-0001, Japan
[b] CREST, JST, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[c] Hokkaido National Fisheries Research Institute, Fisheries Research Agency, 116 Katsurakoi, Kushiro, Hokkaido 085-0802, Japan

ARTICLE INFO

Available online 22 October 2015

Keywords:
Bacterial diversity
Pyrotag sequencing
Phytoplankton sedimentation
Hydrographic events

ABSTRACT

In order to characterize how bacterial communities are propagated over spatial scales in a coastal area, the bacterial community composition was examined along with a transect line set in a bay at an expiry of spring phytoplankton bloom. Four distinctive bacterial communities were found within the bay by a fingerprinting method of 16S rRNA gene amplicons. The most widely distributed one was distributed in the surface and middle layers at whole area of the bay. The water was characterized by low inorganic nutrients concentration and high bacterial abundance, suggesting that the bacterial community had been developed in the bloom. Pyrosequencing analyses of the gene amplicons indicated that *Rhodobacteriaceae* and *Flavobacteriaceae* were abundant in the bacterial community, though the most abundant bacterial taxon was SAR11. The second group was distributed in the bottom water at the coastal side of the bay where considerably high Chl. *a* concentration was observed, probably because of the sedimentation of phytoplankton bloom. The community diversity was high and *Alteromonadaceae*, *Saprospiraceae*, and some families of *Actinobacter* existed more in this community than the others. The third group was distributed in the deep water near the border with the outside of the bay. The ratio of SAR11 was the highest in this community; besides, *Burkholderianceae* and *Rhodospilliraceae* existed in relatively high abundances. Another bacterial community having intermediate characters was observed in the middle to bottom layers around a central part of the bay where vertical water mixing was observed. These findings suggest that spatially different bacterial communities were formed under the influences of phytoplankton bloom and/or hydrographic events such as oceanic seawater intrusion of the bay.

1. Introduction

In coastal areas, biological productivities are often high because of terrestrial nutrient inputs and various marine organisms are brought up there. However, they are easily disturbed by over-loading of nutrients discharged from city area and/or agricultural fields. Artificial architectures such as harbor and flood walls also give substantial effects on coastal environments. To preserve a proper environmental condition, biological diversity should be considered. Bacteria have principle roles in organic matter degradation and nutrients regeneration in marine ecosystems and it can respond directly and quickly to the change of allochthonous nutrient input (Murrell et al., 1999; Paerl et al., 2003). Therefore, bacterial community composition may become a good indicator to assess environmental changes in a coastal marine ecosystem.

Bacterial community diversity is generally examined by using ribosomal RNA genes because most bacteria living in natural environments are unculturable. At coastal areas, it has been revealed that bacterial community composition changes depends on environmental factors such as temperature, salinity, and nutrient concentrations (Kirchman et al., 2005; Fuhrman and Hagström, 2008; Sakami, 2008; Fortunato et al., 2013; Yeo et al., 2013). Phytoplankton blooms and/or eutrophication of water also give effects on bacterial communities (Pinhassi et al., 2004; Tada et al., 2011; Liu et al., 2013). However, it has been difficult to know the exact bacterial community composition including minor species because its diversity is often too large to examine by using ordinal low-resolution methods such as a combination of clone library and sanger-sequencing or some finger printing methods (Gobet et al., 2012). Moreover, environment conditions vary so complicatedly at a coastal area, details of bacterial community variation still remain unknown. Recently developments of pyrosequencing machines and bioinformatics allow us to analyses a massive sequence data. Using these techniques, bacterial community dynamics have been revealed in

Abbreviations: rRNA, ribosomal RNA; Chl. *a*, chlorophyll *a*; T-RFLP, terminal restriction enzyme digestion fragment length polymorphism; DO, dissolved oxygen; SCM, subsurface chlorophyll maximum; DMF, *N*, *N*′-dimethylformamide; MID, molecular identifier; OTU, operational taxonomic unit; PCA, principal component analysis.

* Corresponding author.
E-mail address: sakami@affrc.go.jp (T. Sakami).

http://dx.doi.org/10.1016/j.gene.2015.10.011

relation with the environmental conditions at various coastal environments (Campbell and Kirchman, 2013; Teeling et al., 2012; Wemheuer et al., 2014; Fortunato et al., 2013).

Phytoplankton bloom is one of the most important biological events in marine water, where there are strong interactions between phytoplankton and bacteria. Some bacteria produce chemical substances that promote phytoplankton growth, such as vitamin B12 (Starr et al., 1957) or indole-3-acetic acid that acts as a hormone to a diatom species (Amin et al., 2012, 2015). On the other hand, many kinds of bacteria inhibit the growth of or kill phytoplankton in seawater (Park et al., 2010). The abundance of phytoplankton growth-inhibiting bacteria increases correspondingly with the level of chlorophyll *a* in seawater, suggesting that these bacteria play a significant role in regulating phytoplankton abundance in coastal marine environments (Inaba et al., 2013). Changes of bacterial community composition have been well studied in relation to a phytoplankton bloom. The community composition and dominant species changed accompany with the bloom development as well as bacterial abundance, growth rate, and some enzymatic activities (Pinhassi et al., 2004). The active bacterial species were also different between the inside and the outside of the blooming area (Tada et al., 2011). However, there are few studies about a spatial distribution of such a unique bacterial community in relation with the bloom occurrence. Moreover, bacterial community composition may be different depends on the water depth in a shallow coastal area, because a bacterial community in water may have an interaction with that in the beneath sediment (Yeo et al., 2013; Hamdan et al., 2013). To elucidate bacterial community variation at a coastal area, spatial variations should be clarified as well as temporal variations.

Sendai Bay locates at the north east part of Japan and it has a simple semicircle form with a wide open facing to the Pacific. Some rivers are discharged into the bay to give considerable effects on hydrographic properties, as well as an oceanic water flow into the bay (Kudo, 1971; Kakehi et al., 2012). However, a uniform water mass is formed within the bay in winter, because the reduction of river water discharge and cooling of water surface cause a strong water mixing. Consequently a phytoplankton bloom, which is constituted mainly by diatoms, is developed in the nutrient rich water mass in spring. The purpose of this study is to elucidate the spatial variation of bacterial community composition relating to the phytoplankton bloom. We examined bacterial community composition along with a transect line in the Sendai Bay at the expiry of spring bloom. A terminal restriction enzyme digestion fragment length polymorphism (T-RFLP) method was used to know the distribution of distinctive bacterial communities, and an amplicon-pyrosequencing analysis was done for some representative samples to know details of bacterial community variations.

2. Materials and methods

2.1. Sample collection and DNA extraction

The C line (Fig. 1) which ran from stations C1 to C13 was a cross-shore line located off the Abukuma River. Hydrodynamic profiles of temperature, salinity, dissolved oxygen (DO) were measured by aqua quality sensor AAQ (AAQ-1186, JFE Advantech). Water samples were taken at depths 1 (surface layer), 10, 30 and 50 m (middle layer), and at 0.5 m above the bottom (bottom layer). Water samples were also collected at the sub-surface Chlorophyll maximum (SCM) which was determined from the profile of Chl. *a* measured by the fluorescence sensor of the AAQ. The salinities of the AAQ were calibrated with the salinity measured by the salinometer (AUTOSAL 8400B, Guildline Instruments Ltd). Water samples for Chl. *a* analysis were filtered through a 25 mm Whatman GF/F glass fiber filter and preserved in DMF (*N*, *N*′-dimethylformamide). Fluorometric measurements of Chl. *a* were performed using a Turner Designs fluorometer (10-AU, Turner Designs). Values of fluorescence sensor of the AAQ were calibrated with fluorometrically measured Chl. *a* concentrations. Inorganic nutrient concentrations were determined using an Auto-Analyzer (QuAAtro, BL-TEC). Bacterial abundance was determined using a fluorescent microscopy (Porter and Feig, 1980). Major phytoplankton species were identified from seawater samples fixed with acid Lugol solution (4% final concentration) under light microscopy (Tomas, 1997). DNA water samples were collected at stations C1–5, 7, 10, 12, and 13. Water samples were cooled immediately after collection and kept in dark. Waters of 300 to 500 mL were filtered through a membrane filter of normal pore size 0.22 μm (GS, Millipore) within 10 h after sample collection. Filters were stored frozen at −80 °C until processing. To avoid an inhibitive effect of high concentration of suspended matters in the bottom water, DNA was extracted using a FAST DNA-SPIN kit for soil (MP. Biomedicals) in accordance with the manufacturer's instructions.

2.2. Terminal restriction enzyme fragment length polymorphisms (T-RFLP)

The bacterial community structure in the water was examined using T-RFLP of PCR-amplified 16S rRNA gene fragments. PCR was conducted

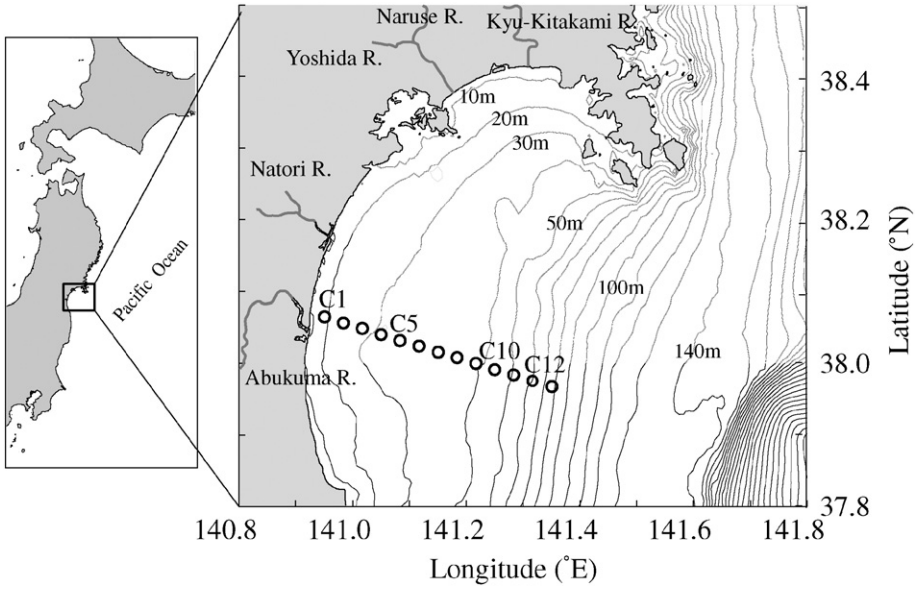

Fig. 1. Locations of observation stations and bathymetry in Sendai Bay.