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Original Article

Gelatin filter capture-based high-throughput sequencing analysis of microbial diversity in haze particulate matter

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Abstract

Airborne particulate matter (PM), especially $PM_{2.5}$, can be easily adsorbed by human respiratory system. Their roles in carrying pathogens for spreading epidemic diseases has attracted great concern. Herein, we developed a novel gelatin filter-based and culture-independent method for investigation of the microbial diversity in PM samples during a haze episode in Tianjin, China. This method involves particle capture by gelatin filters, filter dissolution for DNA extraction, and high-throughput sequencing for analysis of the microbial diversity. A total of 584 operational taxonomic units (OTUs) of bacteria and 370 OTUs of fungi at the genus level were identified during hazy days. The results showed that both bacterial and fungal diversities could be evaluated by this method. This study provides a convenient strategy for investigation of microbial biodiversity in haze, facilitating accurate evaluation of airborne epidemic diseases.

Keywords: airborne particulate matter, gelatin filter, pathogen, high-throughput sequencing

Introduction

We breathe in, more or less, airborne particulate matter (PM), *e.g.*, $PM_{2.5}$ and PM_{10}. As compared to PM_{10} that can be readily removed by mucosal clearance, $PM_{2.5}$[1–3] can be adsorbed into human pulmonary parenchyma owing to their small sizes. Moreover, the higher specific surface area of $PM_{2.5}$ facilitates their role as carrier for numerous contaminants, such as water soluble inorganic ions, heavy metals, polycyclic aromatic hydrocarbons (PAHs), pathogens and bioaerosols[4–10]. After entering the human body, these particles can release antigenic compounds, microbial toxins, and viruses into the bloodstream, leading to serious cerebrovascular and cardiovascular diseases[11–12].

There is evidence suggesting that pathogenic microorganisms bound onto the PM, such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*, are becoming a threat to public health[13]. Inhalation of these pathogens may cause allergic reactions and severe respiratory

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infections. High concentrations of these pathogens also cause skin diseases and even cancers[14]. Accordingly, their potential impacts on the environment and the human health have attracted great concerns.

Up to now, culture-based methods remain the prevalent strategy for the investigation of microbial diversity and evaluation of pathogen risks in airborne samples[15]. For example, Fang *et al* identified 789 airborne bacteria, which were distributed across 55 genera and 184 species according to a culturable method[16]. However, most of the microorganisms cannot be cultured by common growth conditions, resulting in underestimation of microbial complexity in these samples. Therefore, it is urgent to develop culture-independent techniques for the investigation of airborne microorganisms. In terms of airborne sampling methods, traditional sampling processes involve air pump-mediated compression of air, followed by impact of microbes on the surface of quartz filters[17]. However, the impact process may lead to the escape of abundant microbes from the filters, also resulting in undervaluation of microbial integrity[18].

In this paper, we reported an approach by developing a novel gelatin filter-based and culture-independent method for the investigation of the microbial diversity in PM samples. This method involves rapid gas compression by air pumps, thorough particle capture on gelatin filters, convenient dissolution of the filters for DNA extraction, and high-throughput sequencing for analysis of the microbial diversity. Hence, this study provides a new and convenient strategy for the investigation of biodiversity in haze, facilitating accurate evaluation of airborne epidemic diseases.

Materials and methods

Sample collection

The PM samples were obtained each day by MD8 Air Scan sampling device (Air Scan Sartorius AG, Gottingen, Germany) with the average flow rate of 40 L/hour, through the sterile gelatin filters (diameter of 80 mm, pore size of 3 μm, 17528-80-ACD, Sartorius) during November 30 to December 21 of 2016 in different functional regions of Wuqing District, Tianjin (***Table 1***), including: the control groups sampled in clean days (Cle1 from hospitals, and Cle2 from traffic hubs), Group 1 sampled from hospitals (Hosp1, Hosp2, and Hosp3), Group 2 sampled from traffic hubs(Trans1, Trans2, and Trans3), Group 3 sampled from schools (Sch1, Sch2, and Sch3), and traffic hubs (Tran1, Tran2, and Tran3). Initially, we also sampled the materials in clean days from the school as the third control (Cle3). Unfortunately, the sequencing of this sample was failed. Therefore, we did not include this control sample to the manuscript. All the samplers were placed 1.5 m above the ground. The variations of $PM_{2.5}$ and PM_{10} concentrations during the sampling periods were made available online (China national environmental monitoring center). The gelatin filters were stored at −20 °C prior to extraction of total DNA.

Table 1 **Sampling groups of the different functional areas**

Groups	Functional areas	Abbreviations of sampling sites
Control	Clean days of hospitals and traffic hubs	Cle1 (hospital areas), Cle2 (traffic hubs)
Group1	Hospitals	Hosp1, Hosp2, Hosp3
Group2	Traffic hubs	Trans1, Trans2, Trans3
Group3	Schools	Sch1, Sch2, Sch3

Electron microscopy

The morphology of PM samples on the gelatin membranes was characterized by a Phenom scanning electron microscope (SEM) supported with image software (SEM, ProX, Holland).

DNA extraction

The gelatin filters were dissolved in 5 mL sterile water (preheated to 37 °C). The pathogenic microorganisms captured by gelatin filter were lysed by the alkaline lysing liquid (NaOH 50 mmol/L, SDS 1%, Protease K 10 mg/L, RNase 20 mg/L), the total DNA was enriched using magnetic nanoparticles modified by polyquaternary amino salt polymers, and the total DNA was eluted by elution (Jinping Biotech, China) according to the instruction. The extracted DNA was stored at −80 °C for further use.

Amplification of 16S and 18S rDNA gene sequences

The 16S rDNA V3+V4 region was amplified using the forward primer 338F (5′-ACTCCTACGGGAGGC AGCA-3′) and the reverse primer 806R (5′-GGACTAC HVGGGTWTCTAAT-3′), the 18S rDNA ITS region was amplified using primers ITS1 (5′-CTTGGTCAT TTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTT CTTCATCGATGC-3′). The primers were synthesized by Sangon Ltd., Beijing. The PCR reaction procedure is shown in ***Supplementary Table 1***, available online.

High-throughput sequencing of 16S rDNA

The obtained total 16S rDNA and 18S rDNA were

sequenced by double end sequencing (Paired-End) method through FLASH v1.2.7 using the Illumina HiSeq 2500 sequencing platform (BioMarker Technologies Co., Ltd., Beijing, China)[19]. Bioinformatics analysis of sequences was conducted using the QIIME (V1.7.0) software package. Sequences with similarities greater than or equal to 97% were grouped into operational taxonomic units (OTUs). The Shannon index was used to estimate the biodiversity of bacteria in a single sample[20]. The principal component analysis (PCA) and the cluster heatmap analysis were also performed to assess the bacterial composition of the samples[21].

Results

Concentration and diameter of fine particles during the haze events

Fig. 1 shows the variations of $PM_{2.5}$ and PM_{10} concentrations during the sampling periods. The concentrations of $PM_{2.5}$ were higher than 200 μg/m³ in 8 days, and the concentrations of PM_{10} were higher than 200 μg/m³ in 10 days, with the highest PM_{10} concentration being almost 350 μg/m³, which far exceeded the PRC National Standard PM standards:

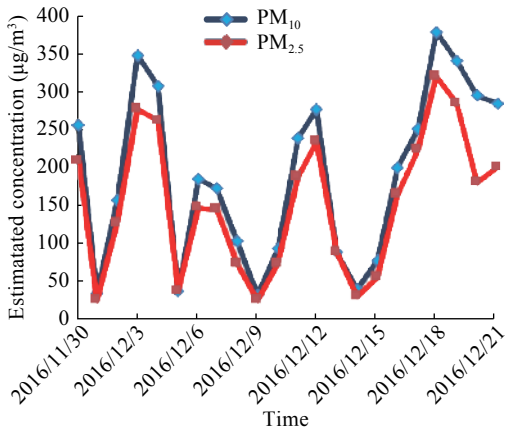

Fig. 1 **Daily average $PM_{2.5}$ and PM_{10} concentrations estimated from the samples collected from November 30 to December 21, 2016.**

50 μg/m³ for PM_{10}, and 35 μg/m³ for $PM_{2.5}$, respectively (PRC National Standard, 2012). As shown in *Fig. 2*, the statistical mean diameters of the sampled particles were in the ranges of 100–900 nm and 1.0–2.5 μm, confirming that the particles were in the scope of fine particulate matter.

Microbial species richness and diversity

Rarefaction curves derived from the observed OTU

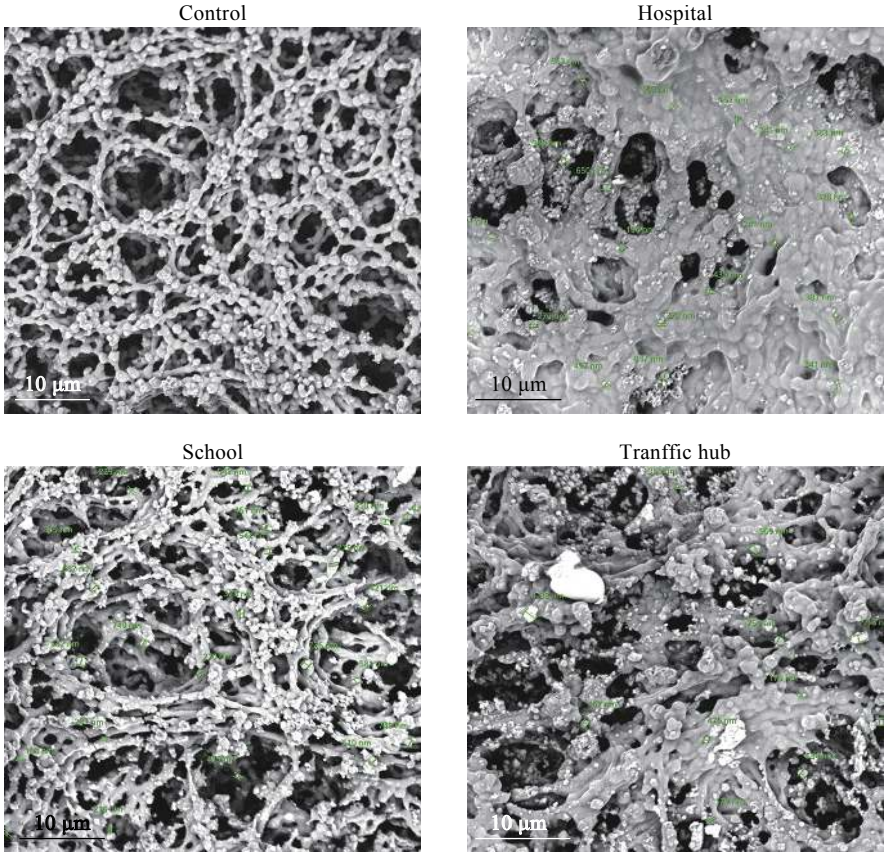

Fig. 2 **Scanning electron microscopy characterization of the gelatin filter after PM sampling in different functional areas and the control group.** The green marks indicated the diameter of fine particles.

number and shannon index were flattened (***Fig. 3***), showing that our sequencing depth was sufficient to cover the vast majority of bacteria and fungi in the samples. The statistics of OTU species in different ranks of the bacteria and the fungi are shown in ***Table 2*** and ***3***, and a total of 584 OTUs of bacteria and 370 OTUs of fungi at the genus level were identified during hazy days.

As shown in ***Fig. 4***, in all samples, *Proteobacteria* was the most abundant phylum, and four other dominant phyla were *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Cyanobacteria* (***Fig. 4A***). Compared to the samples from the clear days, the samples from hospitals and traffic hubs during hazy days contain much higher levels of human health-related bacteria, such as *Acinetobacter*, *Staphylococcus*, *Corynebacterium*, *Lactobacillus*, and *Duganella* (***Fig. 4B***). Especially, *Staphylococcus* and *Corynebacterium* were the predominant pathogens around the high-density-population functional areas, such as hospital and transportation areas. For example, *Staphylococcus* on PM collected around the hospital areas was the dominant bacteria (18% in Hosp2 samples), which may be derived from the high density of patients and health care personnel[22]. Nevertheless, the average abundance of *Staphylococcus* in Sch2 was only 1.2% because of the regular population. The dominant bacteria were *Corynebacterium* (34.3%) in Tran2 and *Lachnospiraceae_Nk4A136* (10.5%) in Tran3. In Sch3 areas, the dominant bacteria were *Lactobacillus* (13.5%) and *Klebsiella* (11.8%). Interestingly, the genus *Sphingomonas* is detected on both the clear days and hazy days, which are the environment-associated bacteria widely found in surface water, the rhizosphere, sediments, and even soils. ***Fig. 4C*** shows that the dominant fungi were *Malassezia* (58.0%) in Hosp1 and (54.9%) in Hosp2, *Alternaria* (55.5%) and *Cladosporium* (9.17%) in Tran3. The genus *Malassezia* has been associated with a number of diseases affecting the human skin, such as pityriasis versicolor, *Malassezia* (Pityrosporum) folliculitis, seborrheic dermatitis and dandruff, atopic dermatitis, and psoriasis. *Malassezia* yeasts are a part of the normal micro-flora, but under certain conditions they can cause superficial skin infection[23]. *Cladosporium*, *Aspergillus* and *Alternaria* could enter the deep lung and cause respiratory diseases[24].

The variation of bacteria in collected samples were analyzed by two methods: the PCA analysis and the clustering heatmap analysis. PCA analysis showed that the high-population-density functional areas (Hosp1, Hosp2, Tran2, and Tran3 but not Tran1) had

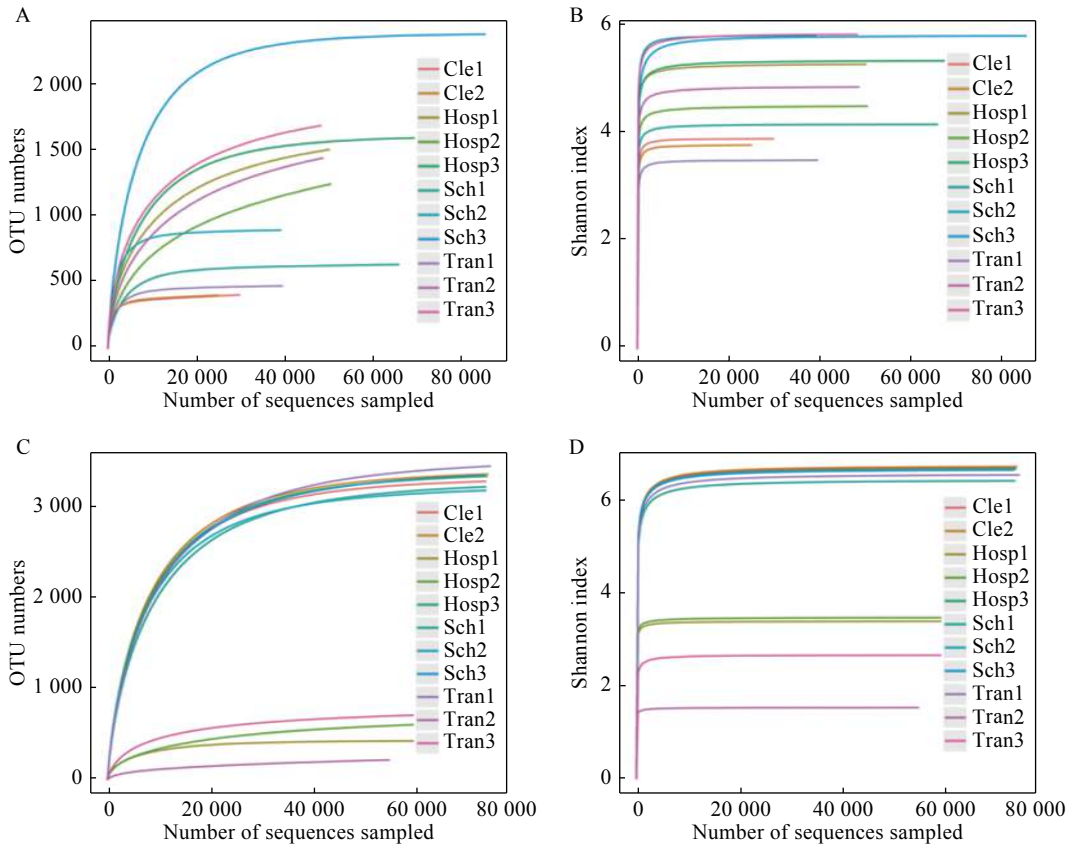

Fig. 3 **Rarefaction curves and Shannon index derived from the observed OTU numbers.** A and B: bacteria; C and D: fungi.

Table 2 **Statistics of OTU species of bacteria in different ranks**

Sample	Kindom	Phylum	Class	Order	Family	Genus	Species
Cle1	1	20	52	83	142	205	143
Cle2	2	20	50	79	135	220	158
Hosp1	2	31	74	120	226	524	362
Hosp2	1	26	55	100	203	507	316
Hosp3	1	31	79	125	243	487	328
Sch1	1	23	52	88	157	261	175
Sch2	1	27	70	114	187	340	240
Sch3	1	39	105	170	318	584	420
Tran1	1	22	50	80	134	221	151
Tran2	1	22	58	112	221	521	336
Tran3	1	26	71	128	253	582	396

OUTs: operational taxonomic units.

Table 3 **Statistics of OTU species of fungi in different ranks**

Sample	Kindom	Phylum	Class	Order	Family	Genus	Species
Cle1	4	18	39	87	209	348	335
Cle2	4	18	41	88	210	339	361
Hosp1	3	8	22	44	93	131	152
Hosp2	3	9	127	63	134	192	254
Hosp3	4	16	41	83	197	334	319
Sch1	4	17	39	81	198	330	313
Sch2	4	16	39	86	197	331	325
Sch3	4	16	39	79	197	345	377
Tran1	4	16	40	87	213	370	373
Tran2	1	6	19	34	58	85	81
Tran3	3	10	28	64	146	223	259

OUTs: operational taxonomic units.

a similar bacterial diversity (***Fig. 4D***). However, the samples from Trans1 and Sch1 displayed little similarity in the bacterial composition. The clustering heat map analysis also demonstrated the similarity of the bacterial composition between the hospital areas and the transportation areas (***Fig. 5***). One cluster was composed of the hospital areas and the transportation areas; another cluster was composed of the samples from control and the school areas (samples Cle1, Cle2, Sch1, and Sch3). The bacterial diversity around school areas (Sch1 and Sch2) is similar to the control group, but not always the case: the sample Sch3 showed partially similar bacterial diversity to the samples from the hubs and hospitals, which may be attributed to transportation of PM from hubs and hospitals.

Discussion

Numerous epidemiologic studies have documented that PM is associated with inflammation-related diseases. For example, PM may result in the alteration of immune functions, such as IgA, IgG, IgM, IgE and lymphocyte profiles ($CD4^+$ T cells, $CD8^+$ T cells, $CD4^+/CD8^+$ T cells) in blood[25], which might link to various adverse health effects, including asthma, pulmonary infectious diseases, diabetes mellitus and obstructive bronchitis[26]. In China, numerous studies have been conducted to monitor regional air pollution[27], and the exposure-response relationships between airborne PM and cardiovascular diseases. All these studies have focused on the particle

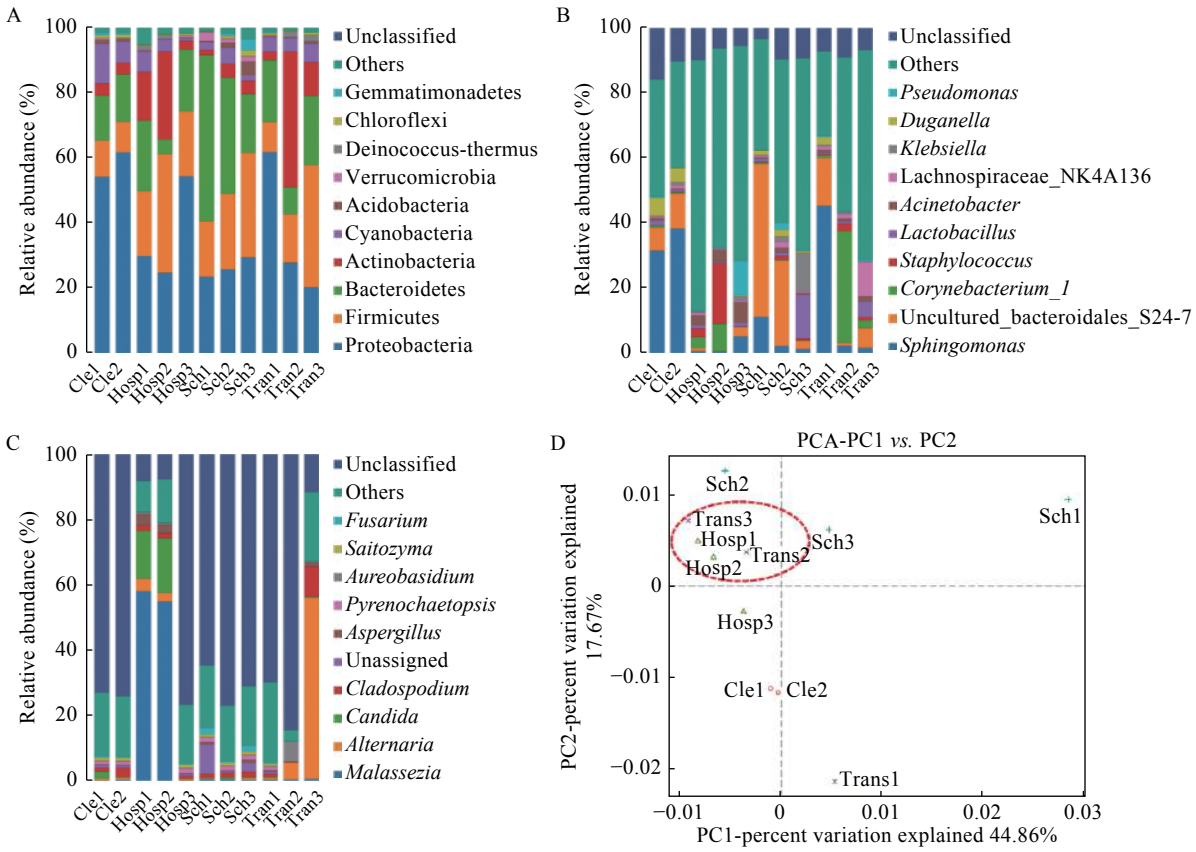

Fig. 4 **Microbial species richness and diversity.** A and B: the bacterial richness and diversity of the 16S rDNA samples at the phylum level and the genus level. C: The fungal richness and diversity at the genus level. Only nodes with the first-tenth relative abundance are labeled. D: The PCA analysis of different functional areas at the genus level.

concentration effects, the airborne gas and other chemical substances[28]. However, little information has been available on the association of particle-bound pathogenic bacteria with the prevalence of infectious diseases and epidemics. In this paper, we found that the amounts of *Staphylococcus* and *Corynebacterium* are high on the PM collected around hospital areas and traffic hubs. *Staphylococcus* may cause both endemics and epidemic infections, such as neumonia, pseudomembranous colitis, pericarditis, and sepsis, especially outbreak of severe acute pneumonia[29]. *Corynebacterium* genus is also able to cause various types of healthcare-associated infections in immunocompromised hosts, such as pneumonia, pharyngitis and epidemic disease[30]. Except *Staphylococcus* and *Corynebacterium*, other prevailing pathogenic bacteria are *Acinetobacter*, *Pseudomonas*, which often cause large multifacility, nosocomial outbreaks and are frequently evolved into drug-resistant bacteria[31]. Our data indicate that densely populated traffic areas show strong evidence of bacteria and the presence of some potentially pathogenic organisms. In addition, $PM_{2.5}$ exposure may alter and impair the normal immune responses of the lung, and decrease the phagocytosis of alveolar macrophages through disrupting the normal physical and immunological function of the lung, induce disorder of inflammatory cytokine[32]. Plus these more active pathogens, all of these effects would lead to the decline in immunity and facilitate infectious diseases.

In conclusions, the pathogenic microorganisms in PM are closely related to their surrounding environmental conditions and population density,

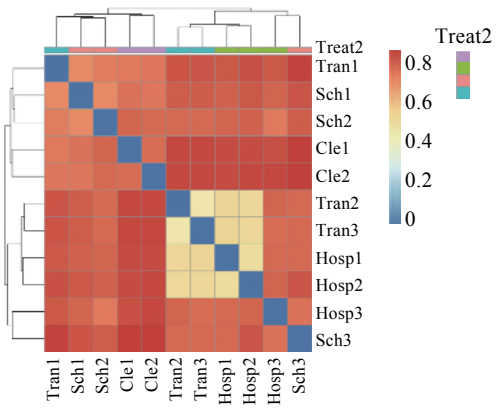

Fig. 5 **The cluster heatmap analysis of different functional areas.**

mobility and activities. In this study, we developed a convenient culture-independent method for the investigation of the microbial diversity in PM samples, which is composed of gas compression, particle capture, DNA extraction, and high-throughput sequencing. The results showed that both bacterial and fungal diversities could be accurately evaluated by this method. The results demonstrated the presence of some pathogenic bacteria, such as *Staphylococcus*, *Corynebacterium*, *Acinetobacter* and *Pseudomonas*, which may affect immunocompromised populations (*e.g.*, the elderly, children and postoperative convalescence patients). This study provides a new strategy for the evaluation and surveillance of airborne epidemic diseases that increasingly threaten urban population.

Acknowledgments

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References

[1] Feng SL, Gao D, Liao F, et al. The health effects of ambient $PM_{2.5}$ and potential mechanisms[J]. *Ecotoxicol Environ Saf,* 2016, 128: 67–74.

[2] Wang FW, Lin T, Li YY, et al. Comparison of $PM_{2.5}$ carbonaceous pollutants between an urban site in Shanghai and a background site in a coastal east China sea island in summer: concentration, composition and sources[J]. *Environ Sci Processes Impacts,* 2017, 19(6): 833–842.

[3] Cao SJ, Kong XR, Li LY, et al. An investigation of the $PM_{2.5}$ and NO_2 concentrations and their human health impacts in the metro subway system of Suzhou, China[J]. *Environ Sci Processes Impacts,* 2017, 19(5): 666–675.

[4] Fang CS, Zhang ZD, Jin MY, et al. Pollution characteristics of $PM_{2.5}$ aerosol during haze periods in Changchun, China[J]. *Aerosol Air Qual Res,* 2017, 17(4): 888–895.

[5] Gao Y, Guo XY, Ji HB, et al. Potential threat of heavy metals and PAHs in $PM_{2.5}$ in different urban functional areas of Beijing[J]. *Atmos Res,* 2016, 178-179: 6–16.

[6] Yeh CF, Lee CL, Brimblecombe P. Effects of seasonality and transport route on chemical characteristics of $PM_{2.5\text{-}10}$ in the East Asian Pacific Rim region[J]. *Aerosol Air Qual Res,* 2017, 17(12): 2988–3005.

[7] Mohammed MOA, Song WW, Ma YL, et al. Distribution patterns, infiltration and health risk assessment of $PM_{2.5}$-bound PAHs in indoor and outdoor air in cold zone[J]. *Chemosphere,* 2016, 155: 70–85.

[8] Cheng Z, Chen LJ, Li HH, et al. Characteristics and health risk assessment of heavy metals exposure via household dust from urban area in Chengdu, China[J]. *Sci Total Environ,* 2018, 619-620: 621–629.

[9] Cao C, Jiang WJ, Wang BY, et al. Inhalable microorganisms in Beijing's $PM_{2.5}$ and PM_{10} pollutants during a severe smog event[J]. *Environ Sci Technol,* 2014, 48(3): 1499–1507.

[10] Wang YC, Fu Y, Wang C, et al. Dissimilar emission characteristics between bioaerosol and suspended particles from gaseous biofilters and bioaerosol health risk evaluation[J]. *Aerosol Air Qual Res,* 2018, 18(7): 1874–1885.

[11] Zeller M, Giroud M, Royer C, et al. Air pollution and cardiovascular and cerebrovascular disease: epidemiologic data[J]. *Presse Med,* 2006, 35: 1517–1522.

[12] Leiva GMA, Santibañez DA, Ibarra ES, et al. A five-year study of particulate matter ($PM_{2.5}$) and cerebrovascular diseases[J]. *Environ Pollut,* 2013, 181: 1–6.

[13] Yoo K, Lee TK, Choi EJ, et al. Molecular approaches for the detection and monitoring of microbial communities in bioaerosols: a review[J]. *J Environ Sci,* 2017, 51: 234–247.

[14] Douwes J, Thorne P, Pearce N, et al. Bioaerosol health effects and exposure assessment: progress and prospects[J]. *Ann Occup Hyg,* 2003, 47(3): 187–200.

[15] Li YP, Fu HL, Wang W, et al. Characteristics of bacterial and fungal aerosols during the autumn haze days in Xi'an, China[J]. *Atmos Environ,* 2015, 122: 439–447.

[16] Fang ZG, Yao WC, Lou XQ, et al. Profile and characteristics of culturable airborne bacteria in Hangzhou, southeast of China[J]. *Aerosol Air Qual Res,* 2016, 16(7): 1690–1700.

[17] Brodie EL, DeSantis TZ, Parker JPM, et al. Urban aerosols harbor diverse and dynamic bacterial populations[J]. *Proc Natl Acad Sci USA,* 2007, 104(1): 299–304.

[18] Azhar EI, Hashem AM, El-Kafrawy SA, et al. Detection of the middle east respiratory syndrome coronavirus genome in an air sample originating from a camel barn owned by an infected patient[J]. *mBio,* 2014, 5(4): e01450–14.

[19] Magoč T, Salzberg SL. Flash: fast length adjustment of short reads to improve genome assemblies[J]. *Bioinformatics,* 2011, 27(21): 2957–2963.

[20] Grice EA, Kong HH, Conlan S, et al. Topographical and temporal diversity of the human skin microbiome[J]. *Science,* 2009, 324(5931): 1190–1192.

[21] Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data[J]. *Nat Methods,* 2010, 7(5): 335–336.

[22] Fitzpatrick F, Roche F, Cunney R, et al. Challenges of implementing national guidelines for the control and prevention of methicillin - resistant *Staphylococcus aureus* colonization or infection in acute care hospitals in the republic of Ireland[J]. *Infect Control Hosp Epidemiol,* 2009, 30(3): 277–281.

[23] Gupta AK, Batra R, Bluhm R, et al. Skin diseases associated with Malassezia species[J]. *J Am Acad Dermatol,* 2004, 51(5): 785–798.

[24] Lee SA, Liao CH. Size-selective assessment of agricultural workers' personal exposure to airborne fungi and fungal

fragments[J]. *Sci Total Environ,* 2014, 466-467: 725–732.

[25] Williams L, Ulrich CM, Larson T, et al. Fine particulate matter ($PM_{2.5}$) air pollution and immune status among women in the Seattle area[J]. *Arch Environ Occup Health,* 2011, 66(3): 155–165.

[26] Xie ZS, Fan CL, Lu R, et al. Characteristics of ambient bioaerosols during haze episodes in China: a review[J]. *Environ Pollut,* 2018, 243: 1930–1942.

[27] Khreis H, Kelly C, Tate J, et al. Exposure to traffic-related air pollution and risk of development of childhood asthma: a systematic review and meta-analysis[J]. *Environ Int,* 2017, 100: 1–31.

[28] Kim SY, Peel JL, Hannigan MP, et al. The temporal lag structure of short-term associations of fine particulate matter chemical constituents and cardiovascular and respiratory hospitalizations[J]. *Environ Health Perspect,* 2012, 120(8): 1094–1099.

[29] Shahsavan S, Jabalameli L, Maleknejad P, et al. Molecular analysis and antimicrobial susceptibility of methicillin resistant *Staphylococcus aureus* in one of the hospitals of Tehran university of medical sciences: high prevalence of sequence type 239 (ST239) clone[J]. *Acta Microbiol Immunol Hung,* 2011, 58(1): 31–39.

[30] Bernard K. The genus Corynebacterium and other medically relevant coryneform-like bacteria[J]. *J Clin Microbiol,* 2012, 50(10): 3152–3158.

[31] Wozniak TM. Clinical management of drug-resistant bacteria in Australian hospitals: an online survey of doctors' opinions[J]. *Infect Dis Health,* 2018, 23(1): 41–48.

[32] Longhin E, Holme JA, Gutzkow KB, et al. Cell cycle alterations induced by urban $PM_{2.5}$ in bronchial epithelial cells: characterization of the process and possible mechanisms involved[J]. *Part Fibre Toxicol,* 2013, 10: 63.