Characterization of Bacterial Communities in Bioaerosols over Northern Chinese Marginal Seas and the Northwestern Pacific Ocean in Spring

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ABSTRACT

Studies of the community structures of bacteria in marine aerosols of different particle sizes have not been reported. Aerosol samples were collected using a six-stage bioaerosol sampler over the Bohai Sea, the Yellow Sea, and northwestern Pacific Ocean in the spring of 2014. The diversity and composition of these samples were investigated by Illumina high-throughput sequencing, and 130 genera were detected in all of the samples; the most abundant bacterial genus was *Bacteroides*, followed by *Prevotella* and *Megamonas*. The Chao1 and Shannon diversity indices ranged from 193 to 1044 and from 5.44 to 8.33, respectively. The bacterial community structure in coarse particles (diameter larger than 2.1 μm) was more complex and diverse than that in fine particles (diameter less than 2.1 μm) in marine bioaerosols from over the Yellow Sea and northwestern Pacific Ocean, while the opposite trend was observed for samples collected over the Bohai Sea. Although we were sampling over marine regions, the sources of the bioaerosols were mostly continental. Temperature and wind speed significantly influenced the bacterial communities in marine aerosols of different particle sizes. There may be a bacterial background in the atmosphere in the form of several dominant taxa, and the bacterial communities are likely mixed constantly during transmission.

1. Introduction

Bioaerosols are airborne particles or large molecules that consist of viruses, bacteria, fungi, pollen, and plant or animal debris as well as fragments and products of these organisms (Ariya and Amyot 2004; Grinshpun and Clark 2005). Bioaerosols can potentially serve as cloud condensation nuclei (CCN; Bauer et al. 2003; Franc and Demott 1998) and ice nucleating particles (INP; Christner 2010; Garcia et al. 2012; Vali et al. 2015) and influence cloud cover and precipitation (Deleon-Rodriguez et al. 2013) and even the global climate (Van Leuken et al. 2016). Bioaerosols also have apparent effects on air quality and human health (Ho and Duncan 2005; Kalogerakis et al. 2005; Ma et al. 2017). Therefore, studies of the composition and structures of microbial communities in aerosols are of great importance for a comprehensive understanding of the effects of aerosols on the climate, environment, and human health.

Many studies have focused on the concentrations and community structures (the composition of a microbial

community and the abundance of its members) of airborne microorganisms, especially terrestrial airborne microorganisms. The concentrations of bioaerosols exhibit significant seasonal variations, and the seasonal variations in different regions are distinctly different (Qi et al. 2014; Dong et al. 2016). Qi et al. (2015) have proposed a fluorescein diacetate (FDA) hydrolysis method for measuring microbial activity in bioaerosols, representing the intensity of microbial metabolic activity, which can be used to indicate the physiological state of microbes. Microbial activity in bioaerosols can be affected by the season, the region, and meteorological factors (Zhong et al. 2016). In the coastal region of Qingdao, microbial activity in bioaerosols is significantly influenced by atmospheric temperature and wind speed, and the seasonal level of microbial activity in bioaerosols from greatest to least is summer, autumn, winter, and spring (Zhong et al. 2016). The structures of bacterial communities change with the presence of haze or dust. The presence of haze can affect bacterial community structures in aerosols. For example, bacterial composition and diversity in the atmosphere vary significantly during dust events, which may carry particles from grasslands (phyllosphere), dry lakes, and

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sandy surfaces, and some bacterial populations, such as Firmicutes and Bacteroidetes, are maintained in the atmosphere for longer time periods (Maki et al. 2017). In addition, pathogenic bacteria detected on hazy days may pose health risks (Ma et al. 2017; Xu et al. 2017).

Studies on microbial communities in aerosols of different particle sizes have also been performed, mainly focusing on terrestrial aerosol microorganisms. Bacterial communities vary significantly among fractions of different sizes during severe occurrences of haze in Jinan, China (Xu et al. 2017). Polymenakou et al. (2008) found that, based on phylogenetic analyses, atmospheric microbial community structure depends on particle size and that the presence of aerosolized bacteria in small size particles may have had significant implications on human health via intercontinental transportation of pathogens during an intense African dust event in the eastern Mediterranean. Particle size distribution can act as a factor affecting the capacity of biological particles to act as cloud condensation nodules (CNs) or INP, thereby influencing their atmospheric transport and processing (Diehl et al. 2001; Morris et al. 2004; Fröhlich-Nowoisky et al. 2016). Particle size can affect the distance, speed, and height of aerosol transmission, which is of great significance to the spread and distribution of microorganisms. The study also found that size matters more than chemistry for the cloud nucleating ability of aerosol particles (Dusek et al. 2006). Therefore, the study of microbial composition of different particle sizes has important environmental and ecological significance.

Oceans cover approximately 70% of the world's surface and are the main source and sink of atmospheric microbes. Marine aerosol plays a role as a main vector for the transport of bacteria across the air–sea interface and the long-distance dispersal of bacterial species (Aller et al. 2005). Marine bioaerosol microorganisms have been studied recently. High-throughput sequencing technology has shown that bacterial communities in marine aerosols are more complex and diverse than previously thought, and the communities over remote marine regions are shaped by the sources of air mass (Xia et al. 2015). Uncultured Betaproteobacteria, *Sphingomonas* sp., and uncultured *Acidovorax* sp. were the dominant bacteria in aerosol samples collected above the Yellow Sea (Wu 2014). Over the East Sea (Korean Peninsula), the abundance of marine aerosol bacteria was found to range from 0.7×10^3 to 1.2×10^3 cells per cubic meter, and the bacterial community was dominated by Gammaproteobacteria and Bacteroidetes (Cho and Hwang 2011). Seifried et al. (2015) found that the most abundant phyla were Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes in marine bioaerosols

sampled during a cruise from the North Sea to the Baltic Sea via Skagerrak and Kattegat. Bacterial concentrations in the marine air were found to be positively correlated with wind speed near the sea surface of the northwestern Pacific Ocean (Hu et al. 2017).

Studies on the community structures of microbes in marine aerosols of different particle sizes are very limited. To preliminarily understand the characteristics of marine aerosol bacterial community structures of different particle sizes and their possible influencing factors, we studied bacterial community structures in marine aerosols with different particle sizes from over the Bohai Sea, the Yellow Sea, and northwestern Pacific Ocean via Illumina high-throughput sequencing, which provide information for future studies.

2. Materials and methods

a. Sample collection

Three sets of bioaerosol samples were collected from the Bohai Sea (BHS) and the Yellow Sea (YS1, YS2) during a ship cruise, while two sets of bioaerosol samples were collected from the Yellow Sea (YS3) and the northwestern Pacific Ocean (NWP) during another ship cruise. All of the samples were collected in the spring of 2014 (Fig. 1). A sterilized sampler and filter membranes were used for sampling. Size-segregated aerosol samples were collected on 0.22- μm polycarbonate membrane filters (Shenghechengxin Membrane Science and Technology Development Center of Beijing, China) for 1 h with a sampling flow rate of approximately 28.3 L min^{-1} using a six-stage microorganism FA-1 cascade impactor (Applied Technical Institute of Liaoyang, China). After the sampler was sterilized with 75% alcohol and 15 min of ultraviolet radiation on a clean bench, the sampler was wrapped in a fresh-keeping bag and placed on the sampling rack for sampling, ensuring that the sampler was sterile before each sampling. The sampler was placed approximately 15 m above sea level. To avoid contaminants from the ship's exhaust, the sampler was fixed in front of the uppermost deck and worked only when the ship was sailing. The aerosol particles were fractionated into the following six stages based on size: 0.65–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7, 4.7–7.0, and $>7.0 \mu\text{m}$, which are referred to as classes 1–6, respectively, in this paper. After sampling, the filters were stored at -20°C for future analysis.

The meteorological parameters (temperature, relative humidity, wind speed, and wind direction) (Table 1) measured by the meteorological observatory on the research vessel were recorded during sampling.

FIG. 1. Sampling transects in the spring of 2014 in the Bohai Sea, the Yellow Sea, and northwestern Pacific Ocean. Each transect represents a total sampling time of 1 h.

b. DNA extraction and Illumina high-throughput sequencing

Genomic DNA was extracted from aerosol samples of each particle size using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The extracted DNA was stored at -80°C prior to further analysis.

The community characteristics of marine bioaerosol bacteria were studied via Illumina sequencing of the 16S rRNA gene. Fragments of the 16S rRNA gene [~ 466 base pairs (bp), V3–V4 region] were amplified using the primer pair 341F ($5'$ -CCTACGGGSGCAGCAG) and 806R ($5'$ -GGACTACGAGGTATCTAAT) (Roggenbuck et al. 2014). Specific primers with barcodes were synthesized before polymerase chain reaction (PCR) amplification. PCR amplification was performed using the KAPA HiFi HotStart ReadyMix (Kapa Biosystems, United States) in a $30\text{-}\mu\text{L}$ reaction containing $15\text{ }\mu\text{L}$ of $2 \times$ KAPA HiFi Hotstart ReadyMix, $0.2\text{ }\mu\text{M}$ forward and reverse primers and approximately 10 ng of template DNA. Touchdown PCR was performed under the following conditions: initial denaturation at 95°C for 3 min; 12 cycles (in which the temperature was decreased by 0.5°C every other cycle during annealing and time was increased by 1 s in every other cycle during extension) of 98°C for 15 s, annealing at $72^{\circ}\text{--}66.5^{\circ}\text{C}$ for 10 s, extending at 72°C for 10–21 s, 94°C for 20 s, annealing from 65° to 59.5°C for 10 s, and extending at 72°C for 10–21 s; 11 cycles of 94°C for 20 s, 58°C for 30 s and 72°C for 30 s; and a final

extension step at 72°C for 2.5 min. The PCR products were analyzed via 2% agarose gel electrophoresis and purified using the AxyPrep DNA Gel Extraction Kit (Axygen, United States). The libraries were quantified using a Thermo NanoDrop 2000 ultraviolet microspectrophotometer and 2% agarose gel electrophoresis. Then, the libraries were quantified using a Qubit 2.0 fluorometer (Invitrogen, Life Technologies), mixed at equal concentration and subsequently sequenced on the Illumina HiSeq 2500 (PE250) platform following the manufacturer's instructions. The 16S rDNA sequencing was performed by the Realbio Genomics Institute (Shanghai, China). Raw sequences were submitted to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) database under the accession number SRP128887.

c. Processing and analysis of sequencing data

A total of 30 samples with different particle sizes were collected from the five sites. Four samples (YS1, $3.3\text{--}4.7\text{ }\mu\text{m}$ and $>7.0\text{ }\mu\text{m}$; YS2, $>7.0\text{ }\mu\text{m}$; and NWP, $3.3\text{--}4.7\text{ }\mu\text{m}$) could not be sequenced because of the low concentrations of the extracted DNA, which possibly reflected the low abundances of microorganisms in these particles. During the sequencing process, both ends of a DNA sequence can be sequenced. First, one end is measured, a read is obtained, paired-end turnaround occurs, and the other end is sequenced to obtain another read. The two reads obtained are paired-end (PE) reads.

TABLE 1. Meteorological parameters at the beginning of sampling.

	Sampling date	Temperature ($^{\circ}\text{C}$)	Relative humidity (%)	Wind speed (m s^{-1})	Wind direction ($^{\circ}$)
BHS	13 May 2014	14.8	85.20	10.5	222
YS1	17 May 2014	13.3	91.90	5.3	339
YS2	30 Apr 2014	13.8	93.20	0.4	235
YS3	18 Mar 2014	11.9	78.70	11	12
NWP	19 Apr 2014	19.8	81.30	6.4	65

PE reads were assigned to the samples based on their unique barcodes, truncated by removing the barcode and primer sequences and then merged using paired-end assembler for Illumina sequences (PANDAseq, version 2.9; Masella et al. 2012). The merged reads were subsequently subjected to quality-based filtering with the analysis platform of the Realbio Genomics Institute. Briefly, average read quality scores below 20 and reads containing more than three ambiguous bases were discarded. The read length range was 220–500 nucleotides (nt). Singletons were filtered, while operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.0.1001, <http://drive5.com/uparse/>) (Edgar 2013); chimeric sequences were identified and removed. All samples were normalized at the same sequencing depth. The OTUs obtained after normalization were used as a basis for calculating metrics associated with alpha diversity and beta diversity. The most abundant sequence from each OTU was selected as the representative sequence for sequence alignment. Sequences were classified based on the Ribosomal Database Project (RDP) database (<http://rdp.cme.msu.edu>) (Cole and Tiedje 2014) at a confidence threshold of 80%. Statistical analyses of the OTU alpha diversity of each aerosol sample based on the Chao1 index (species richness), Good's coverage estimator (coverage), and Shannon diversity index (species diversity) were performed using Quantitative Insights Into Microbial Ecology (QIIME) 1.9.0 software (Caporaso et al. 2010). Richness simply denotes the number of species, as an unweighted measure of species number, while species diversity is a function of the number of species present and the evenness with which the individuals are distributed among these species (Delang and Li 2013; Sanjit and Bhatt 2005).

d. Airmass backward trajectories

Backward trajectories of all the sampled air masses were created using the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPPLIT; Draxler and Rolph 2013). Five-day backward trajectories (Singh et al. 2001; Zhu et al. 2015; Seifried et al. 2015) were calculated for air parcels from the 1000-m air layers based on the GDAS1 meteorological dataset and the model vertical velocity vertical motion method.

3. Results

a. Bacterial community diversity in marine bioaerosols

The Good's coverage estimates for the bioaerosol samples from all of the sites were higher than 99.9%

TABLE 2. Atmospheric bacterial community diversity in marine aerosols.

	Chao1	Shannon	Good's coverage (%)
BHS	1209.2	7.010	100
YS1	1465.4	7.755	100
YS2	1872.1	6.855	100
YS3	2425.0	8.049	99.9
NWP	1400.8	7.347	100

(Table 2), suggesting that the sequencing effort covered a majority of the bacterial communities. The rarefaction curves for the samples gradually became smooth (Figs. 2a,b), indicating that the sequencing capacity was large enough to capture the complete diversity of these communities. The Chao1 index indicated that the richness of the aerosol bacterial communities was highest at the YS3 site and lowest at the BHS site. Bacterial community richness showed the trend, from greatest to least: Yellow Sea, northwestern Pacific Ocean, and Bohai Sea; and the bacterial richness in the south Yellow Sea aerosols was higher than that in the north Yellow Sea aerosols. The Shannon index indicated that the diversity of aerosol bacterial communities was the highest at the YS3 site and lowest at the YS2 site. Aerosol bacterial community diversity showed the trend, from greatest to least: Yellow Sea, northwestern Pacific Ocean, and the Bohai Sea.

b. Bacterial community composition in marine bioaerosols

A total of 724 152 reads were clustered into 4448 unique OTUs with a 97% similarity cutoff. The bioaerosol samples from the five sites had 259 shared OTUs (Fig. 3), accounting for 5.82% of the total OTUs.

All of the OTUs were classified into 32 known bacterial phyla with confidence score thresholds of 80% (Fig. 4a). Nineteen shared phyla were present in aerosol samples from the five sites, and sequences of these phyla from the five sites represented 89.50% of all bacterial sequences. The most abundant bacterial phylum (represented highest proportion of all bacterial sequences) detected was Bacteroidetes (26.99%), followed by Firmicutes (26.72%) and Proteobacteria (21.82%). The relative abundances (percentage of sequences) of these three phyla were more than 80% at the BHS, YS1, and NWP sites, while they were less than 65% at the YS2 and YS3 sites. Among all of the detected phyla, SR1 was detected only at the BHS site, and Elusimicrobia and Lentisphaerae were detected only at the NWP site.

At the genus level (Fig. 4b), 71.03% of all bacterial sequences were assigned to 533 bacterial genera.

FIG. 2. Rarefaction curves of aerosol samples (a) from different sites and (b) of different particle sizes.

We detected 130 of the genera (63.60% of all bacterial sequences) at all of the sites. The most abundant bacterial genus detected was *Bacteroides* (9.56%), followed by *Prevotella* (5.42%) and *Megamonas* (3.22%). The dominant genera in the bacterial communities of bioaerosols from different sites were different. The

dominant bacteria were *Bacteroides*, *Prevotella*, and *Paracoccus* at the BHS site; *Bacteroides*, *Prevotella*, and *Sphingomonas* at the YS1 site; *Pseudomonas* and *Bacteroides* at the YS2 and YS3 sites; and *Bacteroides*, *Alistipes*, *Prevotella*, and *Barnesiella* at the NWP site.

FIG. 3. Venn diagram of OTUs in marine bioaerosols from different sites.

c. Bacterial community structures of marine bioaerosols with different particle sizes

The number of OTUs for 26 successfully sequenced samples of different particle sizes ranged from 154 to 975. The Chao1 indices ranged from 193 to 1044, and the Shannon diversity indices ranged from 5.44 to 8.33 (Fig. 5a). Through double factors—no repeated variance analysis—we found that there was a significant difference among the richness of different sites ($p < 0.05$), there was no significant difference in the richness among different particle sizes ($p > 0.05$), and there were significant differences in the diversity among different sites ($p < 0.05$) and different particle sizes ($p < 0.05$).

Particles larger than $2.1 \mu\text{m}$ in diameter were referred to as coarse particles (c) in the sampler stages, and those less than $2.1 \mu\text{m}$ in diameter were referred to as fine particles (f). The richness and diversity of the bacterial community in the coarse particles ($>2.1 \mu\text{m}$) from the Bohai Sea site were lower than those in the fine particles ($<2.1 \mu\text{m}$) (Fig. 5b), while the richness and diversity of the bacterial communities in the coarse particles at the other four sites were higher than those in the fine particles.

The dominant bacteria in the bacterial communities of bioaerosols from different sites were different, and the bacterial compositions of the coarse and fine particles from the same site also varied (Fig. 6). For example, *Paracoccus* was clearly a dominant genus in fine particles from the Bohai Sea site. The bacterial community structures in fine particles from the YS3 site were similar to the bacterial community structures in aerosols of

most particle sizes from the YS2 site, and among these particles, *Pseudomonas* was the dominant genus. The dominant genera of the coarse particle sizes at the same site were generally more similar, but the dominant genera of fine particle sizes were quite different except for those of the YS3 site. Shared species at different classification levels for each particle size sampled are shown in Table 3. The genera shared by the 26 samples accounted for 20.30% of all of the assigned genera. Shared bacteria accounted for 84.60% of all bacterial sequences at the phylum level and 38.11% at the genus level.

To compare bacterial community compositions among samples of different particle sizes, jackknifed beta diversity and hierarchical clustering analyses based on unweighted (Fig. 7a) and weighted (Fig. 7b) UniFrac distance metrics were performed. In the analysis based on unweighted UniFrac distance, the difference of the bacterial taxa contained in the aerosols at the same site was relatively small. In the analysis based on weighted UniFrac distance, all of the samples were divided into three branches. The bacterial community composition at the YS2 site was similar to that in fine particles at the YS3 site. In the remaining two branches, both samples from the same site and samples with the same particle sizes did not exhibit obvious similarities. In particular, samples with coarse particle sizes and fine particle sizes from the Bohai Sea site were clustered into two separate branches.

d. Influence of meteorological factors on marine bioaerosol bacterial community structures

Analysis of the community data showed that canonical correspondence analysis (CCA) was more suitable for analyzing the relationship between the community composition of airborne bacteria and environmental factors. CCA (Fig. 8) and permutation tests were performed to examine the relationships between meteorological factors and bacterial community structures in marine aerosols.

Based on the relationship between environmental factors and bacterial community composition in samples of different particle sizes (Fig. 8a), the environmental factors on the first- and second-ordinal axes accounted for 68.54% of the cumulative variance between species and environmental factors. Temperature ($p = 0.001$) and wind speed ($p = 0.019$) had significant effects on the bacterial community composition of aerosols of different particle sizes from different sites.

Based on the relationship between environmental factors and bacterial community structures in fine (Fig. 8b) and coarse (Fig. 8c) particles, the first two axes accounted for 69.78% and 64.04%, respectively, of all the variance, as explained by CCA. Four meteorological factors

FIG. 4. Relative abundances of microbial groups in marine aerosols from different sites at the (a) phylum and (b) genus levels.

(temperature, relative humidity, wind speed, and wind direction) had no significant effects on bacterial community structure in both fine and coarse particles ($p > 0.05$). Wind direction had a greater impact on bacterial community structures in fine particles, and wind speed had the greatest impact on bacterial community structures in coarse particles.

e. Backward trajectory analysis

Analysis of the backward trajectories of the air masses showed that samples from the sites in the Bohai and Yellow Seas were collected mainly from continental air masses (Figs. 9a–d), and samples from the NWP

site were collected from mixed continental and oceanic air masses (Fig. 9e).

4. Summary and discussion

Bacteria are important components of bioaerosols and are widely distributed in aerosols. Studies have found that the structures of bacterial communities in marine bioaerosols are complex and diverse (Seifried et al. 2015; Xia et al. 2015). However, studies on the community structures of bacteria in marine aerosols of different particle sizes have not been reported. We obtained 4448 OTUs from 26 aerosol samples of different

FIG. 5. Diversity of bacterial communities in marine aerosols (a) of different particle sizes and (b) in the coarse ($>2.1 \mu\text{m}$) and fine particle ($<2.1 \mu\text{m}$) sizes.

particle sizes from five sites using Illumina high-throughput sequencing. Ma et al. (2017) found that the Chao1 and Shannon indices of bacterial communities in bioaerosols sampled in winter in the coastal region of Qingdao ranged from 78.6 to 287.4 and from 4.31 to 7.00, respectively. The bacterial community structures in marine bioaerosols of different particle sizes were more complex and diverse than the bacterial communities in winter aerosols from Qingdao.

The bacterial community structures in the coarse particle were found to be more complex and diverse than those in fine particle in marine bioaerosols from the Yellow Sea and the northwestern Pacific Ocean, which was consistent with the results of a study on the community structures of bioaerosols from the Qingdao coastal region (Ma et al. 2017). It has been reported that microbes are mainly distributed in the coarse particle size (Dong et al. 2016; Li et al. 2011), and the bacterial richness in fine particulate matter ($\text{PM}_{2.5}$) has been

FIG. 6. Relative abundances of microbial groups in marine aerosols of different particle sizes at the genus level.

shown to be lower than that in coarse particulate matter ($PM_{2.5-10}$) (Bowers et al. 2013); however, the bacterial richness and diversity in the coarse particle was lower than that in the fine particle from the BHS site. The Bohai Sea is a semienclosed inland sea known to have a high population density and significant emissions of fine particulate matter and gaseous precursors (Van Donkelaar et al. 2010). Fine particles in Bohai Sea aerosols have been found to be mainly from continents, and aerosols over the Bohai Sea were relatively enriched in fine particles from anthropogenic sources compared with those over the Yellow Sea (Fu et al. 2015), which results in the bacterial community structure of the fine

particle size in Bohai Sea aerosols being more complex and diverse. In the aerosols of fine particle size over the Bohai Sea, *Paracoccus* was an obvious dominant bacterial genus, which may mainly originated from the adjacent continent.

In our study, among the different sites, the 5.82% shared OTUs accounted for 59.18% of all the bacterial sequences; the 19 shared phyla accounted for 89.50% of all the bacterial sequences; and the 130 shared genera accounted for 63.60% of all the bacterial sequences. The main groups of bacteria in the different sites were consistent, but the proportions of the different species varied; for example, *Bacteroides* was a dominant genus at

TABLE 3. Species shared by marine aerosol samples of all particle sizes.

Classification level	Total number of species shared by samples of all particle sizes	Species shared by samples of all particle sizes
OTU	13 (20.30% of all the bacterial sequences)	OTU120, OTU2, OTU4, OTU6, OTU7, OTU50, OTU12, OTU13, OTU10, OTU11, OTU16, OTU681, OTU30
Phylum	6 (84.60% of all the bacterial sequences)	Euryarchaeota, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria
Genus	18 (38.11% of all the bacterial sequences)	<i>Bacteroides</i> , <i>Barnesiella</i> , <i>Parabacteroides</i> , <i>Alloprevotella</i> , <i>Prevotella</i> , <i>Alistipes</i> , <i>Lactobacillus</i> , <i>Clostridium XIVa</i> , <i>Roseburia</i> , <i>Faecalibacterium</i> , <i>Ruminococcus</i> , <i>Phascolarctobacterium</i> , <i>Megamonas</i> , <i>Megasphaera</i> , <i>Veillonella</i> , <i>Paracoccus</i> , <i>Escherichia/Shigella</i> , <i>Acinetobacter</i>

FIG. 7. (a) Cluster of each particle sample based on unweighted UniFrac distance metrics and (b) beta diversity heat map of bacterial community composition in marine aerosols based on weighted UniFrac distance metrics.

all sites. Studies have indicated that there may be a bacterial background in the atmosphere in the form of several dominant taxa (Gandolfi et al. 2013). Seifried et al. (2015) found that 37 OTUs in marine aerosols constituted more than 75% of all of the sequences, and the dominant OTU in all of the samples, accounting for 17% of all the bacterial sequences, was assigned to the genus *Sphingomonas*, which is known to be ubiquitous in the environment (Fahlgren et al. 2010; Harrison et al. 2005; Li et al. 2010; Zweifel et al. 2012). Our study also supports the hypothesis that there may be a bacterial background in the atmosphere in the form of several dominant taxa. However, among the aerosols of different particle sizes at all of the sites, 13 shared OTUs accounted for 20.30% of all the bacterial sequences, 6 shared phyla accounted for 84.60% of all the bacterial

sequences, and 18 shared genera accounted for 38.11% of all the bacterial sequences. Seifried et al. (2015) pointed out that there may be steady “bacterial core communities” in the atmosphere, possibly from strong regional sources, such as tropical rain forests, big grass fields, or soil areas, which are constantly distributed and mixed over long distances. Some bacteria, which were the dominant species in the present study, such as *Bacteroides*, *Prevotella*, *Pseudomonas*, and *Bacillus*, were also dominant bacteria in other bioaerosol studies that investigated marine/coastal ecosystems (Seifried et al. 2015; Cho and Hwang 2011; Xia et al. 2015; Ma et al. 2017). In addition, *Megamonas*, which is mainly isolated from human or animal intestines, was a dominant bacterial genus in the present study. The relative abundances of these bacteria of different particle sizes varied.

FIG. 8. CCA showing the relationships between environmental factors and the community structures of marine airborne bacteria in (a) aerosols of each particle size, (b) fine particles, and (c) coarse particles.

FIG. 9. Backward trajectory analysis of air masses at the start positions for sampling areas: (a) BHS, (b) YS1, (c) YS2, (d) YS3, and (e) NWP.

Our study further indicated that there may be a bacterial background in the atmosphere in the form of several dominant taxa and that bacteria in aerosols are mixed constantly during transmission, which contributes to the redistribution of bacteria among the aerosols of different particle sizes.

The dominant phyla in the aerosols at different sites were consistent, but the dominant genera were different. The dominant phyla were Bacteroidetes, Firmicutes, and Proteobacteria. These three phyla have been reported to be dominant in marine aerosols, but the dominant phyla over different sea areas were different (Seifried et al. 2015; Cho and Hwang 2011; Xia et al. 2015). The dominant genera in bioaerosols from different sites were different. Xia et al. (2015) also found that the general bacterial communities in the aerosol over different sea areas were markedly different. *Bacteroides* was a dominant genus at all sites, but its relative abundances at the different sites were different. *Bacteroides* species were detected in a variety of environments (Elmir et al. 2009; Green et al. 2011; Ma et al. 2017; Sattar 2014). The bacterial compositions of the coarse and fine particles from the same site also varied. The dominant genera of the coarse particle sizes at the same site were generally more similar, but the dominant genera of fine particle sizes were quite different except for the YS3 site. Fine particles (diameter < 2 μm) have a long residence time in the atmosphere (days to weeks) and can be transported over long distances (Dueker et al. 2011). Long-distance transport of terrestrial aerosols may affect the composition and size distribution of bacterial communities in marine aerosols.

Studies have shown that the differences in bacterial community composition among the sampling locations were affected by the sampling locality itself and their adjacent ecosystems (Seifried et al. 2015). The bacterial taxa in the aerosols were obviously affected by geographical location, which may be the joint role of the sampling location itself and the adjacent ecological ecosystems. The relative abundances of these taxa of different particle sizes varied, leading to the differences in community structures of different particle sizes at different sites. Cho and Hwang (2011) inferred that the occurrences of different major OTUs in air over space and time could not be due to successful adaptations of rare phylotypes to air, but rather to the variation of their sources and emissions affected by local meteorological conditions, as the inference of significant bacterial growth in air is unlikely (Burrows et al. 2009a). The differences in community structures of different particle sizes would be due to the continuous mixing of bacteria in the transmission process and variations in bacteria in

the sources; their emission into the air would be affected by the local meteorological conditions.

Among the observed meteorological factors, temperature had the greatest impact on bacterial community structure. Temperature significantly influenced ($p = 0.001$) the bacterial communities of marine aerosols of different particle sizes. It has been reported that bacterial communities in both terrestrial and marine aerosols can be clearly affected by temperature (Gandolfi et al. 2013; Seifried et al. 2015; Zhen et al. 2017). Samples were collected in the spring of 2014, with atmospheric temperatures ranging from 11.9° to 19.8°C. An increase in temperature can increase aerosol bacterial input and accelerate the movement of convective air, thereby increasing the dispersion of bacteria in the atmosphere (Zhen et al. 2017). In addition, temperature can affect the particle size distribution of aerosols (Lin 2009), thus affecting the particle size distribution of the bacteria in aerosols.

Wind speed had the greatest impact on bacterial community structure in coarse particles (though not significant) and a significant influence ($p = 0.019$) on bacterial communities in marine aerosols of the different particle sizes. Zhen et al. (2017) found that under conditions of high wind strength in the spring, bacterial communities can be affected by dust from various surfaces. Hu et al. (2017) discovered that strong winds over sea surfaces can increase the contribution of oceanic emissions to the concentrations of viable bacteria in aerosols, and the concentration of bacteria is significantly higher when the wind speed is greater than 5.4 m s^{-1} (exceeding a Beaufort force of 3, at which wave breaking can occur). The wind speeds at the BHS, YS3 and NWP sites were all greater than 5.4 m s^{-1} , which could increase the contribution of surface seawater sources, especially for coarse particles (Wu 1992; Veron 2015), thereby increasing the differences in bacterial community composition between the coarse and fine particles.

In our study, bacteria associated with CCN and IN were detected, and the more abundant genera were *Pseudomonas*, *Sphingomonas*, and *Bacillus*. *Sphingomonas*, *Pseudomonas*, and *Bacillus* are CCN bacteria and IN bacteria (Maki et al. 1974; Šantl-Temkiv et al. 2015; Hirano and Upper 2000). *Pseudomonas* strains can use surfactants to facilitate their activation from aerosols into CCN (Ahern et al. 2007). *Bacillus* strains can also produce surfactants and have certain effects on the formation of CCN (Maki et al. 1974; Šantl-Temkiv et al. 2015; Hirano and Upper 2000). Particles can be transported from Earth's surface to the troposphere by convective transport (Burrows et al. 2009b). Studies have found high proportions of bacteria that typically live on

plant surfaces in cumulonimbus clouds, a particular type of a convective cloud (Šantl-Temkiv et al. 2012, 2013). Microorganisms of small particle sizes have a long residence time in the atmosphere and can be transferred to the upper troposphere to reflect or absorb incident light, which affects the formation of clouds and changes the chemical reactions in clouds (Yin et al. 2012; Franzetti et al. 2011). *Pseudomonas*, *Sphingomonas*, and *Bacillus* all exist in fine particles, with possible effects on CCN and INP. Further studies are needed to understand the effects of marine aerosol microorganisms on CCN and INP.

5. Conclusions

This study indicates that bacterial community structures in marine bioaerosols of different particle sizes are complex and diverse. The bacterial taxa in these aerosols were obviously affected by geographical location. The dominant species were different over different sea areas. The bacterial compositions of the coarse and fine particles from the same location also varied. The compositions of these communities can be affected by the differences in both sampling site and particle size. Among the environmental factors examined, temperature- and wind-speed-influenced bacterial communities in marine aerosols of different particle sizes. Our results further confirmed that the bacteria in aerosols have a background that is formed by several species and that the bacteria in aerosols are mixed constantly during transmission. In our next study, we will determine the chemical composition and continue to delve into the problems of aerosol microbial transmission and mixing.

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