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Microbial Community Composition in the Marine Sediments of Jeju Island: Next-Generation Sequencing Surveys[S]

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Marine sediments are a microbial biosphere with an unknown physiology, and the sediments harbor numerous distinct phylogenetic lineages of Bacteria and Archaea that are at present uncultured. In this study, the structure of the archaeal and bacterial communities was investigated in the surface and subsurface sediments of Jeju Island using a next-generation sequencing method. The microbial communities in the surface sediments were distinct from those in the subsurface sediments; the relative abundance of sequences for *Thaumarchaeota*, *Actinobacteria*, *Bacteroides*, *Alphaproteobacteria*, and *Gammaproteobacteria* were higher in the surface than subsurface sediments, whereas the sequences for *Euryarchaeota*, *Acidobacteria*, *Firmicutes*, and *Deltaproteobacteria* were relatively more abundant in the subsurface than surface sediments. This study presents detailed characterization of the spatial distribution of benthic microbial communities of Jeju Island and provides fundamental information on the potential interactions mediated by microorganisms with the different biogeochemical cycles in coastal sediments.

Keywords: Marine sediment, 16S rRNA gene, Archaea, Bacteria, Jeju Island

Introduction

Marine sediments are one of the most extensive habitats for microbes, covering more than two-thirds of the surface of the earth. Quantitative assessments of the microbial populations in sediments indicate that prokaryotes constitute a huge biomass portion of the earth, and that marine sediment processes may therefore substantially contribute to the global biogeochemical cycle (*e.g.*, nitrogen, carbon, and sulfur) [30, 49]. Moreover, because the microbes in sediments degrade organic matter and release elemental nutrients (*e.g.*, nitrogen and phosphorus) into the water column, these microbes, in part, facilitate the growth of primary producers (*i.e.*, algae). Thus, microbial-mediated biogeochemical processes in sediments are involved in the transformations of biogenic elements, which raise questions about the underlying functional contributions of microbes

in marine sediments. To determine the composition of diverse microbial communities and to identify specific niches, phylogenetic markers such as the 16S rRNA gene are sequenced and analyzed by a variety of methods, from a traditional clone library to high-throughput technology. Ultimately, the studies analyzing the 16S rRNA gene show that marine sediments that harbor microbial communities are global in occurrence and represent a wide diversity of habitats, including hydrothermal vents, methane seeps, and contaminated (organic-rich) coastal sediments [31].

As increasing numbers of microbial communities in the various environments are massively sequenced by the next-generation sequencing (NGS) methods, diversity estimates of those continuously rise into the millions [32]. Additionally, integrated metagenome and metatranscriptome analyses based on NGS were recently used to demonstrate the essential functions of microorganisms in sediments [18, 24].

Recently and dramatically, numerous largely unknown bacterial and archaeal phyla were revealed using NGS technology and single-cell genome sequencing [2, 3, 15, 16]. Moreover, Spang *et al.* [38] reported that a new group of the domain *Archaea* (*i.e.*, *Lokiarchaeota*) represented a missing link in the origin of the domain *Eukaryotes*.

Jeju Island, located southwest of the Korean Peninsula, is a volcanic landscape full of craters and cave-like lava tubes formed by the eruptions of lava [50]. Compared with other regions of the west coast, the coast of Jeju Island is not of well-developed coastal sediment (*i.e.*, tidal flats). Interestingly, the coastal sediments of Jeju Island might originate from the island and from Chinese rivers (*i.e.*, Changjiang River) [17]. Therefore, in this study, to estimate the diversity and abundance of the Archaea and the Bacteria in the coastal marine sediments of Jeju Island, a massive amplicon analysis was performed using the GS-FLX pyrosequencing platform. We also tried to identify microorganisms contributing to biogeochemical cycles (*e.g.*, nitrogen, carbon) in coastal sediments of Jeju Island. Our study provides insights that increase the understanding of the structure of the microbial communities in the coastal marine sediments of Jeju Island.

Materials and Methods

Collection of Marine Sediments and Extraction of DNA

Marine sediments were collected from one site (33° 13′ 57′′ N, 126° 14′ 12′′ E) in July 2014, using a core sampler. The core samples were sectioned (surface, approximately 0–10 cm; and subsurface, approximately 10–30 cm) and each section was transferred to sterile plastic tubes for storage at −80°C until analysis. The properties of the surface sediments were as follows: sediment type, muddy sand; total organic carbon, 0.55%; total organic nitrogen, 0.04%, total carbon, 1.43%; and total nitrogen, 0.07%, according to previous studies [28, 40]. A Power Soil DNA kit (Mo Bio Laboratories, USA) was used to extract the genomic DNA from the samples. After genomic DNA was extracted from two independent samples, the DNA from each of the sections was pooled for amplicon pyrosequencing.

PCR Amplification of Archaeal and Bacterial 16S rRNA Genes, Followed by Pyrosequencing

The detailed information on the method of pyrosequencing was described previously [12, 27]. In brief, the V1 and V3 hyper-variable regions of the 16S rRNA genes were amplified from the bacterial and archaeal 16S rRNA genes, respectively. The PCR amplifications of the 16S rRNA genes were performed using the 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 338R (5′-TGCTGC CTCCCGTAGGAGT-3′) primer set for Bacteria and the 514F (5′-GGTGBCAGCCGCCGCGRKAAHACC-3′) and 758R (5′-GGA CTACCCGGGTATCTAATCC-3′) primer set for Archaea. These amplicons were pyrosequenced using a Roche 454 GS FLX pyrosequencer (Macrogen, Korea), according to the manufacturer's instructions.

Pyrosequencing Data Analyses

Before the analysis, both the proximal and distal sequences and the barcode sequences were trimmed from the raw reads. Then, to increase the quality of the pyrosequencing results, the raw reads were processed further to remove the low-quality sequences (short reads <300 bp and reads longer than the size of the expected PCR product). For the pyrosequencing analyses, the modified pipeline described on the mothur website was used (http://www.mothur.org/wiki/454_SOP). The archaeal and bacterial sequence reads were compared with the reference databases of 16S rRNA genes obtained from the Ribosomal Database Project (RDP). The archaeal and bacterial sequences were assigned taxonomically based on the RDP classifiers [4]. The diversity indices (operational taxonomic units (OTUs), the Shannon and Simpson indices, and the Chao1 nonparametric richness index) and the rarefaction curves were determined using the mothur package [36]. During the analysis, the chimeric sequences and those unassigned and/or related to nonmicrobial species, such as from chloroplasts and mitochondria, were discarded. A dissimilarity level of 3% between sequences was used to calculate the diversity estimators. The phylogenetic trees developed with representative reads (OTUs) and reference sequences were constructed using MEGA 6 [41] with bootstrap values based on 1,000 replications [6]. The relative proportion of the reads representing each taxonomic group was calculated, with the exclusion of the reads identified as an unclassified group, unless otherwise stated.

Nucleotide Sequence Accession Numbers

The nucleotide sequences obtained in this study were deposited at the EMBL-EBI European Nucleotide Archive under the study accession number PRJEB9926.

Results and Discussion

Estimation of Microbial Diversity in the Marine Sediments

A total of approx. 54,300 sequences were used for the analyses of abundance and diversity and the taxonomic comparisons. After the preprocessing of the raw read sequences from both sediments, the number of validated reads was 45,436. The total number of archaeal and bacterial OTUs was approximately 5,700 and 7,600, respectively, at a level of 97% sequence similarity (Table 1). The Chao1 index (an estimate of species richness) for Bacteria indicated that the richness was relatively higher than that of Archaea. Additionally, the Shannon and Simpson diversity indices showed that Bacteria were slightly more diverse than Archaea, which was consistent with previous studies [1, 3,

Table 1. An overview of the surface and subsurface sediments of Jeju Island and estimates of sequence diversity and phylotype coverage of the 454 pyrosequencing data.

| Sample	Target domain	Analyzed reads	Observed OTUs	Shannon	Shannon lci[a]	Shannon hci[a]	Chao	Chao lci	Chao hci	Simpson	Simpson lci	Simpson hci	Good's coverage
Surface	Archaea	11,257	2,838	6.56	6.53	6.60	6,276.55	5,830.87	6,788.60	131.25	123.34	140.25	0.85
Subsurface		11,581	2,863	6.70	6.67	6.74	6,854.62	6,335.02	7,451.97	243.27	230.79	257.17	0.85
Surface	Bacteria	10,117	3,374	7.21	7.18	7.24	8,538.32	7,917.87	9,243.50	437.72	409.78	469.75	0.79
Subsurface		12,481	4,227	7.41	7.38	7.44	10,199.84	9,560.89	10,915.33	451.721	424.36	482.85	0.79

Diversity was estimated using operational taxonomic units (OTUs) and was defined as groups with ≥97% sequence similarity. Diversity indices and richness estimators were calculated using the mothur package (of the mothur project; http://www.mothur.org).

[a]*lci* and *hci* are rarefied 95% low and high confidence intervals (provided by the mothur application), respectively.

34] (see Table 1). The lower diversity in Archaea might be related to the energetic costs of metabolism, and support for this explanation was provided by Valentine [46], who suggested that chronic energy stress was the primary selective pressure in the evolution of Archaea. Moreover, the archaeal and bacterial diversity indices (OTUs, Chao1, Shannon, and Simpson) of the subsurface sediments were slightly higher than those for the surface sediments. This result might be explained by the anoxic conditions, because microorganisms can grow by fermentation or anaerobic respiration and can use a variety of electron acceptors (nitrate, sulfate, or iron) [9, 14]. In Table 1, the sequencing reads, the diversity indices, and the coverage of the marine sediment samples of the study are summarized.

Archaeal Community Composition in the Marine Sediments

The composition of the archaeal communities in the surface and subsurface sediments was determined with 454 pyrosequencing. Of the archaeal sequences, 11,257 (before trimming 12,457) and 11,581 (13,047) reads were analyzed from the surface and subsurface sediments, respectively. Recently, using NGS methods, the number of new genomes and 16S rRNA gene sequences has increased dramatically, and the proposal of new archaeal groups has led to a reconstruction of the archaeal tree [3, 8, 37]. In this study, we also identified several new, proposed archaeal groups from the marine sediments (see below). In the surface sediments, most of the archaeal sequence reads were associated with *Thaumarchaeota* (46.8%), whereas most were associated with *Euryarchaeota* (68.6%) in the subsurface sediments (Fig. 1A). *Woesearchaeota* was also identified as one of the major phyla in both sediment types (20.0% and 15.6%, respectively). Based on the sequences, *Aenigmarchaeota*, *Crenarchaeota*, and *Pacearchaeota* were identified as minor archaeal groups using the RDP database. Notably, *Aenigmarchaeota* was found in the subsurface sediments.
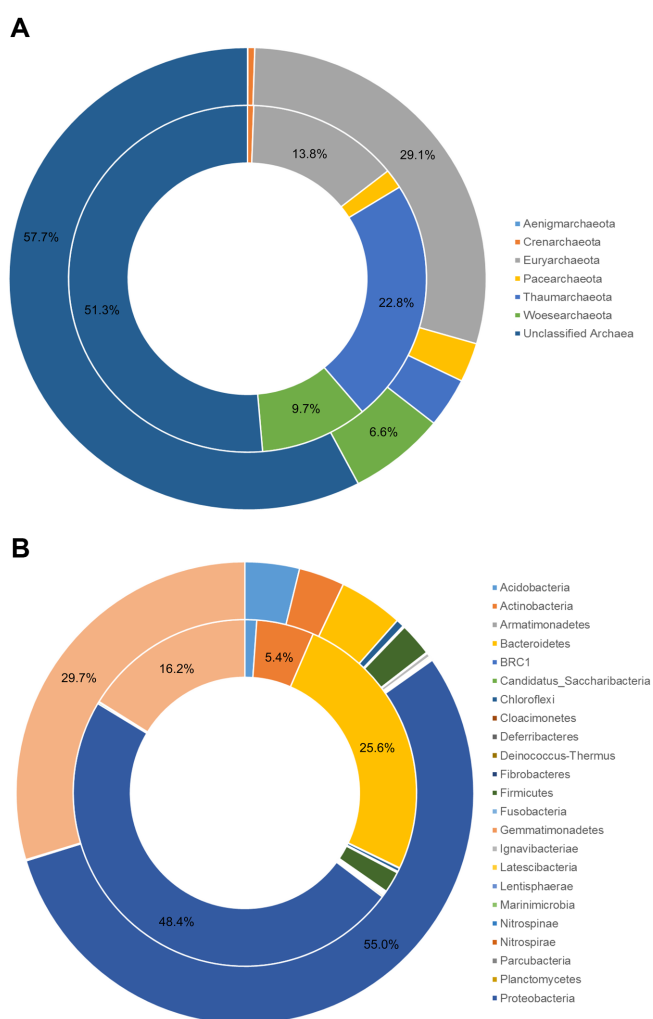
Fig. 1. The abundance of the phyla in Archaea (**A**) and Bacteria (**B**) in the marine sediments.

The archaeal and bacterial 16S rRNA gene sequences were assigned to each phylum using the mothur package and a reference database of 16S rRNA genes obtained from the Ribosomal Database Project. Inner and outer circles show the surface and the subsurface sediments, respectively. Above 5% proportions of the total are shown.

Additionally, the reads assigned to the phyla *Euryarcheaota* (68.6%) and *Pacearchaeota* (6.5%) were significantly more abundant in the subsurface sediments than those in the surface sediments (28.3% and 3.7%, respectively). By contrast, the abundances of the phyla *Thaumarchaeota* (46.8%) and *Woesearchaeota* (20.0%) in the surface sediments were higher than those in the subsurface sediments (8.2% and 15.6%, respectively). Notably, *Woesearchaeota* are identified in terrestrial environments (*i.e.*, groundwater) and considered as symbiotic and fermentation-based lifestyles [3]. It is also possible that the present study site has been continuously exposed to freshwater runoff from Island land (*e.g.*, groundwater). On the other hand, in marine surface sediment, Thaumarchaeota-related sequences have been identified as a major member. It might explain that some thaumarchaeotal strain as an ammonia oxidizer has a metabolic trait for aerobic respiration [references in 39]. None of the reads classified to the *Aigarchaeota* or the *Parvarchaeota* were associated with the DPANN superphylum [37].

To determine the detailed phylogenetic positions [44] of the archaeal reads, we selected the top 500 OTUs as high-ranked taxa with aenigmarchaeotal reads. Using this phylogenetic analysis, the archaeal reads were separated into 12 archaeal groups (Fig. S1). However, the marine benthic group B/deep-sea archaeal group, the deep-sea hydrothermal vent euryarchaeotal group, and the South African gold mine euryarchaeotal group were not found.

The dominant archaeal groups (with more than 10% of the total abundance) of the surface sediments were groups I.1a (31.1%) and I.1b (25.6%) (as ammonia-oxidizing archaea, AOA), and *Woesearchaeota* (13.1%). In the subsurface sediments, the dominant groups were the marine benthic group D (MBG-D, 56.2%) and the terrestrial miscellaneous euryarchaeotal group (TMEG, 19.2%). The other archaeal groups, the marine benthic group C (MBG-C), the miscellaneous crenarchaeotic group (MCG), the methanogens, and the haloarchaea, were identified as minor groups (with abundance ranging from 0 to 9.0%). An unclassified Euryarchaeota group was identified only in the subsurface sediments with an abundance of 0.9% (Fig. 2). Notably, the haloarchaea that live in hypersaline environments (salinity range from 25% to 37%) were found in both sediment types (3.5% and 0.4%, respectively); most haloarchaeal strains were previously isolated from hypersaline environments, including solar saltens and salt lakes. However, Purdy *et al.* [33] isolated haloarchaea that grew slowly at the salinity of seawater (2.5%), and several molecular studies also identified haloarchaea in low-salinity environments [47]. Thus, the diversity and the ecological roles of the haloarchaea are remarkably higher and much wider than previously suggested.

Unexpectedly, although the methanogens and the MCG were identified from organic-rich or subsurface sediments as the dominant, heterotrophic anaerobes [reviewed in 43, 45], as noted previously, the two groups were classified as
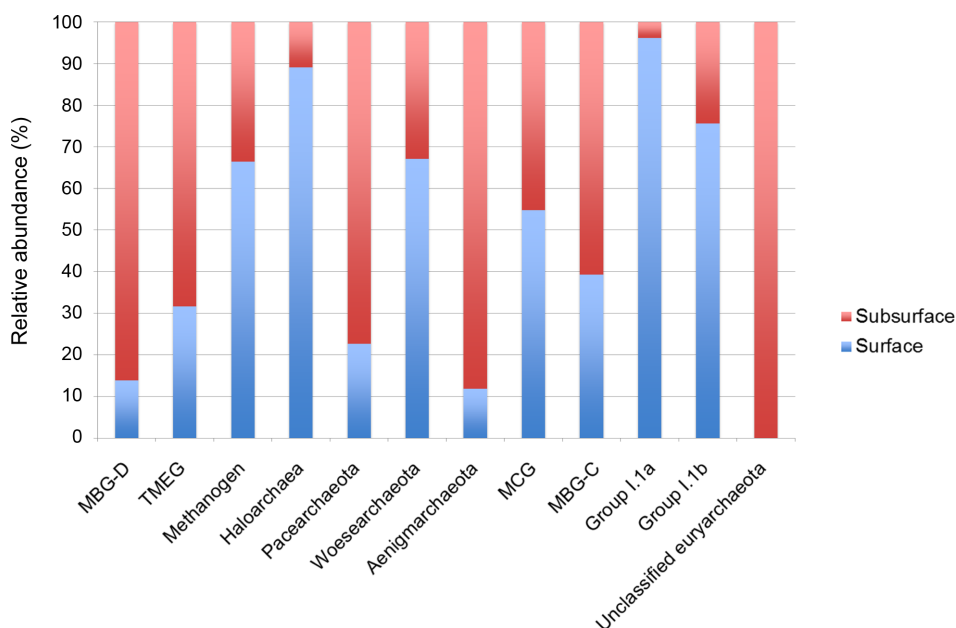

Fig. 2. The relative abundance of archaeal reads at the phylum and subgroup levels in the surface and subsurface sediments.

minor groups in this study. Moreover, the relative abundance of the two groups (7.1% and 0.6%, respectively) in the surface sediments was slightly higher than that (3.6% and 0.5%, respectively) in the subsurface sediments. Therefore, the marine sediments of Jeju Island might possibly have an extremely narrow oxic-anoxic zone [29] or the sampling site might be affected by oligotrophic conditions.

Bacterial Community Composition in the Marine Sediments

The bacterial community structure in the surface and subsurface sediments was estimated by 454 pyrosequencing. Of the bacterial sequences, 10,117 (before trimming 14,603) and 12,481 (14,355) reads were analyzed from the surface and the subsurface sediments, respectively. Overall, 26 phyla, including an unclassified bacterial group, were found in the surface and subsurface sediments. With the exception of the unclassified taxonomic group, most of the bacterial sequence reads were associated with *Proteobacteria* and *Bacteroidetes*, followed by *Actinobacteria*, *Acidobacteria*, and *Firmicutes* (Fig. 1B). The relative sequence abundances (above 1% of the total number of sequences) of *Actinobacteria* and *Bacteroides* were higher in the surface than those in the subsurface sediments, whereas *Acidobacteria*, *Firmicutes,* and *Proteobacteria* were more abundant in the subsurface sediments. Within the phylum *Proteobacteria*, the classes *Alphaproteobacteria*, *Deltaproteobacteria*, and *Gammaproteobacteria* were the most abundant in both sediment types. The *Alphaproteobacteria* and the *Gammaproteobacteria* had higher sequence abundances in the surface than in the subsurface sediments, whereas the *Deltaproteobacteria* was the dominant class in the subsurface sediments (Fig. 1B).

With the exception of the sequence reads assigned to the unclassified taxon, the scrutiny portions (calculated from the total proportion of each taxon at class or phylum level) of the bacterial taxonomic affiliations were estimated to identify a phylogenetic shift within the same lineage between the surface and the subsurface sediments. In the phylum *Acidobacteria*, a total of 10 acidobacterial subgroups (Gp3, 4, 6, 9, 10, 16, 17, 18, 22, and 23) were identified from both sediment types. To date, as the most dominant phylum in soilborne microbial communities [10], 26 subgroups of the phylum *Acidobacteria* are recognized [1]. However, the subgroups are rarely cultured, and consequently, the *Acidobacteria* remains a poorly studied phylum. In this study, we found that only two acidobacterial subgroups (Gp22 and Gp23) predominated, with similar abundances in both sediment types. Wang *et al*. [48] reported that the acidobacterial subgroups Gp10 and Gp22 were higher-abundance ranked taxa in marine sediments. In recent

studies, the abundance and/or diversity of *Acidobacteria* were affected by environmental factors (*i.e.*, pH, soil types, and abiotic soil factors) [21, 22]. In the class *Actinobacteria*, *Ilumatobacter* and *Propionibacterium* were identified as a major and a minor genus, respectively, in both sediments. Additionally, the genera *Sporichthya*, *Streptomyces*, *Terrabacter*, and *Rhodococcus* were identified as minor taxa. The genus *Ilumatobacter* was predominant in the surface sediments, whereas the abundance of *Propionibacterium* was slightly higher in the subsurface than that in the surface sediments. Fujinami *et al*. [7] found that the genome of *Ilumatobacter coccineum* harbored a limited number of secondary metabolic enzymes; that is, type I polyketide synthases that produce the polyketide commercially used as an antibiotic or animal growth promoter [11]. In the phylum *Bacteroidetes*, the classes *Flavobacteria* and *Sphingobacteria* were predominant with high relative abundances in the surface sediments, whereas the class *Cytophaga* was dominant in the subsurface sediments. The *Bacteroidetes* can digest a variety of complex substrates (*e.g.*, cellulose and chitin) [13]. Although only three classes (*Anaerolineae*, *Caldilineae*, and *Dehlococcoidia*) in the phylum *Chloroflexi* were identified as minor taxa in both sediments, the *Chloroflexi* are (an)aerobic heterotrophs that decompose a variety of substrates and are often observed with other microbes (syntrophy) for growth [51]. From these results and the related information, it was deduced that the decomposition activities for complex substrates are higher in the surface sediments under aerobic conditions than those in the subsurface sediments, and subsequently, in the subsurface sediments, the anaerobes use the digested materials for anaerobic respiration and/or fermentation. Additionally, in the phylum *Firmicutes*, the genera *Bacillus*, *Lactobacillus*, *Trichococcus*, and *Clostridium* are well-known fermentation microorganisms, and these groups dominated in both sediments. In the class *Alphaproteobacteria*, four genera (*Erythrobacter*, *Jannaschia*, *Loktanella*, and *Paracoccus*) composed 64.0% of the abundance of the total reads, and higher, in the surface sediments, whereas *Pelagibius* and *Sphingomonas* were the dominant genera (composed 47.2%) in the subsurface sediments. Unexpectedly, in the class *Deltaproteobacteria*, the sulfate reducers (sulfate-reducing bacteria, SRB) were identified as the predominant population and composed 89.2% and 98.0% of the class in the surface and subsurface sediments, respectively. This result might be correlated with the many reduced sulfur compounds found in volcanic and/or hydrothermal vent sediments (*i.e.*, Jeju Island is a volcanic island; see Introduction) [23, 52]. Moreover, in previous studies, the SRB were identified as a predominant group in marine sediments with high

concentrations of sulfate [20, 34]. In the class *Gammaproteobacteria*, of the 28 genera, the abundance of the genus *Hailea* was the highest in the surface sediments, whereas the genus *Acinetobacter* was predominant in the subsurface sediments.

Functional Groups of Microorganisms

Although the phylogenetic positions based on the 16S rRNA gene were loosely correlated with functional traits, we found reads that were affiliated with functional genera of bacteria as minor populations, including nitrogen-fixing bacteria (*Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium*), methylotrophs (*Methylobacterium*, *Methylophilus*, *Methylotenera*, *Methyloversatilis*, and *Methylophaga*), and nitrite-oxidizing bacteria (NOB). Two phyla, *Nitrospinae* and *Nitrospirae*, were identified in both and in subsurface sediments only, respectively. The genera *Nitrospina* and *Nitrospira* within the *Nitrospinae* and *Nitrospirae*, respectively, are well-known NOB [5, 42]. In this study, we identified only eight reads that were affiliated with the *Nitrospina* lineage, with 87.2% 16S rRNA gene sequence similarity with the isolated *Nitrospina* species (Fig. S2), and these reads were separated into four OTUs (OTU1325 in the surface sediments and OTU0219, 0220, and 2924 in the subsurface sediments). As chemolithoautotrophs, the NOB contribute to the global nitrogen cycle via the oxidation of nitrite to nitrate, the second step of nitrification. Notably, the distributions of *Nitrospina* and the AOA were correlated in some coastal and open ocean habitats [19, 35]. Moreover, *Nitrospina* and the AOA of sediments were observed in an enrichment culture [26]. These results might indicate a metabolic link between these nitrifiers [25]. However, in this study, we did not detect a codistribution of the groups I.1a and I.1b as AOA with NOB.

To summarize, a massive analysis was performed on the microbial communities of the coastal sediments of Jeju Island using a pyrosequencing method. The total number of OTUs obtained for Archaea and Bacteria from both the surface and subsurface sediments were approximately 5,700 and 7,600, respectively. The diversity indices indicated that the bacterial diversity was significantly higher than the archaeal diversity. Additionally, the microbial diversity of the subsurface sediments was slightly higher than that of the surface sediments. This study had some limitations. First, the number and data set of the analyzed samples were small compared with other studies, although some candidate divisions were also found. Second, we could not obtain additional environmental parameters for the two sediments; this information would have provided more insights into the relationships between the structure of the

microbial community and the physical and chemical parameters of the sediments. Additionally, although many validated reads were classified at the phylum level, the number of reads affiliated with unclassified taxa increased from the phylum to the species level. This outcome might be a common problem with high-throughput sequencing, but these results will provide basic information for further studies to estimate ecological and functional roles of the microbial community in sediments. In conclusion, this study provides primary information on the microbial diversity to facilitate an understanding of the microbial activity in the coastal sediments of Jeju Island.

Acknowledgments

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