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The major chemical constituents of $PM_{2.5}$ and airborne bacterial community phyla in Beijing, Seoul, and Nagasaki

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HIGHLIGHTS

- Associations between bacterial communities with $PM_{2.5}$ chemical components were investigated.
- NO_3^- and SO_4^{2-} were correlated positively with some airborne bacterial species.
- Spatial variability of relative abundance of phyla was significantly impacted by air parcel movements.
- Local emissions and environments impacted on the diversities of bacterial communities.

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ABSTRACT

Ambient particle ($PM_{2.5}$) samples were collected in three East Asian cities (Beijing, China; Seoul, South Korea; Nagasaki, Japan) from December 2014 to November 2015 to quantitatively investigate airborne bacteria at the phylum level. Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Cyanobacteria represented the top five airborne bacterial phyla in all three cities. The most dominant airborne phylum, Proteobacteria, was more prevalent during the winter (at rates of 67.2%, 79.9%, and 87.0% for Beijing, Seoul, and Nagasaki, respectively). Correlations among airborne bacteria and environmental factors including $PM_{2.5}$, its major chemical constituents, and meteorological factors were calculated. Temperature correlated negatively with Proteobacteria but positively with Firmicutes and Bacteroidetes. The abundance of Cyanobacteria correlated positively with particulate NO_3^- and SO_4^{2-} levels in Beijing ($R = 0.46$ and $R = 0.35$ for NO_3^- and SO_4^{2-}, respectively) but negatively in Seoul ($R = -0.14$ and $R = -0.19$ for NO_3^- and SO_4^{2-}, respectively) and Nagasaki ($R = -0.05$ and $R = -0.03$ for NO_3^- and SO_4^{2-}, respectively). Backward trajectory analysis was applied for 72 h and three clusters were classified in each city. Five dominant bacteria and other bacterial groups showed significant differences ($p < 0.05$) in local clustering, as compared to the long-range transport clusters from Beijing. The proportions of the five bacterial phyla in Seoul were significantly different in each cluster. A local cluster in Nagasaki had higher ratios of all major airborne bacterial phyla, except Proteobacteria.

1. Introduction

Bacteria are ubiquitous and can influence natural ecosystems. They are also known to have important potential effects on human health (Bowers et al., 2011; Gao et al., 2017). Airborne bacteria not only affect chemical reactions in the atmosphere but also affect various human health conditions such as various allergies and respiratory diseases (Jahne et al., 2015; Du et al., 2018). In addition, even if people spend more time indoors, airborne bacteria originating from ambient sources can penetrate indoor spaces, which could result in individuals being exposed (Oh et al., 2015). The atmosphere not only serves as a natural habitat for bacteria but also

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enables their transportation (Hiraoka et al., 2017). Wind-borne bacteria can only move within a distance of 1 km from the source; however, some dust-associated bacteria are transported over 5000 km (Kellogg and Griffin, 2006; Yamaguchi et al., 2012). Several previous studies provided evidence suggesting that airborne bacteria (especially those in dust from the Gobi Desert or the desert area in China) could be transported to Korea and Japan (Yeo and Kim, 2002; Maki et al., 2011; Hiraoka et al., 2017). Correlations between bacterial communities in particulate matter (PM) and meteorological factors have been investigated in many studies to assess the migration of airborne bacteria (Gao et al., 2017; Lee et al., 2017; Liu et al., 2018). Research on the diversity and composition of airborne bacteria has also been carried out recently (Gao et al., 2017). In addition, several studies have analyzed correlations between airborne bacteria and PM, assuming that PM and its chemical constituents also significantly affect the growth and survival of airborne bacteria (Xie et al., 2018; Zhong et al., 2019).

Studies on airborne bacteria have been performed in China, which has serious air pollution problems. Gao et al. (2017) identified the major airborne bacteria associated with $PM_{2.5}$ collected in Beijing, Tianjin, and Hebei. Soil-associated bacteria were found to be highly abundant in the atmosphere in northern China (Gao et al., 2017). Further, Liu et al. (2018) analyzed bacterial distributions among total suspended particles (TSP), PM_{10}, and $PM_{2.5}$ in Hangzhou, China and concluded that pathogenic bacteria could survive in heavily polluted air, even though other airborne bacteria probably died. No correlation was found between pollution levels and bacterial abundances in $PM_{2.5}$ collected in Beijing (Du et al., 2018); however, the relative abundances of major bacteria became highly dominant when the $PM_{2.5}$ mass concentrations were high during haze events in Guilin, China (Zhong et al., 2019). Airborne bacteria have been studied not only in China but also in South Korea and Japan. The abundances of bacteria differed between dust events and non-dust events and Proteobacteria were predominant in $PM_{2.5}$ during non-dust events, as measured in Gwangju, South Korea (Aziz et al., 2018). Moreover, the results of Hiraoka et al. (2017) suggested that the bacterial community varies according to air mass trajectories since the relative abundances of bacteria varied by season in Tokyo, Japan.

In several studies, the chemical constituents of $PM_{2.5}$ were analyzed simultaneously in different countries to compare air pollution levels. Salameh et al. (2015) investigated characteristics of $PM_{2.5}$ and its chemical constituents in five European Mediterranean cities for about one year from 2011 to 2012 and found that fractions of chemical constituents of $PM_{2.5}$ between the cities were markedly different even for similar $PM_{2.5}$ mass levels. Heo et al. (2017) evaluated spatial and temporal variations of chemical components and source contributions of $PM_{2.5}$ observed simultaneously in eleven cities of the Middle East over a 1-year period from January to December 2007. They found that although significant seasonal and spatial variability of $PM_{2.5}$ levels were observed between the cities, the most abundant chemical component and dominant source of $PM_{2.5}$ were carbonaceous aerosols and secondary sulfate, respectively, in the study region. Airborne bacteria associated with $PM_{2.5}$ have also been measured, but comparative studies between countries are limited. This necessitates the comparison of airborne bacterial community diversity in $PM_{2.5}$ samples collected in other countries. In a previous study conducted with the same samples as this study, Lee et al. (2017) used PCA analysis to identify similarities and differences between cities and seasons, of airborne bacteria in $PM_{2.5}$ samples measured in Beijing, Seoul, and Nagasaki. The correlations between the number of species of airborne bacteria and meteorological factors (humidity, wind speed, and temperature) were also investigated. In this study, we conducted the study assuming that the relative abundances of airborne bacteria species will differ, not only by seasonal and meteorological factors, but also by $PM_{2.5}$ and its major chemical constituents. Considering the need to research airborne bacteria, this study was performed (1) to analyze and compare the major chemical constituents and bacterial communities in $PM_{2.5}$ obtained in Beijing, Seoul, and Nagasaki, (2) to study correlations between the major chemical and bacterial species in $PM_{2.5}$ and meteorological factors, and (3) to classify the relative abundances of airborne bacteria by clusters in order to investigate the effects of local or long-range transport.

2. Methods

2.1. $PM_{2.5}$ sampling

$PM_{2.5}$ samples were collected in Beijing, Seoul, and Nagasaki from December 2014 to November 2015. The three cities were selected to see the possibility of long-range transportation of airborne bacteria by simultaneously measuring $PM_{2.5}$ in an airshed (Park et al., 2018). Thirty-nine p.m.$_{2.5}$ samples collected in Beijing, 33 in Seoul, and 34 in Nagasaki were used to analyze airborne bacterial species in $PM_{2.5}$. The sample site in Beijing was located on the roof of the Department of Occupational and Environmental Health Sciences at the Peking University School of Public Health, which is surrounded by major roads and commercial buildings. The sample site in Seoul was installed on the roof of the School of Public Health at Seoul National University, which is adjacent to commercial buildings and roads. The sample site in Nagasaki was installed on the roof of the Institute of Tropical Medicine at Nagasaki University. Nagasaki has been less urbanized than Beijing and Seoul, but residential areas, commercial facilities, and roads were located around the sampling site, which was considered suitable for measuring $PM_{2.5}$.

$PM_{2.5}$ was measured from December 2014 to November 2015 in all three cities. Although different numbers of samples were obtained in different seasons, all four seasons were included during the sampling period. A three-channel sampler, composed of a filter pack, cyclone, dry gas meter, and pump for each channel, was used for $PM_{2.5}$ measurements. Cyclones can accept particles of different sizes selectively, with a certain flow rate. The first channel, which contained a Teflon filter (47 mm, Pall Life Sciences, 1-μm pore size), was used at a flow rate of 16.7 L per minute (LPM) and analyzed the $PM_{2.5}$ mass concentration. The second channel used a flow rate of 10 LPM with a Zefluor membrane filter (Pall Life Sciences, 1$^{-\mu m\ pore\ size)}$ to analyze water-soluble ionic species (NO_3^-, SO_4^{2-}, and NH_4^+). The last channel had quartz microfiber filter (Pall Life Sciences, PALLFLEX Membrane Filters) and collected $PM_{2.5}$ at a flow rate of 16.7 LPM. $PM_{2.5}$ samples collected on the quartz microfiber filter were used to analyze carbonaceous species and airborne bacterial species by next generation sequencing (NGS) analysis. The extraction and analysis methods of airborne bacterial species including NGS analysis are described in sections 2.3 and 2.4.

2.2. $PM_{2.5}$ chemical analysis

Three kinds of filters (Teflon, quartz, and Zefluor filters) were used to determine the major chemical constituents of $PM_{2.5}$. The mass of $PM_{2.5}$ was measured by weighing the Teflon filters using a microbalance (AND HM-202, Japan; precision: 10^{-2} mg). Each Teflon filter was stabilized for 24 h before being weighed in a desiccator under constant temperature (20 ± 2 °C) and relative humidity (35 ± 5%) conditions. A 47-mm quartz microfiber filter was pretreated at 450 °C for 12 h to decrease the value of the carbon blanks and sterilize for NGS analysis. The carbonaceous species including organic carbon (OC) and elemental carbon (EC) of $PM_{2.5}$ on the quartz microfiber filters were analyzed by the National

Institute of Occupational Safety and Health 5040 method based on thermal/optical transmittance (TOT; Thermal/Optical Carbon Aerosol Analyzer, Sunset Laboratory, Inc., USA) (Birch and Cary, 1996). A 47-mm Zefluor membrane filter was used to analyze water-soluble ionic constituents (NO_3^-, SO_4^{2-}, and NH_4^+), and ion chromatography (Thermo Fisher Scientific, ICS-1100, USA) was used to analyze ionic species. The major $PM_{2.5}$ chemical constituents were measured at the sites of each city, and the filter was sealed in a Petri dish and frozen at −20 °C.

2.3. DNA extraction from $PM_{2.5}$ samples

Airborne bacterial species were analyzed using samples collected on quartz microfiber filters. As mentioned (section 2.2), the quartz microfiber underwent high-temperature sterilization at 450 °C for 12 h in a furnace (Lee et al., 2009). $PM_{2.5}$ samples collected on the filters were scratched with sterilized toothpicks to extract DNA. DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, Calif, USA), and the final volume of extracted DNA in TE buffer was 50 μL (Lee et al., 2017). Schematic diagram of NGS analysis is presented in Fig. S1. 10 to 15 samples were used for DNA extraction during each season in each city but DNA was not detected in some samples. DNA was extracted from at least three samples (the samples that were collected in Seoul during the summer) and up to 12 samples (Beijing samples collected during spring and winter; Seoul samples collected during spring; Nagasaki samples collected during winter). The numbers of samples collected among the three cities in each season are presented in Table 1.

2.4. DNA sequencing and analysis

A detailed description of the NGS analysis performed in this study was published by Lee et al. (2017). Briefly, 16S rRNA genes were amplified using Illumina-adapted universal primers. Fifty-microliter PCR mixtures containing 5.0 μL of genomic DNA were subjected to denaturation (3 min at 94 °C), 35 cycles (45 s at 94 °C, 60 s at 50 °C, and 90 s at 72 °C), and a final extension (10 min at 72 °C). The UltraClean PCR Clean-Up Kit (MoBio Laboratories, Carlsbad, CA, USA) was used to purify the amplified PCR mixtures. Bacterial 16S rRNA genes were sequenced using the MiSeq platform (Illumina San Diego, Ca, USA) with the MiSeq Reagent Kit V3 (2 × 300 cycles).

2.5. Meteorological data

Twenty-four-hour values were used for meteorological factors including temperature, wind speed, and relative humidity. The meteorological station in Beijing is located in the Chaoyang Olympic Sport Center. The station is located approximately 2.5 km from the Department of Occupational and Environmental Health Sciences at the Peking University School of Public Health, the Beijing $PM_{2.5}$ measurement site in this study. The meteorological data for Seoul and Nagasaki were obtained from the Korea Meteorological Administration and Japan Meteorological Agency, respectively (Lee et al., 2017). The meteorological station in Seoul is located 3 km from the $PM_{2.5}$ measurement site and that in Nagasaki is located approximately 6 km from the $PM_{2.5}$ measurement site. Planetary boundary layer (PBL) was calculated and the method is

Table 1
Sampling information, seasonal meteorological data and the major chemical constituents of $PM_{2.5}$ measured in Beijing, Seoul, and Nagasaki.

	Average ± S.E.	Total	Spring	Summer	Fall	Winter
Beijing	**Number of samples**	41	12	6	11	12
	Temperature (°C)	11.5 ± 1.60	15.2 ± 2.09	24.1 ± 0.64	15.1 ± 1.61	−1.63 ± 0.49
	Wind speed (m/s)	4.52 ± 0.41	5.08 ± 0.55	2.80 ± 0.00	3.55 ± 0.53	5.78 ± 1.03
	Relative humidity (%)	54.5 ± 3.46	48.8 ± 5.56	72.1 ± 8.99	69.7 ± 5.47	37.4 ± 3.37
	Planetary boundary layer (m)	494 ± 48.6	609 ± 84.1	747 ± 153	349 ± 34.0	377 ± 95.3
	$\mathbf{PM_{2.5}}$ (μg m^{-3})	81.4 ± 13.1	101 ± 28.7	44.6 ± 11.4	76.4 ± 27.4	85.1 ± 23.3
	OC (μg m^{-3})	24.8 ± 4.20	29.5 ± 9.59	9.75 ± 1.03	15.8 ± 4.44	35.9 ± 8.94
	EC (μg m^{-3})	2.55 ± 0.29	2.39 ± 0.52	1.89 ± 0.32	2.46 ± 0.50	3.14 ± 0.72
	$\mathbf{NO_3^-}$ (μg m^{-3})	8.67 ± 1.68	9.64 ± 3.17	5.68 ± 1.29	8.12 ± 3.43	9.76 ± 3.92
	$\mathbf{SO_4^{2-}}$ (μg m^{-3})	6.40 ± 1.28	8.77 ± 2.87	3.73 ± 0.35	6.01 ± 1.54	5.65 ± 2.85
	$\mathbf{NH_4^+}$ (μg m^{-3})	3.45 ± 0.89	4.77 ± 1.99	1.80 ± 0.40	4.59 ± 2.02	2.09 ± 1.46
Seoul	**Number of samples**	33	12	3	7	11
	Temperature (°C)	9.85 ± 1.83	12.9 ± 1.39	27.2 ± 0.89	16.7 ± 2.06	−2.49 ± 0.82
	Wind speed (m/s)	2.73 ± 0.13	2.88 ± 0.20	2.57 ± 0.44	2.43 ± 0.22	2.79 ± 0.29
	Relative humidity (%)	57.7 ± 2.29	54.8 ± 4.69	69.3 ± 5.24	61.6 ± 4.51	55.2 ± 2.95
	Planetary boundary layer (m)	405 ± 41.2	373 ± 57.3	473 ± 75.2	382 ± 43.5	437 ± 105
	$\mathbf{PM_{2.5}}$ (μg m^{-3})	52.8 ± 7.15	69.3 ± 11.4	34.1 ± 1.90	13.2 ± 3.66	61.5 ± 13.0
	OC (μg m^{-3})	8.23 ± 0.77	9.79 ± 0.95	6.01 ± 0.77	4.45 ± 0.68	9.52 ± 1.72
	EC (μg m^{-3})	0.97 ± 0.07	1.13 ± 0.09	0.71 ± 0.16	0.73 ± 0.08	1.02 ± 0.17
	$\mathbf{NO_3^-}$ (μg m^{-3})	7.33 ± 1.41	11.2 ± 2.46	4.60 ± 3.18	1.81 ± 0.31	5.08 ± 1.25
	$\mathbf{SO_4^{2-}}$ (μg m^{-3})	6.65 ± 0.93	9.55 ± 1.46	10.9 ± 2.03	2.79 ± 0.65	3.16 ± 0.33
	$\mathbf{NH_4^+}$ (μg m^{-3})	4.72 ± 0.78	6.94 ± 1.21	4.64 ± 0.33	1.11 ± 0.16	4.62 ± 1.64
Nagasaki	**Number of samples**	34	9	7	6	12
	Temperature (°C)	15.1 ± 1.14	15.6 ± 0.84	24.3 ± 1.42	17.6 ± 0.88	7.99 ± 0.63
	Wind speed (m/s)	2.55 ± 0.24	2.91 ± 0.41	1.66 ± 0.14	2.18 ± 0.47	2.98 ± 0.50
	Relative humidity (%)	72.1 ± 1.79	71.6 ± 3.43	84.6 ± 3.32	65.7 ± 3.16	68.6 ± 1.98
	Planetary boundary layer (m)	653 ± 75.1	437 ± 91.9	219 ± 17.5	735 ± 132	1027 ± 114
	$\mathbf{PM_{2.5}}$ (μg m^{-3})	15.5 ± 1.66	21.5 ± 4.77	16.9 ± 3.67	15.2 ± 2.00	10.3 ± 0.95
	OC (μg m^{-3})	3.81 ± 0.59	5.46 ± 1.93	3.92 ± 0.77	2.90 ± 0.37	2.98 ± 0.65
	EC (μg m^{-3})	0.51 ± 0.09	0.68 ± 0.36	0.43 ± 0.06	0.40 ± 0.05	0.48 ± 0.06
	$\mathbf{NO_3^-}$ (μg m^{-3})	1.51 ± 0.22	0.81 ± 0.29	2.15 ± 0.45	1.10 ± 0.11	1.91 ± 0.48
	$\mathbf{SO_4^{2-}}$ (μg m^{-3})	1.59 ± 0.20	2.52 ± 0.56	0.82 ± 0.15	1.17 ± 0.15	1.51 ± 0.22
	$\mathbf{NH_4^+}$ (μg m^{-3})	0.61 ± 0.12	1.30 ± 0.40	0.21 ± 0.07	0.43 ± 0.07	0.48 ± 0.06

described in section 2.6, along with the used model for analysis.

2.6. Trajectories calculation and cluster analysis

In this study, 72-h backward-air trajectories for three cities were calculated using the National Oceanic and Atmospheric Administration Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) 4 model (Draxler and Hess, 1998). The Global Data Assimilation System (horizontal resolution 0.5° × 0.5°) from the National Center for Environmental Prediction was used with the meteorological data to calculate backward trajectories of air flows, with HYSPLIT version 4.9 (https://ready.arl.noaa.gov/HYSPLIT.php). The model was run every hour at start times of 00:00 to 23:00 Coordinated Universal Time (local times of 16:00 to 15:00 for Beijing and 15:00 to 14:00 for Seoul and Nagasaki) on each sampling day. Through the modeling, PBL was also calculated for every hour and 24 h averages of PBL were calculated for every sampling day to use as meteorological data in this study. The endpoint heights were also considered to cover both horizontal and vertical scales of transport with a 1/2 mixing height over the PBL (Shen et al., 2017; Park et al., 2018). We obtained 984, 792, and 816 trajectories for Beijing, Seoul, and Nagasaki, respectively, and trajectory clustering was applied to group similar trajectories using the HYSPLIT 4 model (Lv et al., 2015).

2.7. Statistical analysis

In this study, one-way analysis of variance (ANOVA) was used to analyze the relationships among airborne bacterial species, major chemical constituents of $PM_{2.5}$, and the meteorological factors. One-way ANOVA is an analysis method that is used to compare the mean values of three or more groups. This method was suitable for comparing many different factors, which were analyzed in three different cities in this study. The post hoc test employed was Scheffe's test. Two-tailed tests of significance were employed, and the significance was assumed at $p < 0.05$ (Harder et al., 2001). One-way ANOVA was applied using IBM Statistical Package for the Social Sciences (SPSS) Statistics (version 25). Pearson correlation analysis was computed using the Openair package in R (version 3.5.2).

3. Results and discussion

3.1. Concentrations of $PM_{2.5}$ and meteorological factors

From December 2014 to November 2015, 41 samples from Beijing, 33 samples from Seoul, and 34 samples from Nagasaki were analyzed. The meteorological data, including the average temperature, average wind speed, relative humidity, PBL, and average mass concentrations of $PM_{2.5}$ and its major constituents are summarized in Table 1. The average temperature was highest in Nagasaki (15.1±1.14 °C) followed by Beijing (11.5±1.60 °C) and then Seoul (9.85±1.83 °C). The average temperature in Beijing and Seoul dropped below 0 °C in the winter, whereas the average temperature in Nagasaki was 7.99 °C in the winter. The average wind speed decreased in the following order: Beijing, Seoul, and Nagasaki (3.61±0.14 m/s, 2.73±0.13 m/s, and 2.55±0.24 m/s, respectively). The wind speed in Seoul and Nagasaki did not change substantially between the seasons, but Beijing had an average wind speed of >5 m/s in the winter and spring (5.78 m/s and 5.08 m/s, respectively), which was lower (2.80 m/s) in the summer. The average wind speed during the summer in Beijing was even higher than the average wind speed in Seoul and Nagasaki. The relative humidity decreased in the following order: Nagasaki, Seoul, and Beijing (72.1±1.79%, 57.7±2.29%, and 54.5±3.46%, respectively). All three cities had the highest humidity in summer. The highest average humidity was 84.6% during the summer in Nagasaki and the lowest average humidity was 37.4% during the winter in Beijing. The average PBL was highest in Nagasaki (653±75.1 m) followed by Beijing (494±48.6 m) and then Seoul (405±41.2 m). Seoul displayed no significant changes in the PBL among the seasons during the study period, but Beijing and Nagasaki had higher PBLs in the summer (747 m) and winter (1027 m), respectively, compared to those in other seasons.

The average mass concentrations of $PM_{2.5}$ were 94.8±19.4 $\mu g/m^3$ in Beijing, 57.7±2.29 $\mu g/m^3$ in Seoul, and 15.5±1.71 $\mu g/m^3$ in Nagasaki. Based on the measurement period, the $PM_{2.5}$ 24-h standard of each country was 75 $\mu g/m^3$ for China (Class 2 standard), 50 $\mu g/m^3$ for South Korea (decreased to 35 $\mu g/m^3$ from 2018), and 35 $\mu g/m^3$ for Japan. In Nagasaki, the average measured $PM_{2.5}$ mass concentration was lower than the 24-h standard, whereas those in Beijing and Seoul were higher than their 24-h standards during the study period. The results of a previous study by Park et al. (2018) showed that the mass concentrations of $PM_{2.5}$ measured in Beijing, Seoul, and Nagasaki were 125±6.80 $\mu g/m^3$, 44.6±0.84 $\mu g/m^3$, and 17.4±0.37 $\mu g/m^3$, respectively. In this study, the $PM_{2.5}$ mass concentrations in Beijing and Nagasaki were higher than those of the previous study, whereas those in Seoul were lower. The average mass concentrations of $PM_{2.5}$ in studies by Li et al. (2017) and Batterman et al. (2016), which measured $PM_{2.5}$ in Beijing over a period similar to that of this study were 78.11 $\mu g/m^3$ and 83 $\mu g/m^3$, respectively. The mass concentrations of $PM_{2.5}$ in these two previous studies were slightly lower than those in this study. Seoul had an average mass concentration of 52.8 $\mu g/m^3$ for $PM_{2.5}$ in this study, which was higher than previously reported values (37 $\mu g/m^3$ for Kim et al. (2018) and 39.67 $\mu g/m^3$ for Park et al. (2019)). The average mass concentration of $PM_{2.5}$ measured in Seoul in this study was higher than that in other studies that measured $PM_{2.5}$ in a similar period because for Seoul, DNA was extracted from only three and seven samples, respectively, in summer and fall. More DNA was extracted from the spring and winter samples in Seoul with a relatively higher $PM_{2.5}$ mass concentration than from those from the summer (Table 1). For Nagasaki, Ng et al. (2019) reported a $PM_{2.5}$ average mass concentration of 18.5 $\mu g/m^3$, which was higher than that in this study (15.1 $\mu g/m^3$). The average $PM_{2.5}$ mass concentration and its major constituents from the three cities in this study did not represent the $PM_{2.5}$ in each city since the samples were selected for NGS analysis, and the results of $PM_{2.5}$ suggested that a relationship existed between airborne bacteria and $PM_{2.5}$.

The carbonaceous species, including OC and EC and ionic species (NO_3^-, SO_4^{2-}, and NH_4^+) in $PM_{2.5,}$ were then analyzed. In Beijing and Seoul, analyzed $PM_{2.5}$ constituents decreased in the order of OC > NO_3^- > SO_4^{2-} > NH_4^+ > EC, whereas Nagasaki had a slightly higher mass concentration of SO_4^{2-} (1.59 ± 0.20 $\mu g/m^3$) than NO_3^- (1.51 ± 0.22 $\mu g/m^3$) but they were similar. Beijing had the highest mass concentrations of OC, EC, and NO_3^- in winter and all 5 $PM_{2.5}$ major chemical constituents exhibited lowest mass concentrations in the summer and highest mass concentrations during the spring in Seoul, whereas they were lowest during the fall, except for EC. Further, all except NO_3^- showed the highest mass concentrations during spring in Nagasaki.

3.2. Relative abundances of airborne bacterial communities

In this study, 99.8% of microorganisms present in $PM_{2.5}$ were classified taxonomically as members of the Bacteria domain, with the exception of 0.11% of taxa that were of the Archaea domain and 0.06% unclassified taxa. At the phylum level, among the 36 total analyzed phyla., 12 phyla were unclassified. Therefore, the analysis of this study was conducted with a total of 25 phyla, including 24 classified phyla and one unknown group, which comprised of 12

Table 2
Average relative abundances of airborne bacteria in $PM_{2.5}$ at the phylum level of total (the sum of the results of three cities) and each city.

Airborne bacteria	Relative abundances (%)			
	Total	Beijing	Seoul	Nagasaki
Proteobacteria	**58.6**	**51.9**	**60.2**	**65.2**
Firmicutes	**20.5**	**20.8**	**23.9**	**16.7**
Actinobacteria	**13.6**	**18.7**	**9.75**	**11.3**
Bacteroidetes	**2.76**	**3.04**	**2.66**	**2.52**
Cyanobacteria	**1.85**	**2.16**	**1.06**	**2.23**
Acidobacteria	0.57	0.81	0.39	0.44
Gemmatimonadetes	0.34	0.36	0.26	0.40
Tenericutes	0.33	0.31	0.60	0.10
Chloroflexi	0.28	0.45	0.17	0.18
Armatimonadetes	0.27	0.17	0.36	0.32
Thermi	0.22	0.41	0.09	0.10
Fusobacteria	0.18	0.18	0.21	0.16
Planctomycetes	0.14	0.19	0.10	0.11
Deferribacteres	0.10	0.13	0.08	0.09
Verrucomicrobia	0.08	0.14	0.06	0.03
Unclassified	**0.08**	**0.12**	**0.07**	**0.04**
Spirochaetes	0.03	0.03	0.07	0.00
Chlamydiae	0.03	0.03	0.03	0.03
Elusimicrobia	0.02	0.00	0.00	0.05
Nitrospirae	0.01	0.01	0.01	0.00
Synergistetes	0.00	0.00	0.00	0.01
Fibrobacteres	0.00	0.00	0.00	0.00
Chlorobi	0.00	0.00	0.00	0.00
Lentisphaerae	0.00	0.00	0.00	0.00
Thermotogae	0.00	0.31	0.00	0.00

unclassified phyla. The average relative abundances of airborne bacteria in $PM_{2.5}$ at the phylum level are shown in Table 2. Cyanobacteria were the fifth highest relatively abundant phylum in all three cities, accounting for 2.16%, 1.06%, and 2.23% in Beijing, Seoul, and Nagasaki, respectively, as shown in Table 2. The sixth highest relatively abundant phylum was Acidobacteria in Beijing and Nagasaki (i.e., 0.81% for Beijing, and 0.44% for Nagasaki) and Tenericutes in Seoul (0.60%). Differences in the relative abundances of the fifth and sixth phyla were tested by Mann-Whitney *U* test using SPSS Statistics, and all three cities showed statistically significant differences. Bacteriodetes, which was the fourth highest relatively abundant phylum in all three cities (3.04%, 2.66%, and 2.52% for Beijing, Seoul, and Nagasaki, respectively) was significantly different from the abundance of Cyanobacteria in Beijing and Seoul; however, there was no difference between the two groups in Nagasaki (p-value < 0.197). All statistical tests were two-sided, and p-values less than 0.05 were considered statistically significant. Therefore, the most common phyla were divided based on Cyanobacteria abundance, which was significantly different across all three cities, its relative abundance exceeding 1% in all of them. In this study, the bacteria were divided into seven groups including species in the top five phyla (Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Cyanobacteria), other bacteria (based on the sum of the prevalence of bacterial species not in the top five phyla), and unclassified bacteria.

Similar to that in previous studies, Proteobacteria, Firmicutes, and Actinobacteria were most abundant among the airborne bacterial species at the phylum level (Table 2), and they accounted for more than 90% of airborne bacteria in all three cities (91.4%, 93.8%, and 93.2% in Beijing, Seoul, and Nagasaki, respectively) (Gandolfi et al., 2013; Behzad et al., 2015; Gao et al., 2017; Liu et al., 2018). Proteobacteria and Firmicutes were previously found to be the most abundant phyla in Beijing, Tianjin, and Hebei in China (Gao et al., 2017), Seoul (Cho and Jang, 2014) and Gwangju (Aziz et al., 2018) in South Korea, and Tokyo (Hiraoka et al., 2017) in Japan. The phyla associated with $PM_{2.5}$ and their relative abundances, as measured in China, Korea, and Japan in previous studies and in this study, were not significantly different. Thus, the airborne bacterial communities might be stable (Xu et al., 2019).

For Proteobacteria, which comprised the highest percentage of total airborne bacteria, the abundances were 51.9% in Beijing, 60.2% in Seoul, and 65.2% in Nagasaki (Table 2), and the abundances in Beijing and Nagasaki was significantly different ($p < 0.05$). The relative abundance of Proteobacteria was highest among those of airborne bacterial species at the phylum level in $PM_{2.5}$, similar to previous findings (Liu et al., 2018). Firmicutes were more abundant in Seoul (23.9%), followed by Beijing (20.5%) and Nagasaki (16.7%), whereas the abundances of Actinobacteria were in the order of Beijing (18.7%), Nagasaki (11.3%), and Seoul (9.75%). The ratio of Actinobacteria in Beijing was statistically different from that found in Seoul and Nagasaki ($p < 0.05$). Bacteroidetes accounted for 3.04%, 2.66%, and 2.52% of bacterial species in Beijing, Seoul, and Nagasaki, respectively. Cyanobacteria species were more abundant in Nagasaki (2.23%) and Beijing (2.16%) than in Seoul (1.06%). In general, cyanobacteria are ubiquitous in both brackish water and freshwater, have a deep affinity with minerals. Therefore, they may originate from the emission of sea spray aerosols, mainly from marine environment such as East Sea and East China Sea (Maki et al., 2014; Aziz et al., 2018). They are also found in soil or ambient dust particles. Several studies have found that cyanobacteria tend to dominate microbial populations in yellow dust particles that are long range transported from desert areas (Maki et al., 2014; Aziz et al., 2018; Romano et al., 2019). The highest ratio of cyanobacteria in microbial population in Beijing has been observed when air parcels are moved from the desert areas in Russia and Mongolia designated by the first cluster of back trajectories in Beijing (Fig. 3), representing that cyanobacteria have a deep affinity with mineral dusts.

3.3. Seasonal variations in the relative abundances of airborne bacterial communities

Season is an important factor associated with variations in airborne bacteria (Gandolfi et al., 2015). Relative seasonal abundances of airborne bacteria at the phylum level were measured in Beijing, Seoul, and Nagasaki (Fig. 1). Proteobacteria, the most abundant phylum of airborne bacteria found in this study, was more abundant during the winter in all three cities, and Nagasaki showed the highest Proteobacteria abundance in the winter (67.2%, 79.9%, and 87.0% in Beijing, Seoul, and Nagasaki, respectively). The lowest ratios of Proteobacteria were found during the spring in Beijing (34.3%) and Seoul (47.3%) and during the fall in Nagasaki (41.2%). The ratios of Proteobacteria during the winter in all three cities were significantly higher than those in the spring and fall for each city ($p < 0.05$). The highest ratios of Firmicutes were 33.2% and 36.6% during the spring in Beijing and Seoul, respectively, and 30.0% during the fall in Nagasaki.

In all three cities, Firmicutes ratios were higher during the seasons associated with lower abundances of Proteobacteria. The ratio of Firmicutes in Nagasaki was also higher during the spring, which supports previous observation that Firmicutes comprises soil-inhabiting bacteria (Gandolfi et al., 2013). Proteobacteria were dominant in non-dust-event days, whereas Firmicutes were predominant during dust events in a previous study (Gandolfi et al., 2013). In this study, the ratio of Firmicutes tended to be higher in the spring when high-concentration events including Asian dust events mainly occurred. Actinobacteria, found in soil and freshwater bacterial communities (Bowers et al., 2011; Gandolfi et al., 2013), showed a higher ratio during the spring in Beijing (23.8%), whereas higher abundances were found during the fall in Seoul (15.3%) and Nagasaki (19.5%). The ratios of Bacteroidetes were low

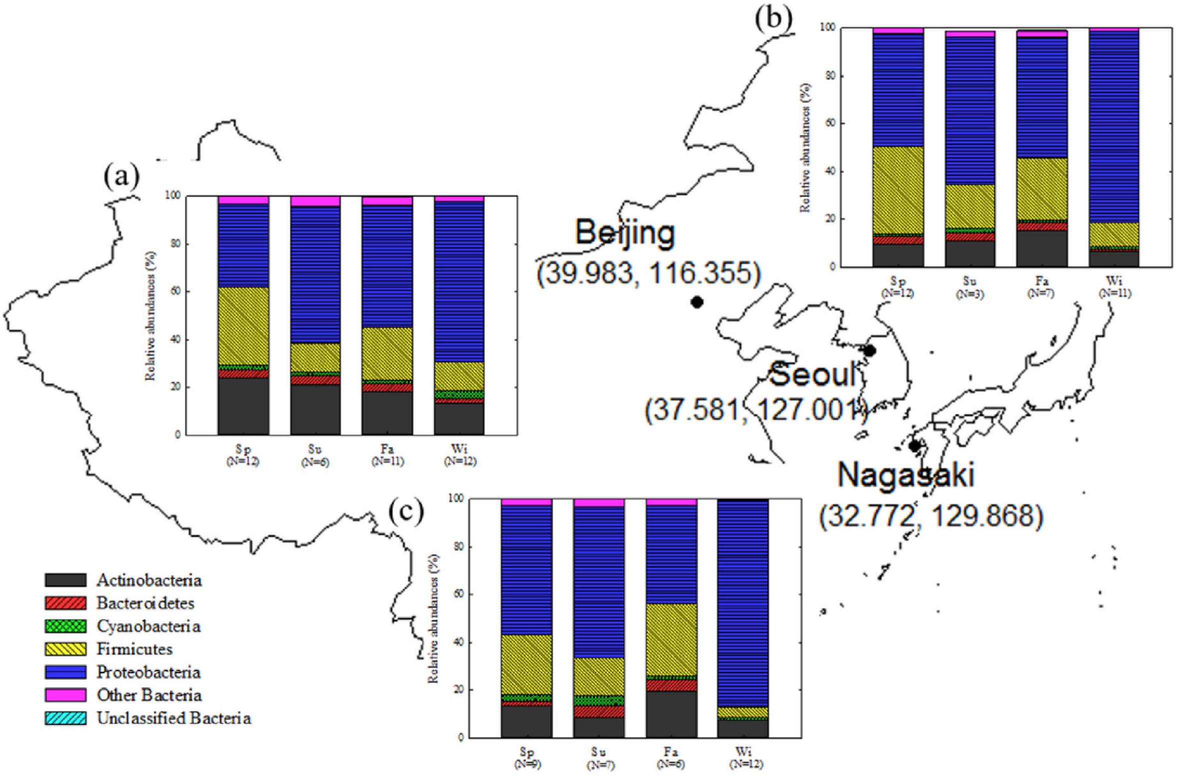


Fig. 1. Seasonal relative abundances of bacterial communities at the phylum level for (a) Beijing, (b) Seoul, and (c) Nagasaki.

during the winter in all three cities (2.12%, 1.33%, and 0.34% in Beijing, Seoul, and Nagasaki, respectively). In Beijing and Seoul, no significant differences were found in the ratios of Bacteroidetes in the spring, summer, and fall, whereas those of Bacteroidetes in the summer (5.17%) and fall (4.82%) were significantly higher than those in the spring (1.81%) and winter (0.34%) in Nagasaki. The ratio of Cyanobacteria was higher during the summer in Seoul and Nagasaki and higher during the winter in Beijing. The abundance of bacterial species accounting for less than 1% of all species (designated here as “other bacteria”) was higher during the summer in Beijing, whereas higher ratios were found during the spring in Seoul and Nagasaki, although the ratios did not differ significantly by season in the three cities.

3.4. Correlation coefficients among $PM_{2.5}$, meteorological factors, and airborne bacterial communities

Correlations of airborne bacteria at the phylum level with $PM_{2.5}$ and meteorological factors in Beijing, Seoul, and Nagasaki were next evaluated (Fig. 2) by one-way ANOVA, as explained in section 2.7. In Beijing, $PM_{2.5}$ and major chemical constituents correlated negatively with temperature, PBL, and wind speed. Meteorological factors, especially PBL and wind speed, correlated negatively with $PM_{2.5}$ and its major chemical constituents, since both factors could lower the levels of air pollution (Liu et al., 2018). Proteobacteria, the most abundant airborne bacterial phylum, showed a negative correlation with $PM_{2.5}$ and its ionic constituents ($R = -0.23$, $R = -0.26$, and $R = -0.33$ for NO_3^-, SO_4^{2-}, and NH_4^+, respectively). Proteobacteria had a relatively higher negative correlation with relative humidity (RH) ($R = -0.46$). Bacteroidetes positively correlated with temperature, whereas Cyanobacteria negatively correlated with this parameter. Positive correlation coefficients were also found between Cyanobacteria and ionic species ($R = 0.46$, $R = 0.35$, and $R = 0.06$ for NO_3^-, SO_4^{2-}, and NH_4^+ respectively). Ionic species were also positively correlated with Actinobacteria in Beijing ($R = 0.23$, 0.25, and 0.26 for NO_3^-, SO_4^{2-}, and NH_4^+, respectively).

All $PM_{2.5}$ values and their major chemical constituents showed significant negative correlations with PBL in Seoul. RH positively correlated with Firmicutes ($R = 0.20$) and Cyanobacteria ($R = 0.29$), whereas Actinobacteria ($R = -0.23$) showed a negative correlation. Proteobacteria and Actinobacteria in Seoul showed slight positive correlations with $PM_{2.5}$, whereas the remaining phyla showed negative correlations. Among airborne bacteria in Seoul, Proteobacteria and unclassified bacteria showed negative correlations with temperature, whereas the other phyla showed positive correlations with temperature. Proteobacteria showed negative correlations with temperature ($R = -0.48$) and SO_4^{2-} ($R = -0.36$), whereas Firmicutes showed positive correlations with temperature ($R = 0.52$) and SO_4^{2-} ($R = 0.38$). Unclassified bacteria group was positively correlated with NO_3^- ($R = 0.50$) and SO_4^{2-} ($R = 0.45$) in Seoul.

Temperature positively correlated with the abundances of most airborne bacterial phyla in Nagasaki, except for Proteobacteria ($R = -0.49$). Among airborne bacterial phyla, the other bacteria group showed the highest positive correlation ($R = 0.61$) followed by Bacteroidetes ($R = 0.42$), Firmicutes ($R = 0.41$), and Cyanobacteria ($R = 0.39$). In contrast, the PBL of Nagasaki showed a positive correlation only with Proteobacteria ($R = 0.31$) but showed negative correlations with the remaining airborne bacterial species including those in the Bacteroidetes ($R = -0.43$), other bacteria ($R = -0.40$), Firmicutes ($R = -0.27$), and Cyanobacteria ($R = -0.25$) groups. Proteobacteria was also associated with different results when compared to those of other airborne bacterial species, when correlating phylum with $PM_{2.5}$. Proteobacteria and $PM_{2.5}$ were negatively correlated ($R = -0.32$), whereas the remaining bacterial phyla showed positive correlations. Bacteroidetes showed a high positive correlation coefficient with $PM_{2.5}$ ($R = 0.50$). The other bacteria group ($R = 0.35$), Cyanobacteria ($R = 0.34$), and Firmicutes ($R = 0.20$) also showed positive correlation coefficients with $PM_{2.5}$. NO_3^- was positively correlated with Proteobacteria ($R = 0.26$) while SO_4^{2-} and NH_4^+ were positively correlated with Bacteroidetes ($R = 0.47$ and 0.54) and other bacteria ($R = 0.33$ and $R = 0.28$) in Nagasaki. Bacteroidetes, which was more abundant during fall in Nagasaki, showed positive correlations with OC ($R = 0.34$), EC ($R = 0.20$), SO_4^{2-} ($R = 0.47$), and NH_4^+ ($R = 0.54$).

In all three cities, wind speed and $PM_{2.5}$ were negatively correlated, whereas airborne bacteria were not significantly affected by wind speed. In Beijing, RH and $PM_{2.5}$ were positively correlated, whereas negative correlations were found in Seoul and Nagasaki. $PM_{2.5}$ and its major chemical constituents were negatively correlated with PBL in Beijing and Seoul. This might be due to the faster diffusion velocity with a higher PBL (Luan et al., 2018). However, $PM_{2.5}$, OC, and EC exhibited negative correlations with PBL, whereas ionic species were positively correlated with PBL in Nagasaki. Proteobacteria showed negative correlations with temperature, whereas Firmicutes and Bacteroidetes showed positive correlations with temperature in all three cities. The correlations between Proteobacteria and humidity were negative in Beijing and Seoul ($R = -0.46$ and $R = -0.08$ for Beijing and Seoul, respectively), whereas Nagasaki was associated with a slightly positive correlation between Proteobacteria and humidity ($R = 0.02$). In a previous study (Gandolfi et al., 2013), the abundances of airborne bacterial species were found to change drastically due to meteorological factors, but the bacterial communities in this study did not show significant correlations with meteorological factors. Firmicutes and SO_4^{2-} were positively correlated in all three cities ($R = 0.01$ for Beijing, $R = 0.38$ for Seoul, and $R = 0.12$ for Nagasaki). As mentioned, the ratio of Firmicutes was higher during Asian dust events, which occur mostly in the spring and can drive long-range particle transport. As such, the relationship between Firmicutes and SO_4^{2-} levels suggest that long-range transport occurred (Chuang et al., 2016).

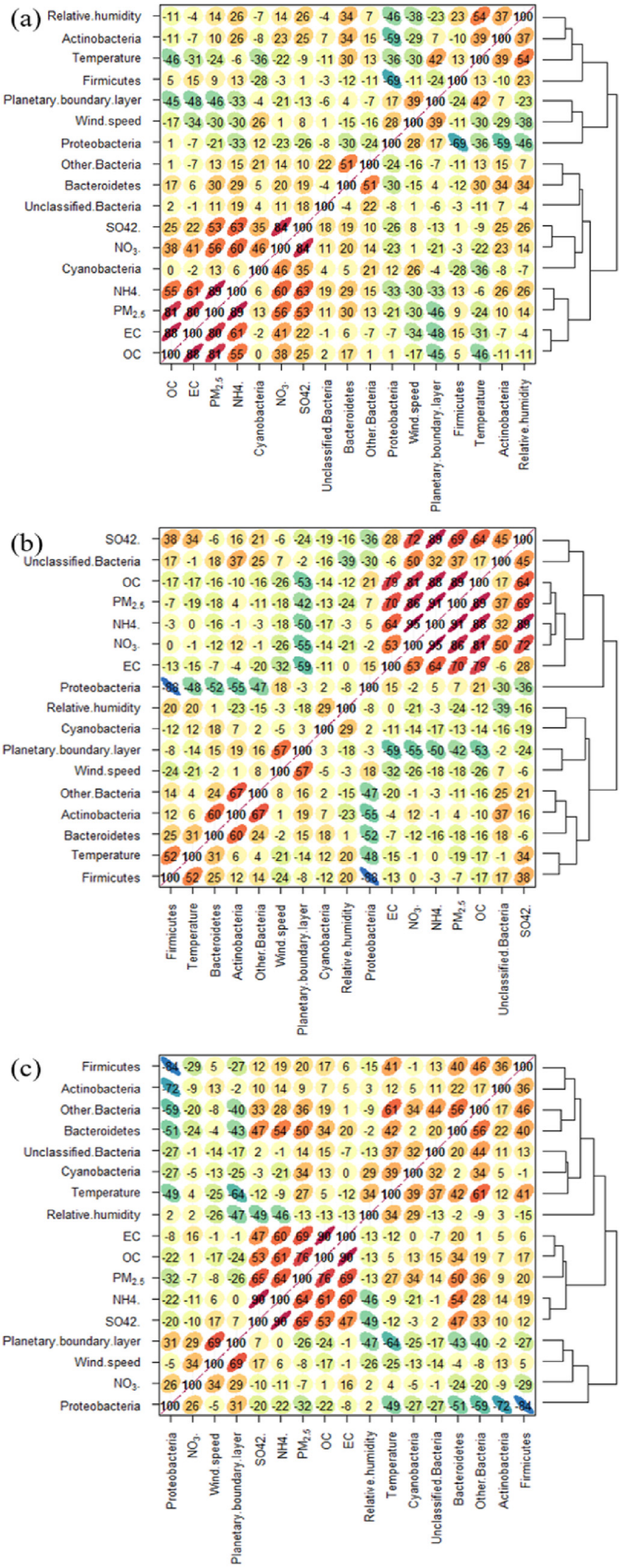


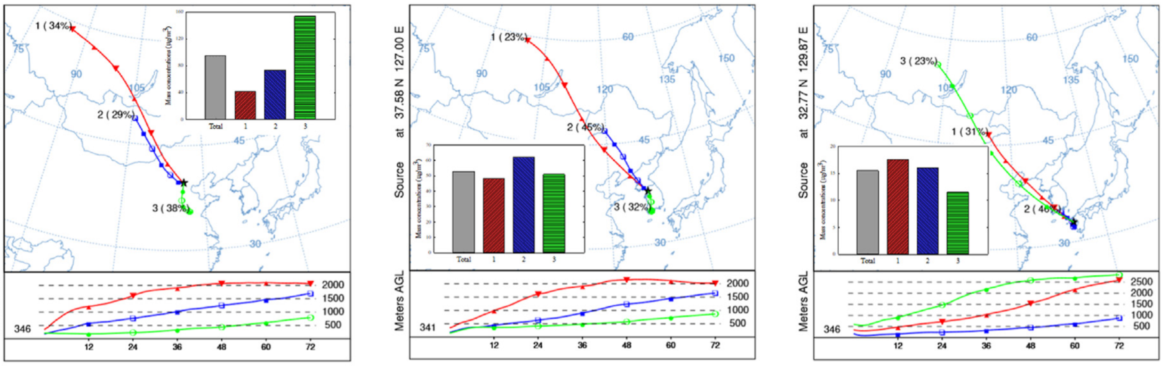
Fig. 2. Correlation plots of $PM_{2.5}$ and its major chemical constituents, airborne bacteria at the phylum level, and the meteorological data of (a) Beijing, (b) Seoul, and (c) Nagasaki.

3.5. *Abundances of airborne bacterial communities associated with trajectory clusters*

Airborne particles, especially Asian dust particles, were previously shown to be transported from desert or arid regions in China and Mongolia to South Korea and Japan (Hiraoka et al., 2017). Xu et al. (2019) found that dust-associated bacterial community was remarkably increased in the air that had travelled long distances from Outer Mongolia and Siberia to China (Xu et al., 2019). Yamaguchi et al. (2012) had earlier stated that air parcels originating in desert areas in the East Asia carried diverse bacterial groups in Japan. The transported particles carried diverse bacterial groups (Yamaguchi et al., 2012). In this study, 72-h backward-trajectory analysis was performed at 1-h intervals. The trajectories of all three cities were divided into three clusters, based on percent changes in the total spatial variance. Cluster analysis results from each city and average mass concentrations of $PM_{2.5}$ in each cluster are presented in Fig. 3.

3.6. *p.m.$_{2.5}$ and its major chemical constituents*

The first cluster of Beijing contained long-range transported air particles from Russia and accounted for 34% of all trajectories. Another long-range cluster, accounting for 29% of all trajectories originated from Mongolia and the third cluster, accounting for 38%, originated locally. The two clusters affected by long-range transport from Russia (41.3 $\mu g/m^3$) and Mongolia (73.0 $\mu g/m^3$) showed lower $PM_{2.5}$ mass concentrations, as compared to the total $PM_{2.5}$ average



(a) Beijing (b) Seoul (c) Nagasaki

Fig. 3. Backward trajectory cluster means of (a) Beijing, (b) Seoul, and (c) Nagasaki (72-h backward trajectories with 1 h time interval) and the average mass concentrations of $PM_{2.5}$ of each cluster. (a)-1: cluster from Russia, 34%, (a)-2: cluster from Mongolia, 29%, (a)-3: local cluster, 38%, (b)-1: cluster from Russia, 23%, (b)-2: cluster from Inner Mongolia, 45%, (b)-3: local cluster, 32%, (c)-1: cluster from Mongolia, 31%, (c)-2: local cluster, 46%, and (c)-3: cluster from Russia, 23%.

mass concentration (94.8 $\mu g/m^3$), whereas the local cluster showed a $PM_{2.5}$ mass concentration (153 $\mu g/m^3$) that was approximately 1.6-fold higher than this total average. ANOVA testing was performed with the measured $PM_{2.5}$ mass concentrations for each cluster, and the $PM_{2.5}$ mass concentrations among all clusters were significantly different for Beijing ($p < 0.05$).

The backward trajectories of Seoul were also divided into three clusters, which were classified as involving long-range transport from Russia (23%), inner Mongolia and northern China (45%), and local transport from the southern part of South Korea (32%). During the study period, the total $PM_{2.5}$ average mass concentration in Seoul was 52.8 $\mu g/m^3$, and different concentrations were found in each cluster; however, the differences were not as clear as those observed in Beijing. In the first cluster involving long-range transport from Russia, the lowest $PM_{2.5}$ mass concentration observed was 48.3 $\mu g/m^3$, whereas the second cluster affected by inner Mongolia and northern China had the highest $PM_{2.5}$ mass concentration of 62.2 $\mu g/m^3$. The results of ANOVA testing showed that the $PM_{2.5}$ mass concentrations from Russia and the local cluster of South Korea (50.8 $\mu g/m^3$) both showed significant differences as compared to the $PM_{2.5}$ mass concentrations of the cluster from inner Mongolia and northern China ($p < 0.05$).

The two clusters affected by long-range transport (among the three clusters of Nagasaki) were influenced by locations similar to the long-range clusters identified in Seoul. The first cluster in Nagasaki was affected by transport from inner Mongolia and northern China, accounting for 31% of the total. The third cluster, which reflected long-range transport from Russia, accounted for 23% of the total. The third cluster of Nagasaki was similar to the first cluster of Seoul, which also involved transport from Russia (23%). Nagasaki showed a higher occurrence of local clustering (46%). Among the three clusters of Nagasaki, the first cluster transported from inner Mongolia and northern China had the highest $PM_{2.5}$ mass concentration (17.5 $\mu g/m^3$). The average mass concentration of $PM_{2.5}$ in the local cluster was 16.0 $\mu g/m^3$, and the cluster affected by long-range transport from Russia had the lowest $PM_{2.5}$ mass concentration (11.5 $\mu g/m^3$). For the local cluster of Nagasaki, the trajectories of air parcels started from the South Sea and moved to the east side of Nagasaki before reaching Nagasaki. The local cluster of Nagasaki might have been affected by ships and vessels, since Nagasaki is a port city in Japan, and it could also have been affected by volcanos in Kyushu, which is at the very southern part of the four islands of Japan (Kerfahi et al., 2017). The $PM_{2.5}$ mass concentration of the cluster, which was affected by long-range transport from Russia, was significantly lower than that of the other two clusters ($p < 0.05$).

3.6.1. *Abundances of airborne bacteria*

Fig. 4 shows the relative phylum-level abundances of bacterial communities in each cluster for Beijing, Seoul, and Nagasaki. Proteobacteria species were predominant, followed by Firmicutes, Actinobacteria, Bacteroidetes, and Cyanobacteria in all clusters of all three cities. The lowest ratio for Proteobacteria was found in cluster 3 of Beijing (51.3%), which was the local cluster in Beijing. The highest ratio of Proteobacteria was identified in cluster 3 of Nagasaki (81.8%), which was affected by long-range transport from Russia. For Beijing and Seoul, the ratios of Proteobacteria tended to be lower in clusters with high $PM_{2.5}$ mass concentrations. The ratio of Proteobacteria in cluster 3 of Beijing, which was the local cluster with the highest average mass concentration of $PM_{2.5}$, was lowest, as mentioned previously. Cluster 2 of Seoul, which was affected by transport from inner Mongolia and northern China, with higher $PM_{2.5}$ mass concentrations, had a low abundance of Proteobacteria (60.4%). It is possible that the ratio of Proteobacteria was lower in Seoul since local pollution from Beijing affects Seoul, or the diversity of airborne bacteria could have increased with increases in the mass concentrations of $PM_{2.5}$. In the previous studies, the relative abundance of Proteobacteria was reported to be lower when the air mass travelled long distances. Moreover, airborne bacterial community was more diverse and richer in westerly long-range-transported air mass (Gandolfi et al., 2013; Xu et al., 2019). In section 3.4, the correlation coefficients between Proteobacteria and $PM_{2.5}$ were shown to be negative for Beijing ($R = -0.21$) and Nagasaki ($R = -0.32$), but slightly positive for Seoul ($R = 0.07$). For the clusters with higher $PM_{2.5}$ mass concentrations in Beijing and Seoul, the ratios of Proteobacteria tended to be lower, showing slightly different results compared to the overall findings presented in section 3.4.

The relative abundances of the top 5 airborne bacterial species and the other bacterial groups were statistically different between the locally affected cluster (cluster 3) and the long-range transported clusters (cluster 1 and cluster 2) for Beijing. In the local cluster of Beijing, the ratio of Actinobacteria was lower (12.6%) than that found in clusters 1 and 2 (22.0% and 21.0%, respectively), whereas the ratio of Firmicutes was significantly higher (29.3%) that that found in clusters 1 and 2 (15.1% and 13.6%, respectively). The abundance of the unclassified bacteria group in Beijing was significantly higher in cluster 1 (0.15%), which was affected by long-range transport from Russia, compared to those ratios in clusters 2 and 3 (0.09% and 0.06%, respectively). Regarding unclassified bacteria in each city, the ratios were higher in Beijing than in Seoul and Nagasaki.

The ratios of top five airborne bacterial species in Seoul were

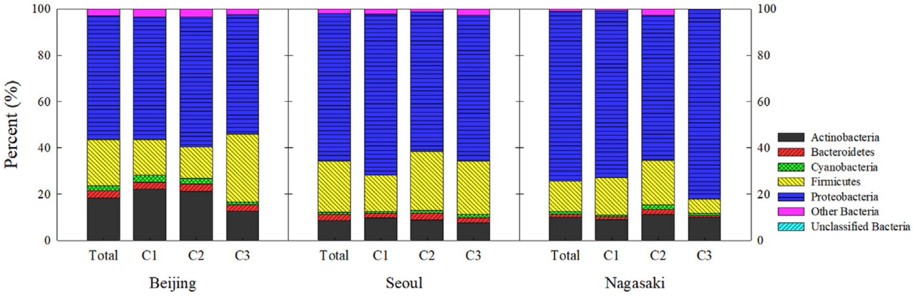


Fig. 4. Relative abundances of bacterial communities at the phylum level associated with the three trajectory clusters of Beijing, Seoul, and Nagasaki.

significantly different in each of the three clusters ($p < 0.05$). Proteobacteria showed the highest abundance (69.4%) in cluster 1, which was affected by long-range transport from Russia, whereas Firmicutes showed the highest ratio in cluster 2, which was affected by transport from inner Mongolia and northern China. The ratios of both the other bacteria and unclassified bacteria groups were higher when Seoul was affected by local emissions (cluster 3). Statistically, the ratios of the two groups of airborne bacteria were significantly lower in cluster 2, which was affected by transport from China.

All airborne bacterial species and groups, except for Proteobacteria in Nagasaki, showed higher ratios in cluster 2, which was affected by local emissions. Firmicutes species, which are known to be elevated during dust events (Gandolfi et al., 2013), showed higher ratios in local clusters in Nagasaki. This finding suggests that the airborne bacteria in Nagasaki were more affected by local emissions (including dust from volcanos) than long-range transported dust. This result is similar to the association between temperature and airborne bacterial species, which showed highly positive correlations in Nagasaki (Fig. 2).

The purpose of this study was to investigate associations between $PM_{2.5}$-bound bacterial communities with $PM_{2.5}$ chemical constituents and to know more specifically the possibility of long-range transport of the airborne bacteria at the phyla level in the study region. Understanding the impacts of airborne bacteria on human health and ecosystems is very important with respect to making effective air quality management strategies. It is hard to address which of bacterial community effects on adverse human health and ecosystems at the phyla level but the genera level is able to address them. Pseudomonas, a genera belonging to the Proteobacteria which occupies the highest abundant of the airborne bacterial phyla, is aeruginosa which is an important species of pathogen infection and can cause various human diseases such as endocarditis, pneumonia, and meningitis (Liu et al., 2018). Other genera of Proteobacteria including vibrio cholera, and Burkholderia cause opportunistic infections and hospital infections (Liu et al., 2018). Bacillus belonging to Firmicutes are pathogens which can cause anthrax in animals and humans and Clostridium belonging to Firmicutes can cause intense poisoning symptoms due to toxins (Spencer, 2003; Warrell et al., 2003). Therefore, identifying $PM_{2.5}$-bound bacterial communities at the genera level is very important to protect human health. Characteristics of seasonal and spatial variability of the bacterial communities at the genera level in the study region will be discussed further in our future works.

4. Conclusions

From December 2014 to November 2015, $PM_{2.5}$ samples were collected in Beijing, Seoul, and Nagasaki, and the major chemical constituents and airborne bacterial species in $PM_{2.5}$ were analyzed in this study. Meteorological factors including temperature, RH, wind speed, and PBL were correlated with airborne bacterial components. Proteobacteria, Firmicutes, and Actinobacteria were the dominant phyla represented by the airborne bacteria, and they accounted for more than 90% of all phyla in all three cities. The relative abundance results for the three cities showed that Proteobacteria was present at a higher average ratio in Nagasaki, Firmicutes was higher in Seoul, and Actinobacteria was associated with a higher abundance in Beijing. Proteobacteria, which was the most abundant airborne bacterial phylum, showed higher ratios during the winter in all three cities. The soil-associated airborne bacteria Firmicutes were more abundant during the spring in Beijing and Seoul and during the fall in Nagasaki. The wind speed was negatively correlated with $PM_{2.5}$, but not with airborne bacteria. The temperature correlated negatively with Proteobacteria in all three cities, but positively with Firmicutes and Bacteroidetes. Significant correlations between the relative abundances of airborne bacteria and meteorological factors were not found in this study Among the chemical constituents of $PM_{2.5}$, NO_3^- and SO_4^{2-} showed positive correlations with some airborne bacterial species in the study region.

The results of cluster analysis considering 72-h backward trajectories showed that the average mass concentration of $PM_{2.5}$ in Beijing was higher when Beijing was locally affected, whereas the average mass concentrations of $PM_{2.5}$ in Seoul and Nagasaki were higher when they were affected by transport from inner Mongolia and northern China. For Beijing and Seoul, in the clusters with higher $PM_{2.5}$ mass concentrations for each city, the ratio of Proteobacteria was lower and the ratios of other airborne bacterial species increased, except for Proteobacteria. It is possible that locally-emitted $PM_{2.5}$ in Beijing affected the airborne bacteria levels in Seoul. However, regarding Nagasaki, the ratio of Proteobacteria was higher in the local cluster rather than in the cluster from inner Mongolia and northern China. These results suggest that airborne bacteria could be affected by long-range transport within $PM_{2.5}$, but that the environment of the sample site is more important for airborne bacteria.

Correlations between airborne bacteria in $PM_{2.5}$ and meteorological factors and the major chemical constituents of $PM_{2.5}$ were analyzed, and the distributions of airborne bacteria by seasons and clusters were also assessed in this study. The classification of airborne bacteria at the phylum level in this study suggested that they might be able to travel long distances but that they are more affected by local environment than long-range transport.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Eun Ha Park: Data curation, Investigation, Writing - original draft. **Jongbae Heo:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing. **Ho Kim:** Methodology, Validation. **Seung-Muk Yi:** Conceptualization, Validation, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.126870.

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