

Bacterial community composition of the sediment in the coastal regions of Kashima-Nada and Kujukurihama, Japan

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Abstract: Environmental monitoring of marine coastal areas is becoming increasingly important because of accelerated global climate change and rapid industrial development. The composition of the bacterial community in bottom sediments is strongly related to the surrounding environment. Thus, surface sediments were collected at the coastal regions of Kashima-Nada and Kujukurihama to elucidate how bacterial communities vary where the confluence of the cold Oyashio and warm Kuroshio Currents occur. The bacterial communities collected at water depths of 10 m were different between the Kashima-Nada and Kujukurihama areas, but were not different at water depths of 30 m. The chlorophyll *a* content in the sediment and bottom water temperature were indicated as the major environmental parameters influencing the composition of the bacterial communities. At 10 m depth in the Kujukurihama area, operational taxonomic units (OTUs) of *Bacteroides*, which are known as high molecular weight organic matter degraders, were abundant, whereas OTUs of Nitrospinae and *Nitrosospira*, which are related to the nitrification process, were less abundant. The taxonomic groups characteristic of the sediment seemed to be related to the properties of organic matter quality in the sediment.

Key words: bacterial community composition, sediment quality, Kashima-Nada, Kujukurihama

Introduction

Terrestrial materials such as soil, inorganic nutrients, and organic matter are discharged into the sea through river water flow or by land erosion, with considerable consequences for the coastal environment (Bergamaschi *et al.*, 2012). In addition to industrial development on land, global climate change, which is predicted to bring about increases in torrential rain and storms (Yagi *et al.*, 2015), will change material inputs from land to coastal areas (Philippart *et al.*, 2011; Viitasalo *et al.*, 2015). To deal with prospective environmental changes, we need to monitor certain environmental parameters precisely. Using physical and chemical monitoring techniques, it is now possible to obtain highly accurate data owing to advances in measuring equipment and information technology (Bean *et al.*, 2017). However,

monitoring physical and chemical parameters is not enough to evaluate integrated environmental conditions for living organisms. As a biological parameter, macrobenthos fauna are often examined in order to monitor coastal environmental conditions. This requires time and labor, and the amount of data is less than what is obtained by physical and chemical monitoring. In contrast, approximately one hundred million bacterial cells can be observed in a single gram of marine sediment (Schmidt *et al.*, 1998). Data collection for bacterial community composition analysis is a mechanical process that augments physical and chemical parameter measurement. Typically, millions of bacterial sequences can be retrieved from one sample, and they can be clustered into hundreds or thousands of species. Therefore, it may be possible to detect certain integrated environmental changes which

2020年12月11日受理 (Accepted on December 11, 2020)

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cannot be detected by physical and chemical parameters alone.

Bacterial community composition of coastal bottom sediments is more diverse than that of seawater (Zinger *et al.*, 2011). Community compositions are often related to environmental conditions such as the particle size of the sediment, and the dissolved oxygen concentration in the bottom water (Zheng *et al.*, 2014), or the organic matter content in the sediment (Qiao *et al.*, 2018). Salinity change in the brackish water area (Vidal-Durà *et al.*, 2018) and anthropogenic contamination, such as metal and polycyclic aromatic hydrocarbon contaminants from land (Sun *et al.*, 2013), can also affect sediment bacterial communities. In land soil, bacterial communities are more affected by the biochemical conditions of the soil, such as organic matter content, than by the origin or geographic location of the soil (Lin *et al.*, 2019). In marine bottom sediments, however, it is not clear what difference the sea area makes. Factors such as the geographical distance from land, or overlying seawater characteristics (the current type), may affect bacterial communities together with the bottom environmental parameters such as particle size or organic matter content.

Kashima-Nada and Kujukurihama are open, large, sandy beaches, which are located at the northern and southern parts of Cape Inubo, respectively, on the Pacific coast of Japan. In these areas, primary production is high, and there are high fishery catches of bivalves and whitebait (Okunishi *et al.*, 2000). The cold Oyashio Current and the warm Kuroshio Current run along the Pacific coast of Japan, and they meet at a confluence point, off Cape Inubo. It is well known that the biological community of the Pacific coast of Japan changes, with Cape Inubo marking the border of the northern and southern fauna and flora (Shimizu, 2001). In addition, the Tone river mouth, which is the largest river in Japan, is north of Cape Inubo. Together with the large amount of river water discharge and the high wind in this area, a strong tidal stream occurs around Cape Inubo, and complex water exchange and sediment transportation occurs (Yagi *et al.*, 2002; Arai *et al.*, 2006; Uzaki *et al.*, 2016). The distribution patterns of macrobenthos fauna vary within a certain band north and south of Cape Inubo,

and the exact dimensions of this band depend also on the year of the survey (Shimizu, 2001). Bacterial communities in sediments may change in different ways from the larger organisms and may help elucidate bottom environmental conditions in more detail.

We examined bacterial community composition in the sediments collected at Kashima-Nada and Kujukurihama by using 16S ribosomal RNA (rRNA) gene sequences. By analyzing relationships between the community composition and sediment quality parameters, such as organic matter content and mud content, we aimed to clarify how the bacterial community varies in this area, and how the sediment qualities influence or reflect these variations.

Materials and Methods

Sample collection

Sediment samples were collected at three sites adjacent to the shorelines at a water depth of 10 m and 30 m, at the Kashima-Nada and Kujukurihama beaches in July, August, and September 2017 (Fig. 1). The annual water temperatures ranged from 25°C (highest; range: 23–27°C) to 12.5°C (lowest; range: 9–14°C) at Kashima-Nada, and from 26°C (highest; range: 25–27°C) to 15°C (lowest; range: 12–16°C) at Kujukurihama area between 2014 and 2018 (Tokyo District Meteorological Observatory). A Smith-

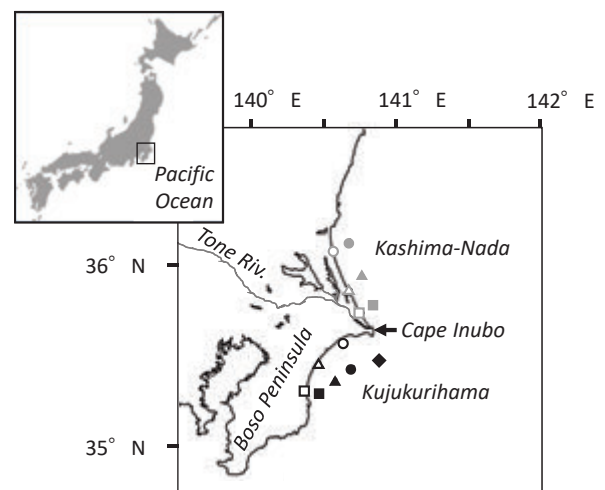


Fig. 1. Sample collection points at the coastal regions of Kashima-Nada and Kujukurihama on the Pacific coast, Japan. Open symbols indicate 10 m depth, and solid symbols indicate 30 m depth.

McIntyre grab sediment sampler (1/20 m², RIGO, Osaka, Japan) was used to collect sediment samples. A surface sediment sample (top 2 cm) was collected using a plastic corer of 23 mm diameter, and the sample was frozen immediately in a sterilized plastic bag. The sediment samples were kept at -60°C until further processing. Temperature and salinity were measured using a multi-parameter handy conductivity temperature depth profiler (RINKO Profiler, ASTD152, JFE Advantech, Nishinomiya, Japan).

Sediment quality analysis

The following variables were analyzed: (i) particle size was determined using a laser diffraction particle size analyzer (SALD-3100; Shimadzu, Kyoto, Japan), (ii) ignition loss was evaluated by igniting the sediment samples at 550°C for 12 h, (iii) chlorophyll *a* content was determined by fluorescence using a spectrophotometer (10AU, Turner Designs, San Jose, USA).

DNA extraction and sequencing

DNA was extracted from a 0.4 to 0.6 g sediment sample using the Fast DNA SPIN Kit for soil (MP Biomedicals LLC., Ohio, USA) according to the manufacturer's instructions. Barcodes were attached to DNA fragments in a 2-step PCR amplification that targeted the variable V1/V2 region of the 16S rRNA gene based on the protocol of Sakami *et al.* (2016). First PCR was conducted using 27Fm and 338R primers (Kim *et al.*, 2013), then the sequences were modified by adding 34 and 36 bp snippets, respectively, in order to later add molecular identifier (MID) tags. Pooled amplicons were paired-end sequenced (2×300 bp) on the Miseq device (Illumina, Tokyo, Japan).

Bacterial community analysis

The sequences obtained by extraction were quality filtered, and chimeras were removed using the UCHIME algorithm (Edger *et al.*, 2011) with alignment to the SILVA database (SSU_Ref_NR ver. 119) reference alignment. Sequences that passed through the filtering process were divided into operational taxonomic units (OTUs) using a 3% similarity threshold and were identified with

taxonomic annotation using the Mothur Miseq standard operating procedure (SOP) (Kozich *et al.*, 2013). Inverse Simpson diversity index was also estimated using this procedure.

The analyses below were done using the statistical package R version 3.5.3 (R Core Team, 2018). Detrended correspondence analysis was performed and produced an axis length of less than four, indicating a linear relationship between community composition and the examined environmental parameters: temperature, salinity, median particle diameter, mud content, ignition loss, and chlorophyll *a* content. Therefore, relationships of the bacterial community composition across the sampling sites were examined using non-metric multi-dimensional scaling (NMDS) analysis, and relationships of the bacterial community composition and environmental parameters were examined using redundancy analysis (RDA). Relative abundance of subphyla of the top three phyla, namely Proteobacteria, Bacteroidetes, and Firmicutes, and phyla whose relative abundance was more than 0.001 were compared among four sampling areas: Kashima-Nada-10 m, Kashima-Nada-30 m, Kujukurihama-10 m and Kujukurihama-30 m. A heatmap figure was made using the 16 taxonomic groups that were significantly different among the four areas (ANOVA, $p < 0.05$).

Results

Environmental parameters

Median particle diameter (d₅₀) was larger in the Kashima-Nada-30 m, and mud content was higher in Kujukurihama-30 m. Ignition loss was higher at Kujukurihama at a depth of 10 m and lower at Kashima-Nada at a depth of 30 m. Chlorophyll *a* content was higher at a depth of 30 m in both areas.

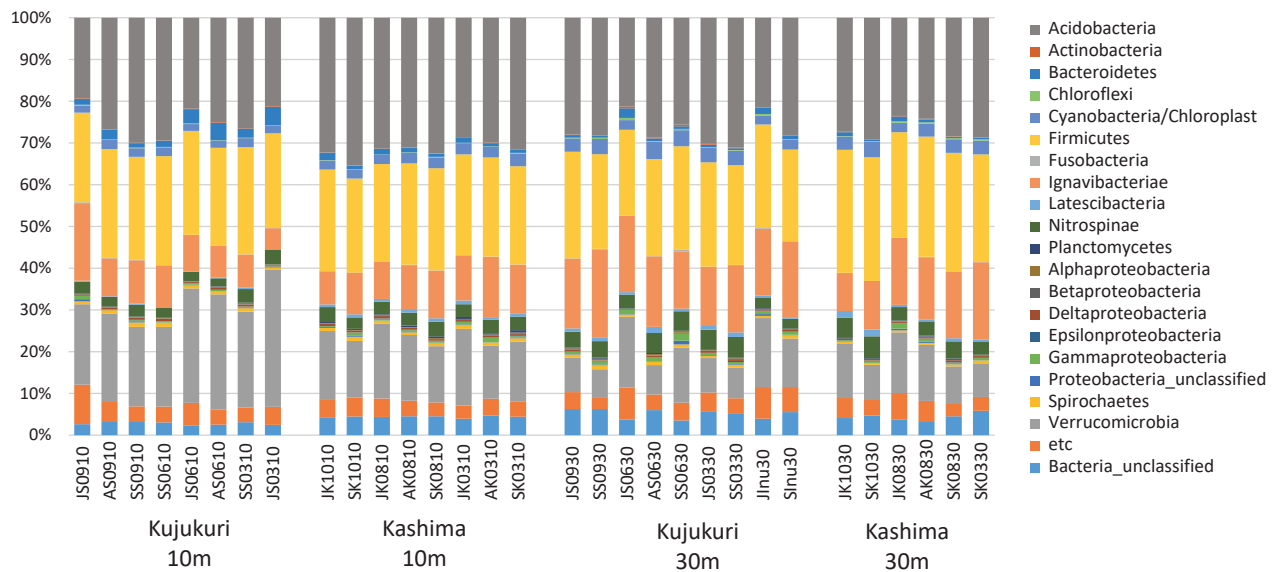
Bacterial community composition

The number of sequences obtained from sediment profiles ranged from 60,000 to 138,000 per sample. The community coverage was estimated to be more than 0.999 for all samples. The sequences were clustered into 803 operational taxonomic units (OTUs). Community diversity (Inverse Simpson index) ranged from 5.8 to 11.4, and it tended to be

Table 1. Average values of environmental parameters and bacterial community diversity at the sampling areas

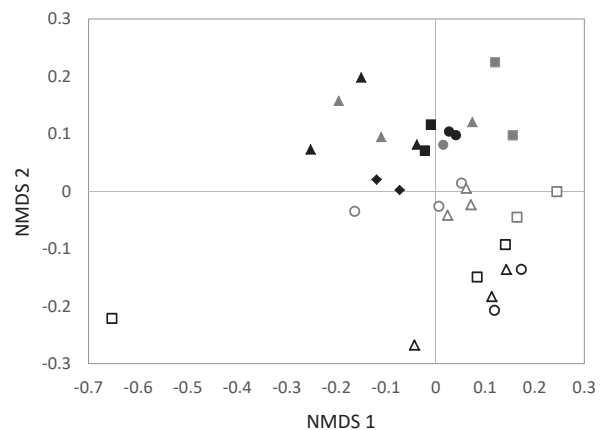
	Temp (°C)	Salinity (psu)	Central particle diameter (um)	Mud Content (%)	Ignition Loss (%)	Chlorophyll <i>a</i> content (ug/cm ²)	Invsimpson index
Kashima-Nada -10m	20.7 ^{ab}	33.7 ^b	187 ^b	0.18 ^b	1.4 ^{bc}	56.3 ^b	6.8 ^b
Kashima-Nada -30m	17.0 ^b	33.8 ^b	239 ^a	0.05 ^b	1.4 ^c	203 ^a	7.4 ^{ab}
Kujukurihama -10m	21.6 ^a	34.0 ^{ab}	166 ^b	0.36 ^b	2.1 ^a	88.8 ^b	8.4 ^{ab}
Kujukurihama -30m	19.3 ^{ab}	34.3 ^a	160 ^b	0.99 ^a	1.8 ^{ab}	167 ^a	8.9 ^a

* Numbers with different superscripts are different at the significance level of $p < 0.05$.

**Fig. 2.** Bacterial community composition in the surface sediment collected at 10 and 30 m depths off the coastal regions of Kashima-Nada and Kujukurihama in July, August and September, 2017.

high at Kujukurihama (Table 1). Of the OTUs, 33% to 53% belonged to the phylum, Proteobacteria, and the major subphyla were Gammaproteobacteria (21%–30%) and Deltaproteobacteria (5%–21%). Other major phyla were Bacteroidetes (7%–33%), Actinobacteria (3%–9%) and Acidobacteria (2%–6%) (Fig. 2).

The community composition of each sample was plotted in two dimensions following NMDS (non-metric multi-dimensional scaling) analysis (Fig. 3). Bacterial communities at a depth of 30 m at both Kashima-Nada and Kujukurihama were plotted together in the top area of the graph. Bacterial communities at a depth of 10 m at Kashima-Nada were plotted below the 30 m communities, and those off Kujukurihama were plotted in the bottom area of the graph.

**Fig. 3.** Non-metric multi-dimensional scaling plot of sediment bacterial communities with environmental parameters. Black symbols indicate Kujukurihama area, and gray symbols indicate Kashima-Nada area. Open symbols indicate a depth of 10 m and solid symbols indicate 30 m depth.

The relationships between the composition of the bacterial community and environmental parameters were examined using redundancy analysis (RDA) (Fig. 4). The X-axis represented 54% of the community variation. Bacterial communities were placed in order of the areas of 30 m depth,

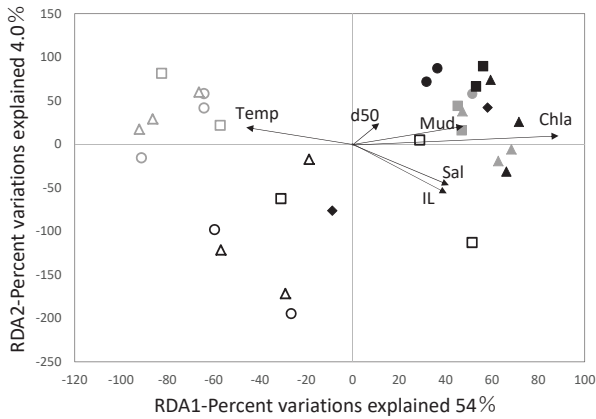


Fig. 4. Redundancy analysis (RDA) of sediment bacterial communities with environmental parameters. Black symbols indicate Kujukurihama area, and gray symbols indicate Kashima-Nada area. Open symbols indicate 10 m depth, and solid symbols indicate 30 m depth. IL, Chl a, d50, and mud represent ignition loss, chlorophyll a content, median particle size, and mud content, respectively.

the 10 m depth at Kashima-Nada, and the 10 m depth at Kujukurihama from the right. The Y-axis represented 4% of the community variation. Bacterial communities at a depth of 10 m at Kujukurihama were placed on this axis. Large directional arrows of chlorophyll a content and water temperature were along the X-axis, implying that these parameters were strongly related to variation in bacterial community composition. Small directional arrows for mud content, salinity, and ignition loss were on the lower right. No obvious directional arrows were observed along the Y-axis.

The relative abundance of the major taxonomic groups, whose abundance was different among the four sea areas, was shown in a heat map (Fig. 5). The taxonomic groups were clustered into two groups (cluster I and II), with the first cluster divided further into three subgroups (cluster Ia, Ib, Ic). Cluster Ia included Acidobacteria and Proteobacteria, except for beta and epsilon types, and abundance tended to be low at a depth of 10 m off the Kujukurihama coast. Cluster Ib included Chloroflexi and Ignavibacteriae, and the abundances tended to be low at a depth of 30 m at Kashima-Nada and at Kujukurihama. Cluster Ic included Betaproteobacteria, Planctomycetes,

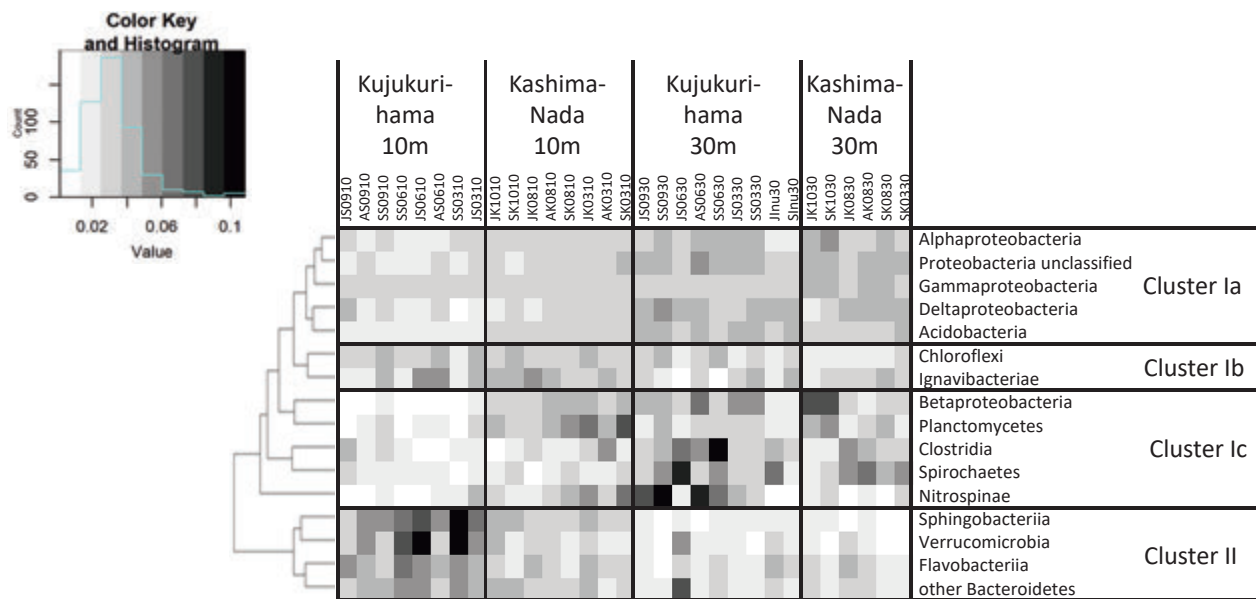


Fig. 5. Hierarchically clustered heatmap of bacterial communities from the four areas at the phylum or subphylum level. Rows represent the relative abundance of each bacterial group, and columns indicate different sediment samples. The relative abundance of each bacterial taxon is depicted by color intensity with the legend indicated at the top of the figure.

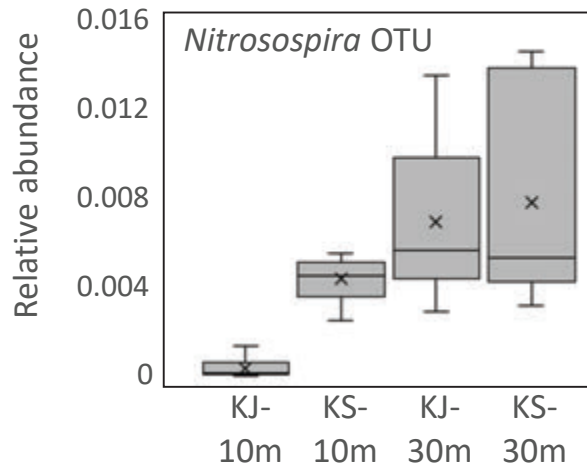


Fig. 6. Relative abundance of a *Nitrosospira* OTU in the four areas. KJ indicates Kujukurihama and KS indicates Kashima-Nada.

Clostridia, Spirochetes, and Nitrospinae, and the abundance tended to be low at 10 m depths in the Kujukurihama area. Relative abundance of an OTU affiliated to genus *Nitrosospira* accounted for 73% of betaproteobacterial OTUs. It was low at a depth of 10 m at Kujukurihama (Fig. 6). Cluster II included Flavobacteriia, Sphingobacteriia, other Bacteroides, and Verrucomicrobia, and the abundance tended to be high at 10 m depth in the Kujukurihama area.

Discussion

It is well known that the fauna and flora change at the border of the northern and southern junction at Cape Inubo, on the Pacific coast of Japan. For example, the distribution of marine bivalves and snails changes from tropical to cold-water varieties at the border of the Boso Peninsula (Shimizu, 2001). The warm Kuroshio Current which runs along the western Japanese Pacific coast changes direction to the east at Cape Inubo, leaving the main island of Japan. Therefore, the Kujukurihama area is influenced by the warm Kuroshio Current. In contrast, a confluence of the warm Kuroshio and the cold Oyashio Currents occurs at the northern end of Cape Inubo, the Kashima-Nada area. Moreover, the four examined areas, *viz.* the 10 m and 30 m depths at the Kashima-Nada and Kujukurihama sites, had different bottom environments as indicated by their particle size and organic matter content data. The

bacterial community composition was different between Kashima-Nada and Kujukurihama at depths of 10 m, but no obvious difference was observed at 30 m. The water depth, chlorophyll *a* content, and mud content was related to the variation in bacterial community composition in addition to the water temperature, indicating that the bacterial communities present in the examined areas varied primarily with the conditions of the bottom environment rather than with the differences in current. On land soil, bacterial community structure and diversity were shown to be affected more by parent material differences or biogeographical separation of the soil than the soil properties, such as organic matter content (Lin *et al.*, 2019). In marine ecosystems, there is evidence that the benthic bacterial community composition is affected more by the geographic location than the ecosystem type and time or upper water productivity (Zinger *et al.*, 2011). Since the bacterial community compositions were affected by sediment qualities rather than the geographical distance, we suggest that there are considerable exchanges of sediment materials between the northern and the southern areas of Cape Inubo.

It has been shown that bacterial community composition in marine bottom sediments changes according to the primary production in the sea (Zinger *et al.*, 2011). The Tone River water discharge to the north side of Cape Inubo, increases primary productivity in both the Kashima-Nada and Kujukurihama areas, and the influence fluctuates widely with precipitation and wind conditions (Arai *et al.*, 2006). The primary productivity in the Kashima-Nada area was estimated to be 0.4 g C/m²/day, and 76% of the production was estimated to depend on the regeneration of the nutrient due to organic matter degradation within the area (Okunishi *et al.*, 2000). Although primary production and a contribution from regeneration production were not indicated at the Kujukurihama area, the taxonomic features observed in the bacterial community composition may indicate different biological production processes between the two areas.

Concerning the abundance of bacterial taxonomic groups in the four sea areas, cluster II (which was abundant at 10 m at Kujukurihama)

included Flavobacteriia, Sphingobacteriia and Verrucomicrobia, which belong to Bacteroidetes. Bacteroidetes are generally considered to be specialists in the degradation of high molecular weight organic matter (Thomas *et al.*, 2011). The total organic matter (ignition loss) was high, and the chlorophyll *a* content was low at the depth of 10 m at Kujukurihama, suggesting that the sedimentary organic matter was relatively poor in fresh material. Flavobacteriia are often observed abundantly at the end of phytoplankton blooms (Teeling *et al.*, 2012; Sakami *et al.*, 2016). They may degrade not only fresh phytoplankton materials, but also labile, high molecular weight organic matter, which is deposited in the sediment. Among Bacteroidetes, Cytophagaceae members often digest macromolecules such as polysaccharides and proteins (McBride *et al.*, 2014), but their abundance did not differ among the four areas with different availability of these nutrients. Verrucomicrobia are also reported to be candidates for the degradation of polysaccharides in Arctic seawater (Cardman *et al.*, 2014). The cluster Ic was less abundant in the shallow area off Kujukurihama, and Betaproteobacteria and Nitrospinae were included in the cluster. More than 70% of the Betaproteobacterial OTUs belonged to *Nitrosospira* which are known as ammonia oxidizers. Because Nitrospinae is also related to the nitrification process, the nitrification process in this area might be less active than in the other three areas.

In conclusion, the composition of bacterial communities in coastal sediments varied mainly with water depth around Cape Inubo, and the differences were concomitant with differences in some environmental parameters. Moreover, the taxonomic groups characteristically found in an area suggest that the difference seems to be related to the properties of the organic matter of the sediment. Information on the benthic bacterial community is useful for monitoring coastal environmental changes because not only does it reflect the environmental conditions, but its taxonomic features indicate the ecological functions of the community. Future longer-term observations may provide detailed information on coastal environment changes in the area.

Acknowledgements

The authors greatly thank Dr. Hiroshi Yagi of the National Defense Academy for fruitful discussions about coastal environment management and the development of new monitoring techniques. The authors also thank the crew of the R.V. Taka-Marui for their support during sample collection. This work was supported by JSPS KAKENHI Grant Number JP17H03317.

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Annotated Bibliography of Key Works

(1) Sakami T., and Kakehi S., 2019: Distribution and community composition of ammonia-oxidizing archaea and bacteria in coastal sediments in response to sediment material gradients at Sendai Bay, Japan. in “Marine Metagenomics” (ed. by Gojobori T., Wada T., Kobayashi T., and Mineta, K.), Springer Nature Singapore Pte Ltd, pp.161–181.

The distributions of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were determined along an environmental gradient from the coastal mud to the offshore coarse sand at Sendai Bay, Japan. The abundance of AOA ammonia monooxygenase alpha subunit gene (*amoA*) was high in the coastal muddy areas and low in the offshore sandy areas. There was a strong positive correlation between AOA-*amoA* abundance and ammonia content in the sediment. The distribution of AOB-*amoA* was similar to that of AOA-*amoA* in July, but remarkably low in the muddy sediments in December. Clone library analysis indicated that the community composition for both types of organisms differed in sandy and muddy sediments and that the diversity was considerably lower in the muddy sediments. These results suggest that the abundance of ammonia-oxidizing organisms was controlled by the ammonia levels in the sediment. However, there are some inhibitive conditions for AOB: presumably, the low organic matter supply to the surface oxic layer during autumn in the muddy sediment in Sendai Bay.