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Charge levels and Gram (±) fractions of environmental bacterial aerosols

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ABSTRACT

Here, we investigated charge levels and Gram (+/−) fractions of environmental bacterial aerosols. The bioaerosols with +/− charges were collected separately over different time periods using an electrostatic sampler. Air samples were cultivated using selective media for G+ and G−, and further confirmed by Gram stain method. In addition, the viable bioaerosol size distributions over a 24 h time period were also obtained using an ultraviolet aerodynamic particle sizer (UV-APS). Finally, the elementary charge units carried by environmental G+ and G− bacterial aerosols were derived based on distribution-weighted particle aerodynamic diameters.

Results showed that G+ had equal abundances with G− regardless of the charge polarity and environments. The outdoor viable bioaerosols were observed to have slightly smaller distribution-weighted particle size (1.64 μm) than that (1.79 μm) of the indoor environment. In general, the outdoor culturable bacterial aerosol charge levels turned to be normally-distributed with a peak around 21–29 elementary charge units, while the indoor ones seemed to be skewed toward 46–92 elementary charge units. Results here suggest that a significant fraction of viable-but-not-culturable (VBNC) bioaerosol particles might be present for indoor environments. Results here can not only help design better electrostatic sampling device, but also assist in bioaerosol exposure assessment.

1. Introduction

It is well accepted that bioaerosol exposure can result in many adverse health effects, including respiratory impairment, microbial infection, allergenic reaction, respiratory sensitization, and toxicological reactions (Douwes et al., 2003; Walinder et al., 2001; Laumbach & Kipen, 2005). To adequately assess the exposure, bio-sampling is one of the most crucial steps (Xu & Yao, 2011). To this end, various samplers of different collection principles, e.g., filtration, liquid impingement, and impaction have extensively been developed and/or investigated (Chen et al., 2004; Tolchinsky et al., 2010; Haatainen et al., 2010; Bundke et al., 2010; Park et al., 2009; Gorner et al., 2006; Zhen et al., 2009). On the other hand, the electrostatic method as an emerging tool attracts great attention due to its low mechanical impaction injuries to the microbial cells and low pressure drop (Han et al., 2010; Yao et al., 2009; Han & Mainelis, 2008; Madsen & Sharma, 2008; Yao & Mainelis, 2006; Mainelis et al., 1999, 2002a, 2002b).

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The electrostatic collection is based on the utilization of bioaerosol electrical charge, and its collection efficiency depends on the aerosol charge level and the electrostatic field strength (Yao & Mainelis, 2006). As a result of its collection principle, the electrostatic method has lower aerosol deposition velocity and less desiccation over the bioaerosol particles, thus electrostatic sampling could more effectively protect the culturability of bioaerosols (Mainelis et al., 1999, 2001, 2002a; Yao & Mainelis, 2006). Under certain experimental conditions, the electrostatic method was shown to have better collection efficiencies than the traditional BIoStage impactor and the BioSampler when sampling airborne bacteria, fungi, endotoxin, and allergens (Yao & Mainelis, 2006; Yao et al., 2009). Accordingly, a number of studies were carried out to investigate the levels of electrical charges of both polarities carried by the bioaerosols for optimizing the electrostatic collection. It was pointed out that the naturally occurring bioaerosols carry electrical charges of both polarities which are enough to ensure their electrostatic collection (Mainelis et al., 2001; Lee et al., 2004; Xie et al., 2009). In one study, the concentration and diversity of total culturable bacterial aerosols both positively charged and negatively charged from office, hotel and outdoor environments were shown to vary with the sampling environments (Shen et al., 2013). In monitoring bioaerosols including bacteria, fungi and other cellular derivatives, use of electrostatic sampler with either positively or negatively charged particle collection would result in underestimates for the other polarity bioaerosols. This underestimate could be as much as 50% (Shen et al., 2013).

In addition to the concentration levels, the bioaerosol charge levels and compositions in each polarity are also important from the health standpoint. It has been reported that the particle electric charge levels could influence their depositions in the human lung (Melandri et al., 1977). For example, 1-μm aerosol particles with 100 elementary electric charges were shown to have a 10 times higher lung deposition efficiency than the electrically neutral particles (Bailey, 1997). The airborne bacterial aerosols consist of both Gram-negative (G−) and Gram-positive (G+) bacteria and their concentration levels vary in different environments (Górny & Dutkiewicz, 2002). Exposure to different bioaerosol compositions could lead to diverse health effects (Eber et al., 2011; Heederik & Mutius, 2012), especially for respiratory diseases such as allergy (Heederik & Mutius, 2012) and asthma (Vartiainen et al., 2002; Mutius & Radon, 2008; Smit et al., 2010). The outer membrane of the Gram-negative bacterial cell wall contains lipopolysaccharide (LPS) which can cause immune activation (Yoshimura et al., 1999). It has been reported that more than 30% of hospital-acquired infections are related to Gram-negative bacteria, e.g., hospital-acquired pneumonia (Peleg & Hooper, 2010). The infections by Gram-positive bacteria also cause a tremendous human toll, e.g., the pneumococcus which is a leading cause of death with a mortality rate of 40% for healthy elderly individuals (Rello et al., 1996). Another example is the infection of *Staphylococcal* which is the dominating cause of bacteremia in US hospitals (Marshall et al., 1998). As both bioaerosol charge level and composition (Gram type) have important implications for human health, it is useful to know the fractions of G− and G+ in bioaerosols with different charge polarity and level.

This study was designed to study the distribution of G− and G+ in both positively and negatively charged bacterial aerosols in natural environments using electrostatic means. The air samplings were conducted from morning to afternoon in both indoor and outdoor environments using an electrostatic sampler designed in our previous study. For each individual environment, different polarity charged bioaerosols were collected by the sampler and cultured on selective agar media for G+ and G−. In addition, the cultivation of G+ and G− bacteria were also confirmed by the Gram stain method. At the same time, viable (fluorescent) bioaerosol distributions were also monitored using an ultraviolet aerodynamic particle sizer (UV-APS) over a 24 h time period in both environments. The distribution-weighted diameter and charge levels for environmental bacterial aerosols were further derived. Information developed here can not only help design better electrostatic sampling device, but also provide important information for assessing bioaerosol exposure risks in different environments.

2. Materials and methods

2.1. Ultraviolet Aerodynamic Particle Size Spectrometer (UV-APS)

A UV-APS (Model 3314; TSI, Inc., Shoreview, MN) device was also used to monitor the viable bioaerosol size distributions both in indoor and outdoor environments. The UV-APS can real-time monitor the airborne bioaerosol particles that are emitting fluorescence (viable bioaerosols) within a size range of ~0.5–20 μm in aerodynamic diameter. It works through the irradiation of bioaerosol particles by the ultraviolet laser beam at a specific wavelength of 355 nm, and the excited fluorescence is detected by the photomultiplier tube (PMT) of the UV-APS (TSI, Inc.). The measured signals are considered to originate from living organisms which have NAD(P)H molecules and riboflavin (Agranovski & Ristovski, 2005).

2.2. Electrostatic sampler used

In this study, the electrostatic sampler designed in a previous study (Yao et al., 2009) was utilized to sample environmental Gram-negative and Gram-positive bacterial aerosols carrying both positive and negative charges. As shown in Fig. 1(B), this sampler is composed of a plastic transparent body, two copper sheets measured as 10 by 27 cm, an air inlet, and an air outlet. The copper sheets spaced at 2.3 cm were connected to a high voltage supply (model 205B-15R from Bertan Associate, Inc., Valhalla, NY), and the samplings were conducted using a vacuum pump connected to the outlet of the

electrostatic sampler. The electrostatic sampler was designed to accommodate two square agar plates (96-well plate size) as illustrated in Fig. 1.

2.3. Selective culturing media for G+ and G− bacteria

In this study, two types of selective media were used to grow Gram-positive and Gram-negative bacterial aerosols, respectively. Phenethyl alcohol (PEA) agar culture medium (Qingdao Rishui Biotechnology Co., China) only permits Gram-positive bacteria to multiply. The detailed ingredients of PEA agar culture medium consist of casein Tryptone 15.0 g/L, protease digestion of Soybean papaya 5.0 g/L, sodium chloride 5.0 g/L and agar 15.0 g/L. On the other hand, the Gram-negative selective medium (Qingdao Haibo Biotechnology Co., China) was used to grow Gram-negative bacteria. The Gram-negative selective medium is composed of six ingredients, which include peptone 5.0 g/L, yeast extract powder 2.0 g/L, crystal violet 0.002 g/L, skimmed milk powder 1.0 g/L, Nisin 0.0016 g/L and agar 15 g/L.

2.4. Experimental procedures

2.4.1. Viable bioaerosol size distributions

For indoor environments, the monitoring was conducted in an office environment with about 16 m^2 space using the UV-APS. For outdoor environment monitoring, we selected the outside of an office building within Peking University campus in Beijing. The sampling data were set to be recorded every 15 min for over 24 h time period.

2.4.2. Bioaerosol sampling

To study bioaerosol concentration levels with either positive or negative charges, aerosol samples were collected using the electrostatic sampler operated at a flow rate of 4 L/min with an electrostatic field strength of 8.7 kV/cm at a voltage of 20 KV provided by the high voltage power supply. Firstly, two square agar plates filled with PEA agar culture medium described above were placed inside the sampler to collect the bacterial aerosols with positive charge. Secondly, the positive and negative electrodes connected to the electrostatic sampler were switched to collect negatively charged bacterial aerosols with two new square agar plates. From a previous study (Yao et al., 2005), it can be inferred that the tested electrostatic field strength and conditions caused little damage to the bacteria including those bioaerosols and those collected on the agar surface.

The aerosol samples were continuously collected using the electrostatic sampler for 40 min in an office (lab) environment and an outdoor environment, respectively, at Peking University located at northwest 4th Ring of Beijing during the winter of 2012, and only two persons were present during the indoor sampling. The collected bioaerosols were cultured using Gram-positive and Gram-negative selective medium, respectively. The humidity level observed by a hygro-

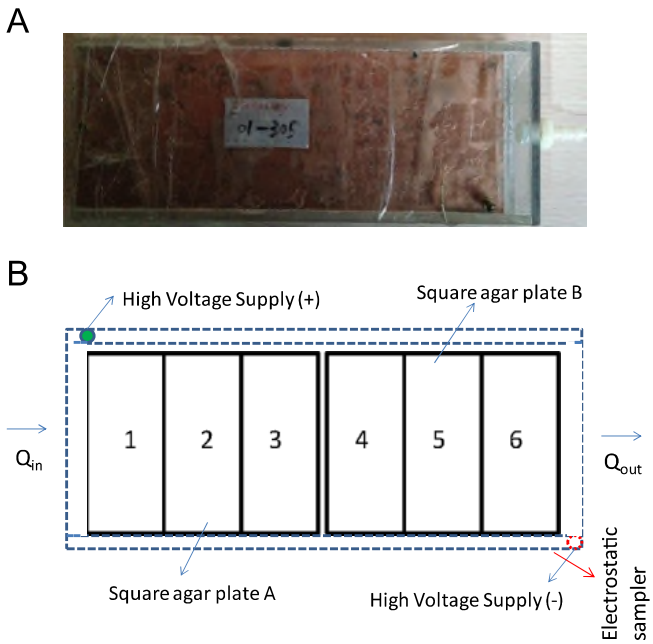

Fig. 1. (A) shows the picture of the electrostatic sampler used in this work, and (B) is the sketch of experimental sampling of bioaerosols carrying negative or positive chagres using an electrostatic sampler developed in a previous study (Yao et al., 2009a, 2009b); two square plates (A and B) were placed between two copper sheets of the electrostatic sampler connected to a high voltage supply during the sampling; for each sqaure plate, its surface agar area was equally divided into three regions (1, 2, and 3 or 4, 5, and 6) as indicated in the figure, and bioaerosols with different charge levels were collected in the different regions.

thermograph (Omega, America) was 42% for indoor and outdoor, and the temperature was measured as 12 °C for the outdoor environment and 15 °C for the office environment. The sampling experiments were conducted three times from morning to afternoon.

2.4.3. Culturing bacterial aerosols

When sampling bacterial aerosols from ambient air, fungal aerosols were also collected. As indicated in Fig. 2(B), fungal aerosols would overgrow bacterial aerosols. In this work, we used nystatin tablets (Zhenyuan Pharmaceutical Co., Ltd., Zhejiang, China) to inhibit the fungal growth. According to the instructions of nystatin tablets, 1,000,000 U nystatin (2 tablets) was added to 1 L trypticase soy agar (TSA) (Becton Dickinson Microbiological System, Sparks, MD) for growing bacteria before autoclaving. To this end, we performed additional sampling tests using a BioStage impactor (SKC, Inc.) to sample bioaerosols with and without use of the nystatin tablet to confirm that use of the tablet does not cause inhibition of bacterial growth. As observed in Fig. 2(B), without addition of nystatin tablets the fungi were overgrown, and in contrast only bacterial colonies were observed when the nystatin tablets were used as shown in Fig. 2(A).

Here, we tested the effectiveness of selective media using the Gram stain method with *Bacillus subtilis* (ATCC 9372) (vegetative cells) and *Escherichia coli* (ATCC 15597), which are representative Gram-positive bacteria and Gram-negative bacteria, respectively. As shown in Fig. 2(C)–(E), *B. subtilis* was inoculated on both PEA agar plates and Gram-negative selective agar plates, and the same experiments were conducted for *E. coli*. The agar plates were cultured at 26 °C for 2–3 days. As observed from the figure, use of selective medium can selectively grow Gram-positive and Gram-negative bacteria. Microscopic images revealed that the Gram stain method can also clearly distinguish between Gram-negative (purple) and Gram-positive (blue) as seen from Fig. 2(E).

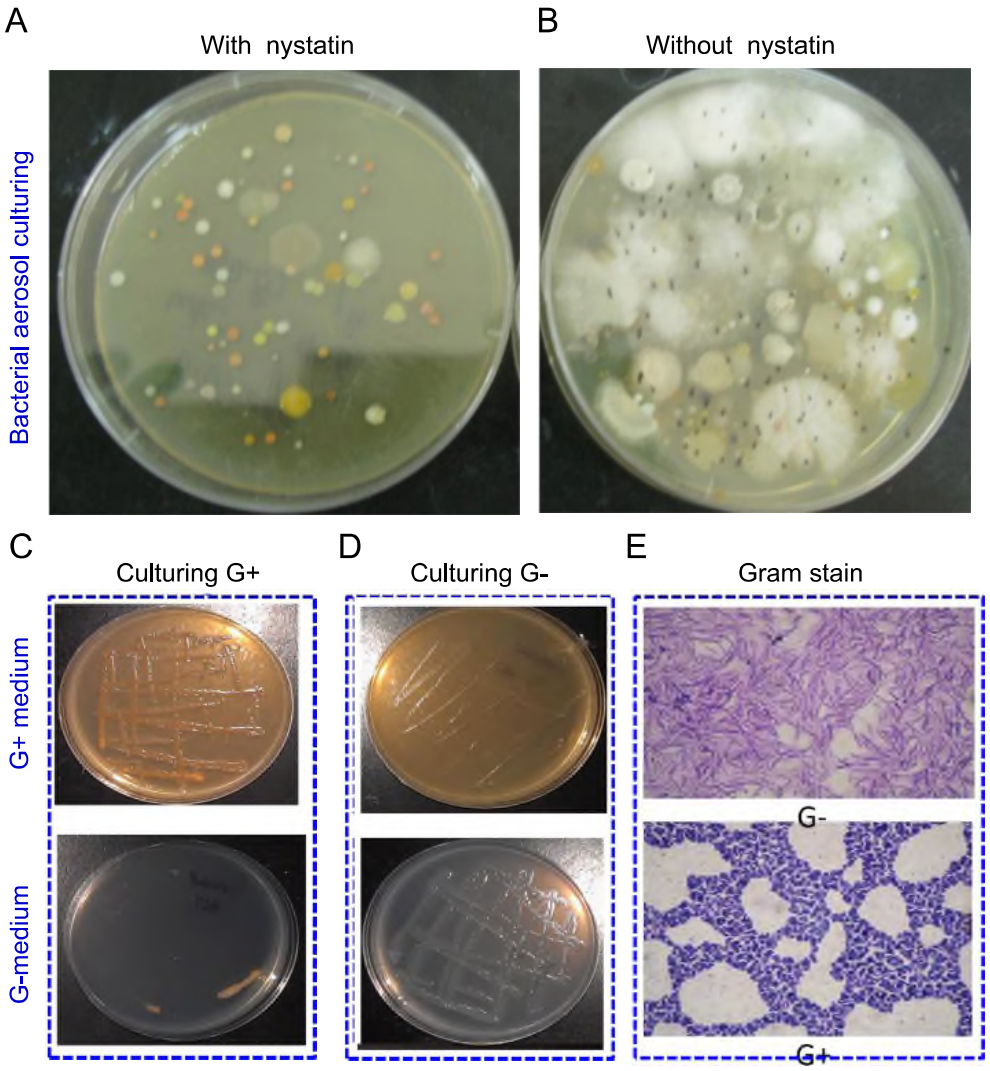

Fig. 2. (A) and (B) shows some examples of culturable bacterial aerosols with and without fungal inhibitor nystatin, respectively; (C) and (D) shows some examples of culturable Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*) growing on selective media; (E) shows examples of microscopic images for Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*) bacteria using Gram stain method. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Accordingly, the collected bacterial aerosols were cultured directly on PEA agar plates and Gram-negative selective agar plates at 26 °C for 3 days in our work. Each of the square agar plates used was prepared using 70 mL autoclaved agar suspension. The CFUs were manually counted for each of the regions (numbers 1–6) divided for the two square agar plates as depicted in Fig. 1, and the culturable bacterial aerosol concentrations of Gram-positive and Gram-negative bacterial aerosols were calculated for each charge polarity for different regions. The total culturable bacterial aerosol concentrations were also calculated using the method mentioned above by summarizing those detected from six regions of the two agar plates used.

2.4.4. Calculating weighed diameter and electric charge level of bioaerosols

In this work, UV-APS was used to measure concentration of bioaerosols indoors and outdoors in 54 different size ranges for 24 h in each environment. From the monitoring data, we calculated the concentration percentages of each size range. Based on this information, we further obtained the weighted diameters of indoor and outdoor bioaersols using the following equation:

$$D_w = \sum_{i=1}^{54} (d_i \times R_i) \tag{1}$$

where D_w is the weighted diameter for indoor or outdoor viable bioaerosols, d_i is the particle diameter, and R_i is the concentration percentage for each size range.

To calculate the electric charge levels of bacterial aerosols collected by the electrostatic sampler, we used the weighted diameters obtained here for indoor or outdoor viable bioaerosols according to the charge level calculation established by a previous study (Xie et al., 2009).

3. Statistical analysis

The difference in concentrations between positively and negatively charged Gram-positive and Gram-negative bacterial aerosols for indoors and outdoors were analyzed using the independent sample *t*-tests through SPSS 16.0. A *p*-value of less than 0.05 indicates a significant difference between groups (confidence level 95%).

4. Results and discussion

In this work, we have studied the electrical charge levels of indoor and outdoor Gram positive (+) and negative (−) bacterial aerosols from both indoor and outdoor environments. As demonstrated in Fig. 2, use of selective media can selectively grow Gram-positive and Gram-negative bacteria. In addition, fungal species can also be effectively suppressed using nystatin tablet as seen from the figure such that bacterial colonies can be clearly seen and counted. As also observed in Fig. 2(E), the Gram-stain method also confirmed the effectiveness of our selective media for growing G+ and G−.

By using the UV-APS, we have obtained the viable bioaerosol size distributions both for indoor and outdoor environments over a 24 h time period as shown in Fig. 3. Overall, it seems that indoor and outdoor environments had a similar bioaerosol size distribution pattern although they are in different magnitudes. The concentrations of indoor viable bioaerosols were found significantly higher than those of outdoor ones regardless of size ranges as shown in Fig. 3. Here, viable bioaerosol particles refer to bio-particles with active metabolic activities which are different from culturable bioaerosol particles which can reproduce. As observed from the figure, the indoor and outdoor ratios (in/out ratios) for all size ranges were found to range from 4 to 12, and the lowest ratios were observed at 1.2–2.3 μm size ranges. Previous studies show that indoor culturable bacterial concentration was significantly higher than for the outdoors (Mentese et al., 2009; Naddafi et al., 2011). These results were consistent with our viable bioaerosol monitoring results, but different from our culturing data. In their work, they used the Andersen-type impactor, while our work used an electrostatic sampler. These two different samplers would result in different culturable bioaerosol concentration levels. Nonetheless, the culturable bacterial concentration could be influenced by many different parameters, and here are some examples in specific environments for certain time periods. As shown in the figure, both indoor environment and outdoor environment had peaks around 1.1–15 μm, and both environments were also observed to have fewer viable bioaerosol particles larger than 10 μm. Using Eq. (1) and the size distributions, the weighted diameters (D_w) of indoor and outdoor viable bacterial aerosols were calculated as 1.79 μm for indoors and 1.64 μm for outdoors. The difference of weighted diameter (D_w) for indoor and outdoor environments may be due to the differences in the species diversity and size distribution for viable bioaerosols. The different bioaerosol emission sources in different environments play a role in the difference detected. Although longer monitoring time would get a more precise estimate for overall profiles of both indoor and outdoor viable bioaerosols, here we set the time resolution to 1 min for UV-APS measurement, and we had a total of about 1440 measurements both for indoor and outdoor environments. Thus, our estimate of the viable bioaerosol particle size distribution was robust with respect to the overall trend.

Figure 4 shows the electric charge quantities of indoor G+ and G− aerosols with positive charge. As observed from the figure, the concentration of positively charged G+ bacterial aerosols were higher than G− bacteria in indoor environment (office) as detected by the electrostatic sampler. Total culturable G+ bacterial aerosol concentration was measured as 90 ± 18 CFU/m^3, and that for G− bacterial aerosol was 79 ± 16 CFU/m^3 yet with a slightly higher variation. For both Gram-

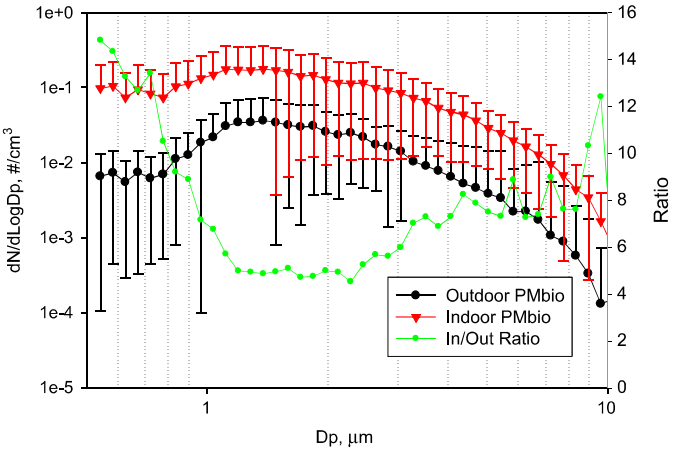

Fig. 3. The size distributions and ratios (in/out ratio) of both indoor (lab office) and outdoor viable bioaerosols (PMbio) monitored by UV-APS; the data points and error bars stand for the averages and standard deviations, respectively, for the monitoring data collected over a time period of 24 h in both environments; data were recorded every 15 min during the time period.

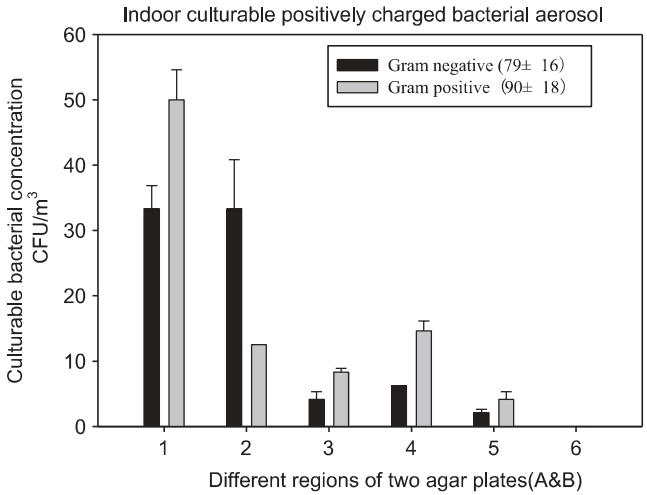

Fig. 4. Indoor culturable Gram-positive and Gram-negative bacterial aerosols with *positive* charges collected in different regions (1, 2, and 3 or 4, 5 and 6) of two agar square plates (shown in Fig. 1); data points and error bars stand for averages of three independent repeats from morning to afternoon; the experiments were conducted around northwest 4th Ring of Beijing during the winter of 2012; charge levels for different regions: 1: > 92 elementary units; 2: 46–92 elementary units; 3: 32–46 elementary units; 4: 23–32 elementary units; 5: 19–23 elementary units; 6: 16–19 elementary units.

positive and Gram-negative, their concentrations both decreased along the sampling regions of the agar plates, which corresponded to decreasing charge levels. For indoor positively charged bacterial aerosols, the concentration of G+ bacteria at each individual region was higher than those of G− bacteria except for region 2. Likewise, the same results for indoor negatively charged bacterial aerosols were also obtained as shown in Fig. 5. Negatively charged G+ bacteria were nearly twice more than G− bacteria in indoor environment detected using the electrostatic field sampler, and the total culturable G+ bacterial aerosol concentration was measured as 106 ± 14 CFU/m^3, and that for G− bacterial aerosol was 63 ± 10 CFU/m^3. Their concentrations generally decreased along with the sampling regions of the agar square plates except for G+ at region 4. Statistical analysis indicated that there were no significant differences detected in concentration levels of G+ and G− in the indoor environment regardless of charge polarity (p-values=0.774 and 0.8334 for negative and positive, respectively; independent sample t-tests). Figures 6 and 7 show negatively and positively charged concentration levels of outdoor G+ and G− bacterial aerosols, respectively, for an outdoor environment. As observed from Fig. 6, the total culturable G+ bacterial aerosol concentration was 310 ± 86 CFU/m^3, and that for G− bacterial aerosol was 335 ± 53 CFU/m^3; for those negatively charged aerosols the total culturable G+ bacterial aerosol concentration was 308 ± 28 CFU/m^3, and that for G− bacterial aerosol was 431 ± 63 CFU/m^3. Different from positively charged ones, the concentration of negatively charged G+ bacteria outdoor approximately increased along with the sampling regions (except for region 4), whereas the concentration of negatively charged G− bacteria outdoor fluctuated, and the highest concentration appeared in region 4 as observed in Fig. 6. Likewise, we did not detect statistically significant differences in G+ and G− concentration levels for the outdoor environment (p-values=0.522 and 0.856 for negative and positive, respectively; independent sample t-tests). The concentrations of outdoor culturable bacterial aerosols were significantly higher than those of indoor culturable bacterial aerosols regardless of their charge

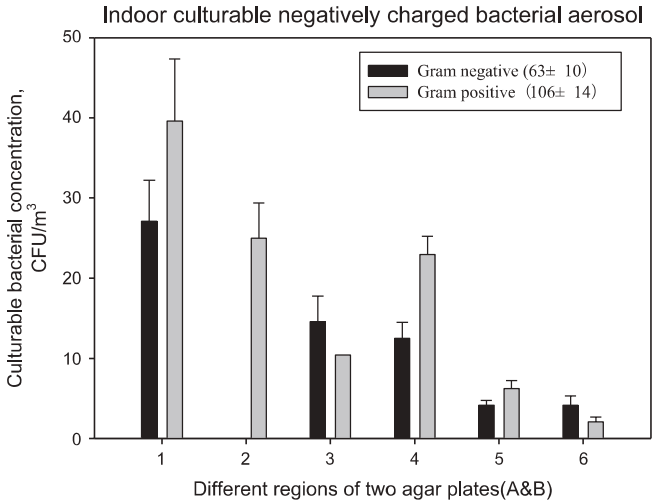

Fig. 5. Indoor culturable Gram-positive and Gram-negative bacterial aerosols with *negative* charges collected in different regions (1, 2, and 3 or 4, 5 and 6) of two agar square plates (shown in Fig. 1); data points and error bars stand for averages of three independent repeats from morning to afternoon; the experiments were conducted around northwest 4th Ring of Beijing during the winter of 2012; charge levels for different regions: 1: >92 elementary units; 2: 46–92 elementary units; 3: 32–46 elementary units; 4: 23–32 elementary units; 5: 19–23 elementary units; 6: 16–19 elementary units.

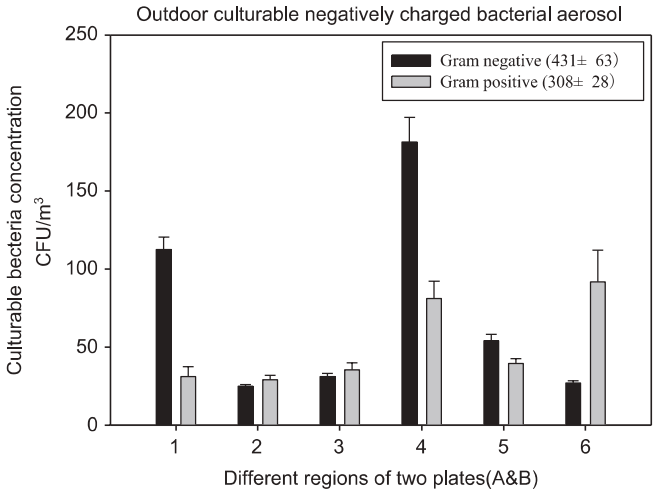

Fig. 6. Outdoor culturable Gram-positive and Gram-negative bacterial aerosols with *negative* charges collected in different regions (1, 2, and 3 or 4, 5 and 6) of two agar square plates (shown in Fig. 1); data points and error bars stand for averages of three independent repeats from morning to afternoon; the experiments were conducted around northwest 4th Ring of Beijing during the winter of 2012; charge levels for different regions: 1: >85 elementary units; 2: 43–85 elementary units; 3: 29–43 elementary units; 4: 21–29 elementary units; 5: 18–21 elementary units; 6: 15–18 elementary units.

polarity, which was in line with the results found in our previous study (Shen et al., 2013). Compared to the UV-APS data, however, the culturable data indicated that a significant fraction of indoor bioaerosols are in the state of viable-but-not-culturable (VBNC) or very sensitive to the sampling stress and cultivate conditions. In this work, we did not compare the differences of G+ and G− with different charge polarity between different collection regions due to the low-resolution bioaerosol electrical charge measurement. Nonetheless, our work provided bioaerosol electrical charge ranges with their appropriate fractions in the ambient air.

Using the weighted diameters obtained, we further calculated the viable bioaerosol electrical charge levels of indoor and outdoor bioaerosols collected on different regions of the two square agar plates as shown in Figs. 4–7. By calculation using data shown in the figure, the indoor bacterial bioaerosols with positive charges collected in the first region accounted for about 20% of the total with elementary charge greater than 92 elementary charge units. The maximum percentage of outdoor bacterial bioaerosols appeared in region 4 with elementary charges from 21 to 29 units. What is more, we can see that except for the first region, the distributions of outdoor bacterial bioaerosols can be approximately considered as normal distribution. The distributions of positively charged bacterial bioaerosol for both environments are also different from that of negatively charged ones. For outdoor bioaerosols, close to 80% carry a charge level of less than 85 elementary units, while for indoor ones approximately 50% carry a charge level of less than 92 elementary units. It is rather difficult to precisely estimate the ambient bioaerosol charge distribution because the bioaerosol particles could have a variety of different sizes,

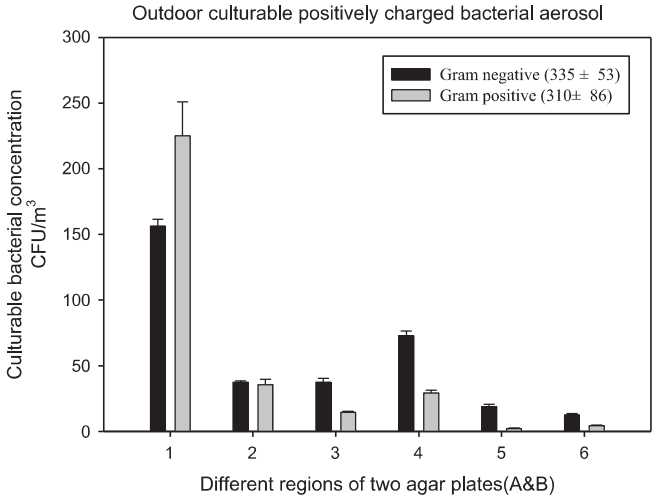

Fig. 7. Outdoor culturable Gram-positive and Gram-negative bacterial aerosols with *positive* charges collected in different regions (1, 2, and 3 or 4, 5 and 6) of two agar square plates (shown in Fig. 1); data points and error bars stand for averages of three independent repeats from morning to afternoon; the experiments were conducted around northwest 4th Ring of Beijing during the winter of 2012; charge levels for different regions: 1: >85 elementary units; 2: 43–85 elementary units; 3: 29–43 elementary units; 4: 21–29 elementary units; 5: 18–21 elementary units; 6: 15–18 elementary units.

and even for the same size they could carry different levels of electrical charges. Use of electrical mobility will come across the same problem assuming the electrical mobility is mainly governed by the electrical charge and particle diameter. For example, a large particle carrying a high electrical charge and a small particle carrying a low electrical charge can have the same electrical mobility. Thus, if they enter the sampler at the same point, both will land at the same spot while having a very different electrical charge. Given this difficulty, we used weighted aerodynamic diameter (using the information presented in Fig. 3, we obtained the weighted diameter for a particular environment as indicated in Eq. (1) by summarizing the product of specific size and its overall fraction). This way we obtained a single size particle for a particular environment, and then we could further calculate its charge levels for a particular collection region. Here, use of the weighted diameter implies that for a particular percentage of the time the ambient particles behave as a particular diameter and at other times they behave as another different size particle, and so on and so forth. Therefore, the use of distribution-weighted diameter can somehow take into account the possibility that large particles having high electrical charge and small particles having low charges would land at the same spot on the agar collection surface. To address the charge level uncertainty, e.g., overlapping over different collection regions, many parameters are needed to be taken into account, e.g., the statistical points of particles entering the device, the charge distribution for a single-sized ambient particle and other environmental factors. In addition, the charge distribution could also be affected by the fluorescence from non-biological particles, especially those smaller ones which however have been registered by the UV-APS (Agranovski & Ristovski, 2005; Huffman et al., 2010). Overall, this work represented an effort in estimating real-world bioaerosol charge levels, and more work, e.g., higher resolution for the charge level, is certainly needed.

The observed differences in the viable bioaerosol particle size distribution and the concentration levels in the indoor and outdoor environments were due to human activities and environmental factors such as temperature, atmospheric radiation and humidity level. For viable bioaerosol particle size distribution, the actual results could vary with different environments and seasons even for the same location. For indoor environment, it could strongly depend on the environmental characteristics and human occupancy. One study pointed out that elevated concentrations of indoor airborne bacteria were attributed to human occupancy, and indoor bacteria are associated with human skin, nostrils, and hair (Hospodsky et al., 2012). Indoor dominant bacterial taxa were found to be *Proprionibacterineae*, *Staphylococcus*, *Streptococcus*, *Enterobacteriaceae*, and *Corynebacterineae* (Hospodsky et al., 2012), and each occupant contributed about 37 million gene copies per hour (Qian et al., 2012). In another study, it was indicated that activity strength is a robust measure for relating human activities to indoor bioaerosol levels (Chen & Hildemann, 2009). It was found that with the presence of smokers, bacteria and dust particles formed aggregates compared to single particles without smoking (Górny et al., 1999). In this work, we observed viable bioaerosol peaks at 1.1–1.5 μm in the indoor environment. In a recent work, a viable bioaerosol peak was detected at 1.7 μm using the UV-APS unit in an automobile cabin (Li et al., 2013). In addition to human sources, air conditioner duct, shower curtain, humidifier, toilets as well as any water-damaged carpets could be emission sources of various bioaerosols upon disturbance under high humidity level for indoor environment. Therefore, different indoor characteristics could greatly affect the bioaerosol emission rates and size distributions.

For outdoor bioaerosol size distribution, we also observed peaks at 1.1–1.5 μm here, which were identical to those of outdoors but with different magnitudes. Similar to indoor environments, the outdoor bioaerosol size distribution could be significantly impacted by different emission sources. For example, an outdoor environment which is close to a wastewater treatment plant would be expected to be different from those without the plant that is producing significant amounts of

bioaerosols. Among others, atmospheric irradiation could also impact the viability of the outdoor bioaerosols (Li et al., 2010). In our work, we found significant temporal variations in bioaerosol concentration levels regardless of the environments. Different bioaerosol size distributions could represent different exposure risks because of different particle deposition efficiencies in human lung. Nonetheless, observed viable bioaerosol size distributions are some examples in different environments tested, and might not exactly represent those under different climatic and ecological conditions. Besides, the actual viable or culturable bioaerosol concentration levels could fluctuate depending on location, season and monitoring time, thus those levels described in this work should be used as a reference.

In addition to particle size distributions, the bioaerosol compositions are also important with respect to exposure risk. Bacteria can be classified into Gram-positive and Gram-negative according to their membrane types. As observed in Fig. 2(E), Gram-positive bacteria are purple-colored, while Gram-negative bacteria appear to be pinkish. For Gram-negative bacteria, the dominant membrane component is endotoxin which has been shown to cause a variety of adverse health effects (Heine et al., 2001; Morgenstern et al., 2005; Nilsson et al., 2004). In addition, it was shown that among 10,337 isolates from sputum samples collected from 39,920 lower respiratory tract infection patients, 68.72% were Gram-negative bacteria, 20.65% were Gram-positive bacteria, and 10.62% were fungi (Wang et al., 2009). High levels of environmental endotoxin (the composition of Gram-negative bacteria's cell wall), and (1,3)-β-D glucans (the composition of fungi's cell wall) exposure could induce a series of inflammations and toxic reactions to reduce human immune ability (Yoshimura et al., 1999; Yao et al., 2009). In addition, it was also found that Gram-negative bacteria tend to become more drug resistant (Wang et al., 2009). Thus, it is important to determine the relative abundance of these different types of bacteria. In this work, we did not find a statistically significant difference in the abundances of culturable Gram-positive and Gram-negative bacteria detected using an electrostatic sampler. However, it is surprising to learn that more than 80% of the genera detected from automobile cabin filters using high throughput gene sequence were Gram-positive bacteria regardless of geographical locations (Li et al., 2013). The differences might be due to different methods of detection. Here, we only detected culturable fraction of bacteria, while high throughput gene sequence detected all bacterial species including those non-culturable ones.

At the same time, the charge level of bacterial aerosols also plays an important role in their lung deposition efficiency, thus directly impacting human health. It has been indicated that the cell wall structures of bioaerosol and the process of friction and dispersing in atmosphere would lead to electric charge on the surface of bioaerosol (Mainelis et al., 1999). In addition, bacteria could transform their own cell wall structures dynamically in response to the variations of temperature, osmotic pressure, salinity and pH value (Cronan & Gelmann, 1975), and these changes would have influences on the electric charge of bioaerosols. During the process of bacteria aerosolizing and dispersing, they could obtain as high as 13,000 elementary electric charges (Mainelis et al., 2001, 2002c, 2002d). Melandri et al. (1983) reported that the deposition ability of particles with the diameter range of 0.3–1.0 μm will increase along with the augment of elementary charges according to the human test. Cohen et al. (1998) found that the deposition ability of singly charged one was 2.3 times higher than that of charge-neutralized aerosols and 6.2 times higher than zero-charge particles. Previously, most studies determined charge levels for lab-generated bacterial aerosols (Xie et al., 2009; Mainelis et al., 2001, 2002a), and in one study the charge measurement method was improved by utilizing the qPCR and electrostatic sampling technique (Xie et al., 2009). Here, by using a bioaerosol distribution weighted aerodynamic diameter obtained by the UV-APS, we estimated environmental bioaerosol charge distributions for both indoors and outdoors using the electrostatic sampler. In general, our results revealed regardless of charge polarity that outdoor aerosol charge levels turned to be more normally distributed with peaks at 21–29 elementary charge units as shown in Figs. 6 and 7, while indoor aerosol charge levels seemed to be more skewed toward peaks at 46–92 elementary charge units. As discussed previously, bioaerosol concentration level fluctuates with time and environment, and results shown here, e.g., charge and concentration levels, might only represent basic profiles in each of the environments tested.

In addition to the health impacts, bioaerosol charge level and polarity also impact their electrostatic collection (Grinshpun et al., 2007; Hogan et al., 2004; Li & Wen, 2003; Mainelis et al., 2002b). In our recent study, we have also investigated culturable bioaerosol diversity and concentration levels with different charge polarity in different environments (Shen et al., 2013). It was found that for hotel and outdoor environments bioaerosols with both charge polarities had similar concentration levels, while the office environment tested had more negatively charged bacterial aerosols (Shen et al., 2013). Here, we detected no statistically significant differences in positively and negatively charged culturable bacterial aerosols for both environments (office and outdoors) tested ($p=$values >0.7). These results suggest that bioaerosol charge level also changes dynamically in response to environmental characteristics. Nonetheless, the information regarding bioaerosol charge level with different polarity is important to the design of further electrostatic sampler. Based on our data, electrostatic sampler for either positively or negatively charged particle collection would underestimate as much as 50% of the culturable bioaerosol load in the environments. The information from the ambient and indoor bioaerosol charge levels obtained here is also useful for selecting appropriate electrostatic field strength.

5. Conclusions

In this work, we investigated Gram-positive and Gram-negative fractions in those aerosols with positive or negative charges using the electrostatic sampling technique and selective culturing media. In addition, we also studied the charge level of ambient and indoor bacterial aerosols. Consistent with previous studies, positively and negatively charged culturable bacterial aerosols appeared to be in equal quantities in the environments investigated. In addition, equal

abundances were also observed for culturable G+ and G− bacterial aerosols regardless of the charge polarity and environments tested. Using the UV-APS, outdoor viable bacterial aerosols were shown to have smaller distribution-weighted particle size (1.64 μm) than that (1.79 μm) in indoor environment. Besides, this work also found that outdoor culturable bacterial aerosol charge levels turned to be more normally distributed at 21–29 elementary charge units, while indoor ones seemed to be more skewed toward 46–92 elementary charge units. Our results from the UV-APS unit also imply that a significant fraction of indoor bioaerosols might be in the state of viable-but-not-culturable. Information developed here can not only help design better electrostatic sampling device, but also provide important information for assessing bioaerosol exposure risks in different environments.

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