Invited Article

Chinese Science Bulletin

Environmental Sciences

September 2013 Vol.58 No.26: 3169–3176
doi: 10.1007/s11434-013-5852-9

Negatively and positively charged bacterial aerosol concentration and diversity in natural environments

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Received December 5, 2012; accepted January 23, 2013; published online May 31, 2013

Bioaerosol charge information is of vital importance for their electrostatic collection. Here, electrostatic means and molecular tools were applied to studying bioaerosol charge dynamics. Positively or negatively charged bioaerosols were collected using an electrostatic sampler operated with a field strength of 1.1 kV cm^{-1} at a flow rate of 3 L min^{-1} for 40 min. Those with fewer or no charges bypassing the sampler were also collected using a filter at the downstream of the electrostatic sampler in one environment. The experiments were independently conducted three times in three different environments. The collected bacterial aerosols were cultured directly on agar plates at 26°C, and the colony forming units (CFU) were manually counted. In addition, the CFUs were washed off from the agar plates, and further subjected to polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) for culturable diversity analysis. The results revealed remarkable differences in positively and negatively charged culturable bacterial aerosol concentration and diversity among the studied environments. In the office environment, negatively charged culturable bacterial aerosols appeared to dominate (P = 0.0489), while in outdoor and hotel environments both polarities had similar concentration levels (P = 0.078, P = 0.88, respectively). DGGE patterns for positively charged culturable bacterial aerosols were shown strikingly different from those of negatively charged regardless of the sampling environments. In addition, for each of the environments positively charged culturable bacterial aerosols collected were found to have more band pattern similarity with those positively charged for respective regions of agar plates than those negatively charged, and *vice versa*. The information developed here is useful for developing efficient electrostatic sampling protocols for bioaerosols.

positive charge, negative charge, bacterial aerosol, culturable diversity, PCR, DGGE, gel pattern similarity

Citation: Shen F X, Kai W, Yao M S. Negatively and positively charged bacterial aerosol concentration and diversity in natural environments. Chin Sci Bull, 2013, 58: 3169–3176, doi: 10.1007/s11434-013-5852-9

It is well accepted that exposure to biological aerosols can cause numerous adverse health effects [1–3]. Accordingly, a variety of sampling techniques including filtration, liquid impingement, and impaction have been investigated in monitoring bioaerosols [4–8]. Recently, electrostatic sampling techniques for bioaerosols have also increasingly been studied [9–18]. Previous studies have shown that the electrostatic sampling performed better than the BioStage impactor [15] and the BioSampler [12] when sampling airborne culturable bacteria and fungi, endotoxin and allergens, respectively, under certain conditions. Due to its lower deposition velocity and less desiccation, the electrostatic sampling has been shown to be a less stress causing collection method for culturable bioaerosols [15–18].

It has been shown that airborne biological agents carry certain amount of electrical charges in both charge polarities [10,18,19]. The electrostatic sampling technique is a collection method which utilizes the electrostatic field to collect the charged bioaerosols. During the collection, the charged airborne bio-particles are precipitated by the electrical force, thus getting deposited onto a collection medium. Therefore, its collection efficiency greatly depends on the bioaerosol charge level and polarity. When the electrostatic sampling is applied, the amount and polarity of aerosol charges and electrical mobility together determine the physical collection efficiency of the particles. Likewise, electrical charges

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on the particles also play a role in their physical depositions in the lung [20−23]. Additionally, the charge levels and polarity of the bioaerosols can greatly impact their removal efficiencies and detection by electrostatic means [24−28]. While the level of charges results in different amount of electrostatic force imparted on the aerosols, the charge polarity determines the direction of the electrical force. In a previous study, four square agar plates were placed on two sides of an electrical sampler for simultaneous collection of airborne bacteria with both positive and negative charges [15]. The charge polarity is a critical factor to ensure the bioaerosols with either charge polarity to be collected onto the agar medium. In natural environments, environmental bioaerosols are to carry various levels of electrical charges with different charge polarity. Many studies have been conducted to investigate microbial aerosol diversity both in indoor and outdoor environments [29−32]. However, information about the diversity of those bacterial aerosols with either negative or positive charges in various environments is not available despite of their importance in both electrostatic sampling and many health-related issues as discussed above.

To fill this literature gap, this study was designed to investigate the culturable bacterial aerosol diversity and concentration for those both positively charged and negatively charged in office, hotel and outdoor environments using electrostatic means and PCR-DGGE analysis. The bacterial aerosols with different charge polarity (negative and positive) were collected using an electrostatic sampler, and further cultured directly on square agar plates at room temperature. The CFUs grown were subjected to PCR-DGGE analysis for culturable bacterial aerosol diversity. The information developed here is useful for developing appropriate electrostatic sampling protocol as well as assessing human biological inhalation exposure.

1 Materials and methods

1.1 Electrostatic sampler used

In this study, the electrostatic sampler designed in a previous study [12] was used to sample the environmental bacterial aerosols with both positive and negative charges. The sampler is composed of a plastic body, two copper sheets measured as 8.5 by 25.5 cm, an air inlet, and an air outlet. The copper sheets spaced at 8 cm are connected to a high voltage supply (model 205B-15R from Bertan Associate, Inc., Valhalla, NY) which can provide a voltage up to 50 kV. The electrostatic sampler was designed to accommodate two square agar plates (96-well plate size) as illustrated in Figure 1.

1.2 Bioaerosol sampling and concentration

In this study, the electrostatic sampler was operated at a flow rate of 3 L min^{-1} with an electrostatic field strength of 1.1 kV cm^{-1} obtained by applying 8 kV to the sampler, and two square agar plates were placed inside the sampler for the sampling. Here, the electrostatic field of 1.1 kV cm^{-1} was the highest one that can be achieved in the sampling environments without the occurrence of air dielectric breakdown. Under the tested conditions (1.1 and 3 L min^{-1} sampling flow rate), our sampler should have more than 40% collection efficiency for the particles of 0.3 μm and bigger [10], and complete collection efficiency profile can be obtained from Xie et al. [10]. It can be inferred from a previous study [25] that the tested electrostatic field strength and conditions caused little damage to the bacteria including airborne and agar-surface borne.

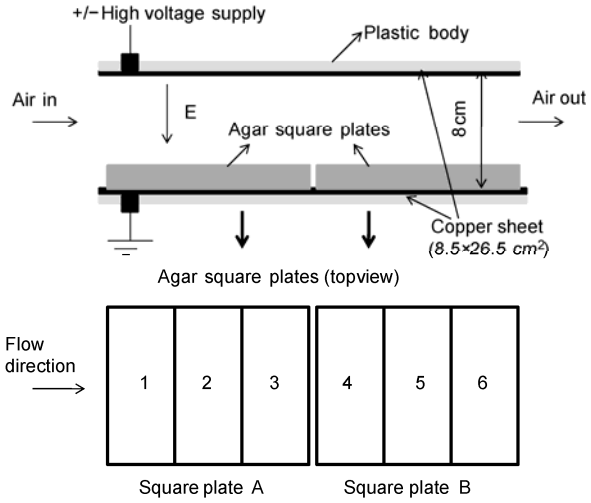

Figure 1 Two square agar plates placed inside the electrostatic sampler for environmental bacterial sampling. 1−6 represent different regions divided for the two plates; E stands for the electrostatic field inside the sampler.

The air samples were collected for 40 min in an office environment, an outdoor environment (outside of 2 story building), and a hotel room within Peking University located at northwest 4th Ring of Beijing during the winter of 2010. The hotel building has a central air conditioner and the office environment does not have a ventilation system. Only one occupant was present during the sampling. The connection cable of the high voltage supply was switched to collect either positively charged or negatively charged bacterial aerosols. For positively and negatively charged bacterial aerosols in each of three different environments studied, three independent air samples were collected. The air samples were taken alternately for positively and negatively charged bacterial aerosols with approximately 1 h apart on the same day. Based on the experimental parameters used, for bacterial aerosols of 1−3 μm flowing through the sampler from the top edge, 10−35 elementary units of charges estimated by an Aerosol Calculator developed by Paul Baron are generally required to allow aerosols to be collected, and those with fewer or no charges will exit the sampler. Therefore, mixed cellulose ester (MCE) filters of 25 mm in diameter with a pore size of 0.45 μm (SKC, Inc., Eighty

Four, PA) were also placed at the downstream of the electrostatic sampler for sampling bacterial aerosols with fewer or no charges in the hotel room environment.

The collected bacterial aerosols were cultured directly on Trypticase Soy Agar (TSA) (Becton, Dickson and Company, Sparks, MD) plates at 26°C for 3 d. Each of square agar plates were prepared using 70 mL agar suspension obtained by dissolving 10 g Tryptone, 10 g Soytone, 5 g Sodium Chloride, 15 g Agar powder into 1 liter of deionized water (Millipore). In this study, the two square agar plates were divided into six equal regions shown in Figure 1. The division of the two square agar plates would allow us to investigate the distribution of culturable bacterial aerosols with different levels of electrostatic charges of either positive or negative polarity. The CFUs were manually counted for each of the regions (numbers 1−6) divided for the two square agar plates as depicted in Figure 1, and the culturable bacterial aerosol concentrations were calculated for each charge polarity for different regions. The total culturable bacterial aerosol concentrations were also calculated using those from six regions of the two plates used. The humidity level was observed approximately 40% for all environments, and temperature was measured about 17°C for the outdoor environment, and 15°C for office and hotel environments in which the samplings were conducted.

Based on the dimension of the electrostatic sampler and the sampling flow rate, the face velocity for the sampler was about 0.78 cm s^{-1}. This resulted in a maximum residence time of 33 s for the bacterial aerosols passing through the sampler. However, the settling velocity by gravity would be less than 0.03 cm s^{-1} for particles of 3 μm or smaller, which however requires 4.4 min to allow the aerosols to land onto the agar plates. Therefore, the gravity itself played a minor role in bacterial aerosol collection in this study. Due to bacterial aerosol fluctuations in natural environments, it is difficult to estimate the overall physical collection efficiency for them.

1.3 Diversity of positively and negatively charged culturable bacterial aerosols

(i) DNA sample preparation. For bacterial aerosol diversity study, the CFUs from different regions (numbers 1−6 shown in Figure 1) of the two square agar plates for the air samples cultured were washed off using DI water to a bacterial suspension of 4−5 mL. In this study, pooled CFUs from all regions for both positively charged and negatively charged bacterial aerosols in the environments studied were also prepared. In addition, total air samples were also prepared for all environments by mixing those positively and negatively charged CFUs for overall diversity analysis.

(ii) PCR amplification. For various microbial suspensions prepared, 1 mL of the bacterial suspension after centrifugation at 10000 r min^{-1} for 1 min (Centrifuge 5804R, Eppendorf) was used for DNA extraction by a bacteria DNA extraction kit (TIANamp Bacteria DNA Kit, Tiangen, Beijing) following the manufacturer's instruction. The DNA extraction kit utilizes silica membrane technology and special buffer system with lysozyme for a wide range of both Gram-negative and Gram-positive bacteria (Tiangen). The extracted DNA samples were further resuspended into 100 μL TE buffer (Tiangen). The V3 region of the 16S rRNA gene was amplified using primers P2 (5′ATTACCGCGGCTGCTGG-3′) and P3 (5′-GCclampCCTACGGGAGGCAGCAG-3′) as described by Muyzer et al. [33]. PCR reaction mixture (total volume was 50 μL) included 5 μL DNA template, 2 μL primer P2 (10 μmol L^{-1}), 2 μL primer P3 (10 μmol L^{-1}), 25 μL 2×Master Mix (10×*Taq* Buffer, dNTP Mixture, *Taq* (2.5 U μL^{-1})) (Tiangen) and 16 μL dd H_2O. The cycle conditions were: 94°C for 3 min, 30 cycles of (94°C for 30 s, 55°C for 30 s and 72°C for 1 min), and 72°C for 10 min. DI water was used as the negative control in the PCR experiments. For comparison of indoor and outdoor culturable bacterial aerosol diversity, smaller amount of DNA template (2 μL) was also tested for the PCR amplification.

1.4 Bacterial aerosol diversity analysis by DGGE

In this study, the DGGE analysis of PCR amplicons from the aerosol samples was performed using a Bio-Rad Dcode TM mutation detection system (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. DGGE analysis is a simple, but with high mutation detection rate for those DNA segments of <500 bp, especially after adding GC-clamp to the primers. In details, approximately 20 μL PCR product was transferred to each well of 8% polyacrylamide (acrylamide/bisacrylamide 35.7:0.8) gels containing a linear 30%−65% gradient of urea and formamide increasing in the direction of electrophoresis. The electrophoresis was conducted in 1× TAE buffer for 540 min at a constant voltage of 100 V at 60°C. DNA bands in gels were stained by GelRed solution (10000×diluted with DI water) (Biotium, Hayward, CA) and photographed (Molecular Imager Gel DocTM XR system, Bio-Rad) under ultraviolet lamp at the wavelength of 254 nm. Data were recorded and analyzed with Quantity One software. The DGGE bands for different bacterial aerosol samples (bacterial suspensions) were further compared using similarity dendrograms obtained using the BioRad built-in software functions "band detection" and "unweighted pair group method with arithmetic mean (UPGMA)" clustering, and the gel band similarity of the culturable bacterial aerosol diversities was analyzed using the scale bar produced by the methods.

1.5 Statistical analysis

Relevant data were analyzed using paired *t*-test component of SigmaPlot 10 and Analysis of Variance (ANOVA) tests. Blank agar plates without sampling were taken to the sam-

pling site serve as negative controls. A *P*-value of less than 0.05 indicated a statistically significant difference.

2 Results and discussion

In general, the culturable bacterial aerosol concentrations with positive and negative charges were found to strongly depend on the sampling environments. Figure 2 shows office positively and negatively charged culturable bacterial aerosol concentrations obtained at different regions of two square agar plates shown in Figure 1. As observed from the figure, the concentrations in general decreased from the sampling inlet to the outlet for both polarities. For positively charged culturable bacterial aerosols, no statistically significant difference was detected for different regions of two square agar plates (P = 0.266), and similar finding was observed for negatively charged culturable bacterial aerosols (P = 0.281) due to high variations observed for different repeats. Total positively charged culturable bacterial aerosol concentration was measured as 594 CFU m^{-3}, and that for negatively charged was 875 CFU m^{-3} but with a slightly higher variation. Paired *t*-test showed that there was a statistically significant difference between the positively and negatively charged culturable bacterial aerosol concentration (P = 0.0489). In this study, the samplings for negatively and positively charged bacterial aerosols were not performed in parallel, thus environmental variations with respect of time in bacterial aerosol concentrations should be taken into account for the actual comparison here. A study suggested that the atmosphere is mainly colonised transiently by microorganisms from local sources, depending on air fluxes [34]. However, another study indicated that the airborne bacterial population variation is small in an outdoor environment [35]. For hotel room environment, negatively charged culturable bacterial aerosol concentration was also observed higher than those of positively charged as shown in Figure S1 (Supporting Information), but not statistically significant (P = 0.88).

For outdoor culturable bacterial aerosol samples, the situation was slightly different as observed in Figure 3. In general, there were statistically significant concentration differences detected between different regions of two square agar plates for both positively charged (P = 0.005) and negatively charged (P = 0.004). The concentration difference observed between the office and outdoor samplings for positively and negatively charged culturable bacterial aerosols obtained for different regions of the two plates might be attributed to the difference between the charge distributions in these two different environments. The total positively and negatively charged culturable bacterial aerosol concentrations in the outdoor environment were observed higher than those respective ones in office and hotel environments. The total positively charged culturable bacterial aerosol concentration in the outdoor environment was measured as 1692 CFU m^{-3} and that for negatively charged was 1331 CFU m^{-3}. Different from the office environment, paired *t*-test showed that no statistically significant difference was observed between two polarity (P = 0.078), although positively charged was shown to slightly dominate. The results from Figures 2, 3, and S1 implied that the bioaerosol concentrations varied with the sampling environment. In a previous study, it was shown that the sampling seasons also play a role in overall bioaerosol concentrations [31]. In this study, the air samples were collected during a winter time in Beijing. Accordingly, the season parameter should be considered when the results here are referenced or discussed with.

Our data revealed remarkable differences in positively and negatively charged culturable bacterial aerosol concentration and diversity among the sampling environments. Figure 4

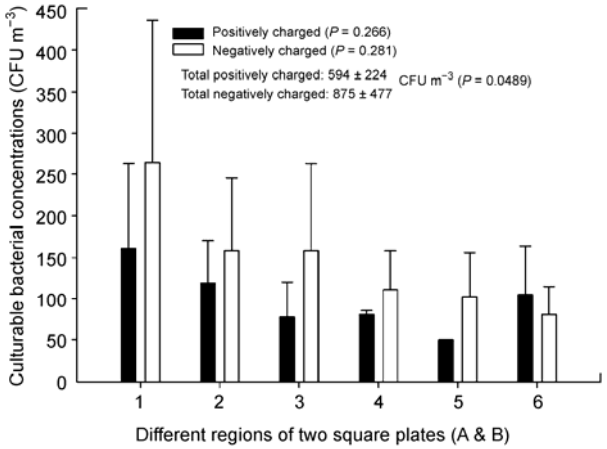

Figure 2 Office positively and negatively charged culturable bacterial aerosol concentrations measured at different regions of two square agar plates shown in Figure 1. Data points represent averages of three independent measurements and errors stand for the standard deviation from three repeats.

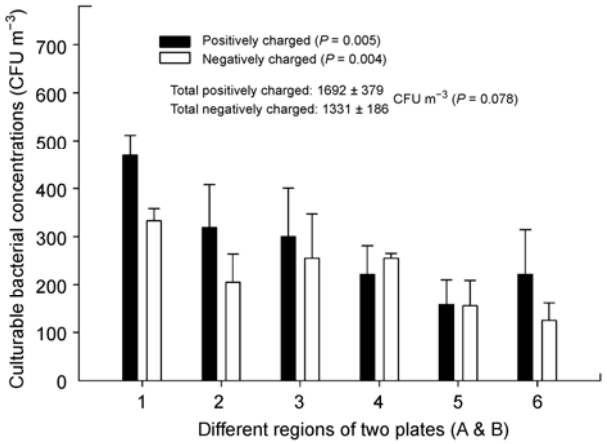

Figure 3 Outdoor positively and negatively charged culturable bacterial aerosol concentrations measured at different regions of two agar square plates shown in Figure 1. Data points represent averages of three independent measurements and errors stand for the standard deviation from three repeats.

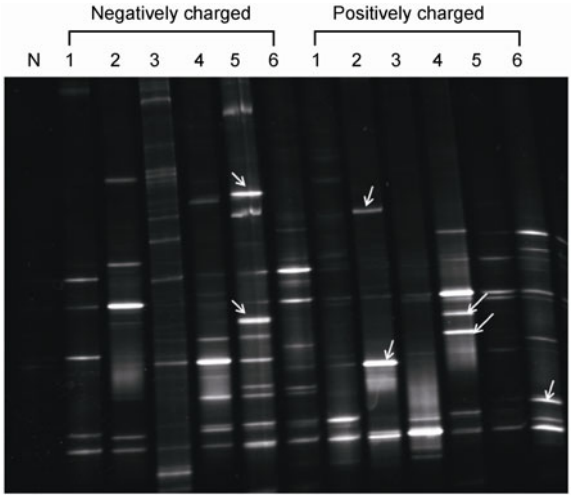

Figure 4 Office positively and negatively charged culturable bacterial aerosol diversity measured for different regions (numbers 1–6) of two agar square plates shown in Figure 1. N represents the negative control; gel bands obtained from pooled samples from three independent samplings.

shows office positively and negatively charged culturable bacterial aerosol diversity measured using PCR-DGGE method for different regions (numbers 1–6) of two square agar plates shown in Figure 1. As observed from Figure 4, more diversity (more bands) was observed for office negatively charged culturable bacterial aerosols than those for office positively charged. For negative charged aerosols, more diversity for pooled samples from three repeats was observed in the numbers 4, 5 regions of two square agar plates. There were some gel bands (marked by arrows in the left) that were found in certain regions, but absent from other regions for the negatively charged DGGE profile. Similar findings were observed for the positively charged office aerosols as marked by the arrows in the right in the figure. These findings suggest that different species might carry different levels of natural charges under atmospheric conditions. In our previous study, we have found that most of aerosolized *B. subtilis* var niger and *P. fluorescens* were collected into numbers 2, 3, 4 regions of the two square agar plates [10]. Previous studies indicated that for aerosolized bacterial species, the charge consists two components: natural charge and the charge imparted by mechanical dispersion [18]. For naturally occurring bacterial aerosols, they mainly carry their natural charges. When comparing the diversity obtained for the negatively charged and positively charged culturable bacterial aerosols for a given sampling region, different gel patterns were observed. For most gel bands, if they were found from a given region in negatively charged gel profile, they were absent from the same region in the positively charged gel profile, but could be found in other regions; and *vice versa*. This suggests that for a particular species, the natural positive and negative charge levels could be also different for a given species under atmospheric conditions. The similarity dendrogram of office positively and negatively charged culturable bacterial aerosol diversity was shown in Figure 5. As observed from the figure, most similarity ranged from 40% to 60%. In general, the diversity of negatively charged culturable bacterial aerosols was shown to have more band similarity with those with the same polarity aerosols regardless of the sampling environments. In our second set of experiments in an office environment, negatively charged culturable bacterial aerosols were also observed to dominate in the air samples collected (Figure S2).

Figures 6 and S3 (second set of experiments) show the outdoor negatively and positively charged culturable bacterial aerosol diversity measured for different regions (numbers 1–6) of the two square agar plates shown in Figure 1. In contrast, more culturable diversity was observed for the positively charged culturable bacterial aerosols than those negatively charged in outdoor environment. Compared to office culturable bacterial aerosol diversity, gel bands for outdoor air samples were more uniform in the DGGE profile, i.e., most regions share similar bands for both polarities. In addition, outdoor culturable bacterial aerosols were

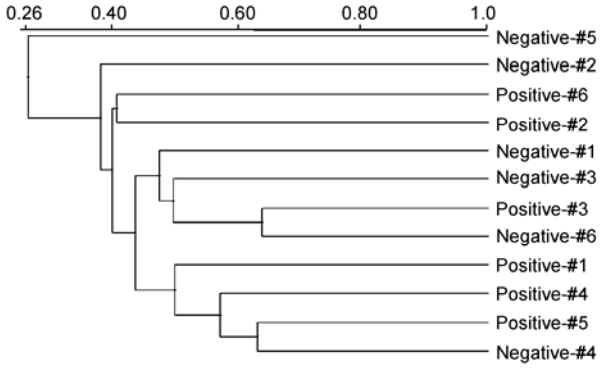

Figure 5 Similarity dendrogram of office positively and negatively charged bacterial aerosol diversity. Negative and Positive represent negatively charged and positively charged, respectively. Numbers 1–6 represent different regions grouped for the two plates shown in Figure 1.

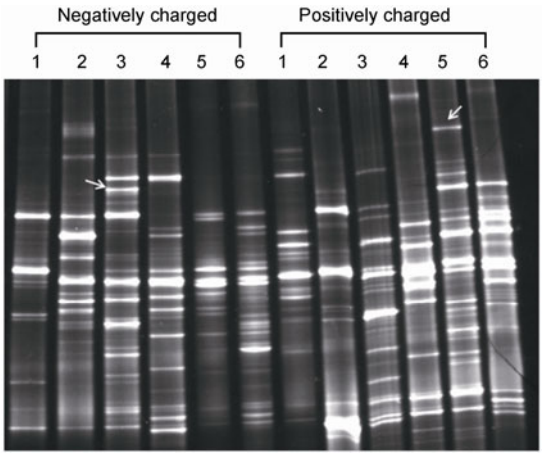

Figure 6 Outdoor negatively and positively charged culturable bacterial aerosol diversity measured for different regions (numbers 1–6) of two agar square plates shown in Figure 1. Gel bands obtained from pooled samples from three independent samplings.

shown to have more diversity than those indoors. Figure 7 shows the similarity dendrogram of outdoor positively and negatively charged culturable bacterial aerosol diversity. Similar to office culturable bacterial aerosol diversity, positively charged aerosols appeared to have higher band similarity with those also positively charged, and negatively charged had higher band similarity with those negatively charged.

Here, diversity differences were also observed for total negatively and positively charges bacterial aerosols in different environments. Figure 8 shows the office and outdoor culturable bacterial aerosol diversity for total positively charged, total negatively charged, and those of both polarities. As observed for the figure, more diversity (more bands) was observed for total outdoor culturable bacterial aerosols than that of the office culturable bacterial aerosols. Cluster analysis indicated that the band similarity between the indoor and outdoor is low, about 50% as shown in Figure 9. Total positively charged culturable bacterial aerosols appeared to have more species richness than those negatively charged in the outdoor environment. Use of different template DNA amount in this study also resulted in the same gel patterns as observed in Figure 8. Similar to the office experiments, cluster analysis showed that total positively charged culturable bacterial aerosols had more similar band patterns with those positively charged, and vice versa. Figures 10 and S4 show the hotel room culturable bacterial aerosol diversity in those samples collected by the electrostatic sampler and those by the MCE filter at the downstream. Similar to the office environment, hotel room negatively charged culturable bacterial aerosols appeared to have more species richness than those positively charged ones. As shown in Figure 10, compared to those collected by the electrostatic sampler, those collected by the MCE filter appeared to have less species richness. Those culturable bacterial aerosols collected by the MCE filter at the downstream of the electrostatic sampler carried fewer or no electrical charges. From the results shown in Figure 10, it seems that the electrostatic sampler collected most bacterial aerosol species.

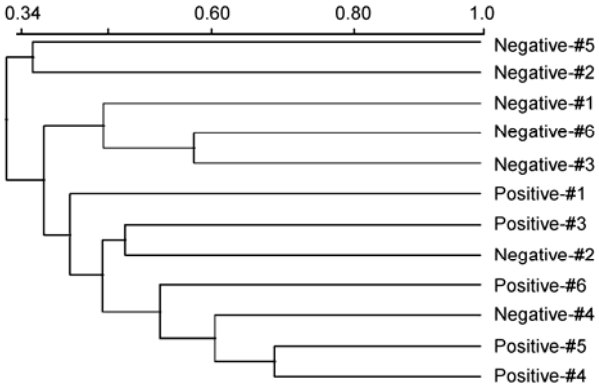

Figure 7 Similarity dendrogram of outdoor positively and negatively charged bacterial aerosol diversity. Negative and Positive represent negatively charged and positively charged, respectively. Numbers 1–6 represent different regions grouped for the two plates shown in Figure 1.

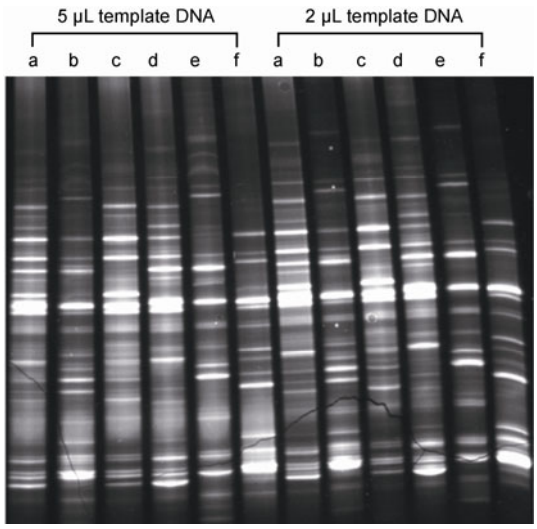

Figure 8 Office and outdoor culturable bacterial aerosol diversity for total positively charged, total negatively charged, and both polarity. a, Outdoor bacterial aerosol (both polarity); b, indoor bacterial aerosol (both polarity); c, outdoor negatively charged bacterial aerosol; d, outdoor positively charged bacterial aerosols; e, indoor negatively charged bacterial aerosol; and f, indoor positively charged bacterial aerosol; gel bands obtained from pooled samples from three independent samplings.

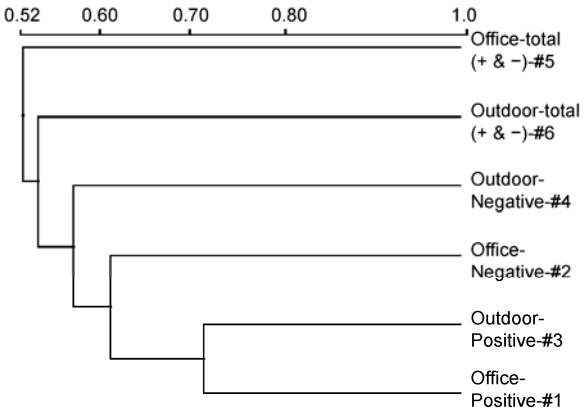

Figure 9 Similarity dendrogram of office and outdoor positively and negatively charged bacterial aerosol diversity for the DGGE profiles show in Figure 8 (5 μL template DNA). Negative and Positive represent negatively charged and positively charged, respectively; "total" includes both negatively and positively charged.

The observed findings were likely due to a result of various types of bacterial aerosols present in different environments. The cell membrane consisting of a lipid bilayer with many proteins is involved in regulating its metabolic activities, where the lipid molecules are oriented with their polar groups [28,36]. There are two major groups of bacterial membrane types: Gram-positive and Gram-negative. For Gram-positive bacteria, they have very different membrane surfaces, which are more hardy than those of Gram-negative ones. In natural environments, the bioaerosols including a diverse set of Gram-positive and Gram-negative

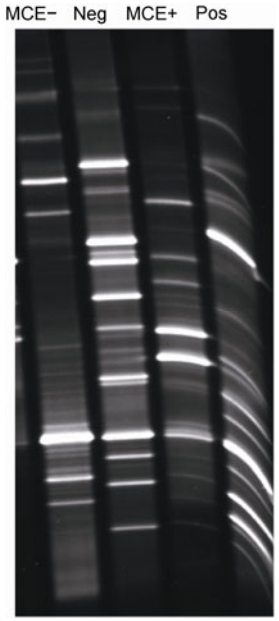

Figure 10 Hotel room positively and negatively charged culturable bacterial aerosol diversity. "MCE–" and "MCE+" stand for culturable bacterial aerosols collected at the downstream when the electrostatic sampler was used for collecting negatively and positively charged bacterial aerosols, respectively; "Neg" and "Pos" stand for the total negatively and positively charged culturable bacterial aerosols collected, respectively.

bacteria could exhibit a range of charge levels with different polarity (positive and negative). Our data might provide certain clues about species-specific charge level and polarity.

This study provided firsthand information about the concentration and diversity of both positively and negatively charged culturable bacterial aerosols in different environments. However, only culturable fractions were investigated in this study. In natural environments, those non-culturable also account for a large fraction, and the total bioaerosol diversity (dead and alive) could be different than these presented here. Besides, when aggregated with biologicals, other non-biological particles present in the sampling environments could affect the collection of the particles even for the same species into specific regions of two square plates, which in turn could influence the gel patterns. Further studies are needed to elucidate those differences between the culturable and non-culturable bioaerosols and to investigate the possible influencing factors. Using the qPCR technology, we have previously investigated the charge distributions of aerosolized *B. subtilis* var niger and *P. fluorescens* with known sizes in a similar experimental setting used here. However, in natural environments, the bioaerosols appear in various sizes, their size distribution needs to be combined with the technique developed here for determining the actual charge levels of naturally occurring ones. This type of information together with charge polarity distribution would facilitate a better understanding of biological aerosols in natural environments. Emergence of molecular methods such as PCR and DGGE greatly improves the understanding of airborne microbial community, but certainly they have their own limitations, e.g., PCR inhibition and intraspecies operon heterogeneities. Thus, the results obtained here might be negatively impacted by these limitations. Besides, additional species composition data would further improve our manuscript, however such efforts are extensive and not the main focus of this investigation. Instead, this work was primarily designed to study the differences of microbial structures in terms with sequences for those negatively and positively charged bacterial aerosols from different environments.

3 Conclusions

In this study, bacterial aerosols both positively charged and negatively charged from office, hotel and outdoor environments were collected and studied. The results have demonstrated that the positively and negatively charged culturable bacterial aerosol concentration and diversity varied with the sampling environments. More culturable bacterial aerosol diversity was observed for outdoor culturable bacterial aerosols than that in the indoor environments. Regardless of the sampling regions of agar plates and environments, positively charged culturable bacterial aerosols were shown to have more band pattern similarity with those with the same charge polarity, and *vice versa*. The information obtained here can be used to develop an appropriate electrostatic sampling protocol and to assess human biological inhalation exposure. Future work might be needed to elucidate the possible roles of gene for the distribution of bacterial aerosol charge polarity in nature environments.

This work was supported by the National Natural Science Foundation of China (21277007, 21077005 and 41121004).

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Supporting Information

Figure S1 Hotel positively and negatively charged culturable bacterial aerosol concentrations.

Figure S2 Office room positively and negatively charged culturable bacterial aerosol diversity obtained on different agar squares plates (A and B) shown in Figure 1 in 2nd set of experiments.

Figure S3 Outdoor positively and negatively charged culturable bacterial aerosol concentrations measured at different regions of two agar square plates shown in Figure 1 in 2nd set of experiments.

Figure S4 Hotel room positively and negatively charged culturable bacterial aerosol diversity obtained on different agar squares plates (A and B) shown in Figure 1.

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