Composition Analysis of Airborne Microbiota in Outdoor and Indoor Based on Dust Separated by Micro-sized and Nano-sized

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ABSTRACT

Airborne microorganisms are associated with human health and awareness of the important influence of nano-sized extracellular vesicles (EVs) on health has risen. Thus, we analyzed the micro-sized microbes (m-MBs) and nano-sized microbial EVs (n-MEVs) of outdoor and indoor air through a field study in Seoul, Korea. We conducted 16S rDNA-based metagenomic analysis of m-MBs and n-MEVs in outdoor airborne dust (OAD), indoor airborne dust (IAD), and indoor dust from carpets (IDC). The dominant taxa in OAD were altered depending upon the outside environment, such as sunny, haze, and rainy. Also, dominant taxa in IAD and IDC were changed depending on the outside environment. In addition, there were differences of microbiome composition and diversity between the m-MB and n-MEV in OAD, IAD, and IDC. m-MB in OAD were more correlated with that of IDC, whereas n-MEVs in OAD were more related to those in IAD. Thus, indoor bioaerosols can be affected by different source according to bioaerosol size. Additionally, risk of bioaerosols can be different according to dominant taxa, and therefore we suggested that further study for risk of dominant taxa according to environments is necessary. We suggested that nano-sized microbial EVs should be included as parameters to manage air quality.

Keywords: Metagenomics, Bioaerosol, Extracellular vesicles, Nanoparticle, Microbiota

1 INTRODUCTION

Most people spend 90% of their life indoors (Hoppe and Martinac, 1998), and the outdoor environment affects the indoor environment. Indoor and outdoor environments are important for health. Many microorganisms such as bacteria and fungi in air and the concentration of bacterial and fungi in aerosols are estimated to be 10,000 bacteria cells m$^{-3}$ and 1,000 fungal spores m$^{-3}$, respectively (Bauer et al., 2002). In general, bacteria such as Staphylococcus, Micrococcus, Corynebacterium, Bacillus, Enterococcus, Streptococcus, Enterobacteriaceae, and Escherichia are present in indoor air (Kim and Kim, 2007). Previous researches on airborne microbial communities have been carried out using culture-based microbiological methods or sequencing-based methods such as Sanger’s sequencing and next-generation sequencing (NGS) (Shin et al., 2015; Yoo et al., 2017). Culture-based studies can directly observe bacteria and fungi in the air and yield colony-forming units. However, there are some limitations to this such as bacteria counted less than 1% of the total in a solid medium agar plate (Fahlgren et al., 2010). Therefore, recent researches on airborne microbial diversity based on NGS have been increasing in various indoor facilities such as childcare facilities, hospitals, and schools (Shin et al., 2015; Fu et al., 2021; Li et al., 2021).
Airborne bacteria are associated with a wide range of negative health effects, including respiratory and infectious diseases, acute toxic effects, allergies, and even cancer (Douwes et al., 2003). Recently, interest has shifted from the composition of the microbiome to understanding the functional roles it plays in human health. An emerging functional component of the microbiome is the role of microbial extracellular vesicles (EVs) in systemic microbiome activity (Choi et al., 2015; Yang et al., 2019a). As awareness of the significant role that EVs play in interkingdom intercellular transport and communication has risen, attention has recently focused on the influence of microbial EVs on health and disease. Some studies have shown that EVs are absorbed into the body and can travel in the blood and affect the organs (Choi et al., 2015). Microbial EVs are spherical, lipid-bilayer vesicles, with nano-sized diameters ranging from 20 to 100 nm, which are involved in intercellular communication that delivers microbial components including DNA, RNA, proteins, and lipids throughout the body (Brown et al., 2015; Lee et al., 2007, 2009). Especially, microbial EVs derived from dust were found to be related to pulmonary disease. In vivo testing has shown that bacteria-derived EVs in indoor dust can induce neutrophilic pulmonary inflammation (Kim et al., 2012) and subsequently, emphysema (Lee et al., 2013; Kim et al., 2015). In addition, lipopolysaccharide in airborne microbial EVs was reported to induce neutrophilic asthma (Simpson et al., 2007) and systemic injections of microbial EVs induced a powerful antitumor response in the lungs of mice with metastatic carcinoma and melanoma cells (Kim et al., 2017). Overall, many studies of EVs have demonstrated that they can directly affect disease states.

However, no studies have been conducted on microbial EVs in the air, and there has been no in-depth study on the distribution and correlation of microbe and microbial EVs diversity in the ambient air and indoors. Several studies on submicron bioaerosols were conducted (Wei et al., 2019), however, they did not target to microbial EVs. Since size of submicron particle is under 1.0 µm, the other materials are included. In addition, some submicron bioaerosols are culturable (Clauß, 2015), but microbial EVs could not be cultured. Therefore, the aim of this study was to examine the micro-sized microbe and nano-sized microbial EVs microbiota according to the outside environmental conditions and the correlations between the microbiota distribution indoors and outdoors through a field study. The results of this study will increase the understanding of the importance of micro-sized microbe and nano-sized microbial EVs management and emphasize the necessity of management methods considering the microbiome distribution in the characteristics of indoor environments for indoor air quality management.

2 METHODS

2.1 Sampling

Air samples of outdoor airborne dust (OAD) and indoor airborne dust (IAD) for bioaerosol analysis were collected in an office building for one week in sterilized Petri dishes using the gravitational settling method (Aydogdu et al., 2010). The sampling sites and duration were described in Fig. 1(A) and Fig. 1(B), respectively. Representative samples were taken at six outdoor sites and six indoor sites. To collect outdoor samples, we considered wind direction and we selected sampling sites that are south-north, east-west, and center of the rooftop. In addition, we considered windows, working space, and center of the rooms to analyze indoor airborne samples. Samples for indoor dust were collected from carpets (IDC) using a vacuum for five minutes at three sites. Outdoor and indoor sampling was conducted on rooftops and top floor of one office building in the office blocks where there are many large buildings, in Sangam-dong, Mapo-gu, Seoul, Korea, respectively. Sampling was conducted four times from May to July 2016 when there were generally not special events, such as rainy season and yellow dust, for this pilot study.

2.2 Environmental Data

The indoor temperature was maintained from 24 to 26°C using air conditioner, and the indoor relative humidity was maintained at 30 to 60% using automatic HVAC system. Hourly measures of outdoor PM_{10} and PM_{2.5} were obtained from a monitoring station in Mapo-gu through the official website AIRKOREA (www.airkorea.or.kr) of the Korea Ministry of Environment. Daily outdoor information on the mean temperature (mT), maximum temperature (maxT), minimum temperature (minT), mean relative humidity (RH), rainfall (RF), sunshine hours (SH), cloudiness,
haze, and wind speed were provided by the Korea Meteorological Administration. Monitoring sites of these data is in Mapo-gu where it is the nearest station of monitoring stations in Seoul (Fig. 1(A)).

2.3 Isolation, Sequencing, and Assignment of Microbes and Microbial EVs

Samples were filtered through a cell strainer after being diluted in 10 mL of phosphate-buffered saline (PBS) for 24 hours at 4°C (Yang et al., 2020a). In addition, two PBS samples were analyzed as a blank (negative control), and a standard material was used to control errors such as contamination and high-level growth. To analyze dust separated into micro-sized and nano-sized, we separated EVs from samples using centrifugation and filtering as in previous study (Yang et al., 2019a; Kim et al., 2013). The pellet and supernatant containing cells and EVs, respectively, in the samples were separated by centrifugation twice at 10,000 × g for 10 min at 4°C. To eliminate cell or foreign particles, the supernatant was filtered through a 0.22-µm filter. To extract DNA from the pellets and supernatant, the filtered samples were boiled and centrifuged as in a previous study (Yang et al., 2019a; Kim et al., 2013). DNA contained within the pellets and supernatant was extracted using a DNeasy PowerSoil kit (QIAGEN, Germany). The DNA extracted from the isolated bacterial cells and EVs contained in each sample was quantified using the QIAxpert system (QIAGEN, Germany). Bacterial genomic DNA targeting the 16S V3-V4 hypervariable regions was amplified with V3 forward and V4 reverse primers as previously described (Yang et al., 2019a). Libraries were prepared using the PCR products, and each amplicon was sequenced using MiSeq (Illumina, San Diego, USA). Taxonomic assignment was done by the profiling program MDx-Pro.
ver.1 (MD Healthcare, Korea) based on the Greengenes database (ver. 13_8) as previously described (Yang et al., 2019a).

2.4 Dynamic Light Scattering (DLS)
EVs separated from samples in method Section 2.3 were diluted with PBS to 500 ng mL\(^{-1}\) and the diameter size distribution was measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) and analyzed by Dynamic V6 software.

2.5 Statistical Analysis
To avoid potential bias caused by differing sequencing depths, the samples were rarefied to a depth of 16,500 reads for the microbes and 13,500 reads for the microbial EVs for subsequent analysis. Significant differences in the outdoor environments and microbiomes between the sampling weeks were assessed using the Kruskal-Wallis and Mann-Whitney tests for continuous variables. The findings were considered significant at \(p\)-values of \(< 0.05\). Alpha diversity of the microbial composition was measured using the Shannon index and Chao1 index and was rarefied to compare species richness and evenness. To analyze beta diversity, principal component analysis (PCA) was used to find clustering of groups based on Euclidean distance and analysis of similarity (ANOSIM) was performed. Correlations between OAD, IAD, and IDC were analyzed using Spearman’s correlation. All statistical analyses were carried out using R version 3.6.1.

3 RESULTS AND DISCUSSION

3.1 Nano-sized Microbial Extracellular Vesicles
We found that outdoor and indoor airborne dust included ultrafine particles with sizes of 34.58 ± 36.30 nm and 27.11 ± 28.22 nm, respectively, and difference of size between outdoor and indoor was not significant (Fig. 2). The maximum ultrafine particle size was 190.1 nm in outdoor and 91.3 nm in indoor air. Previous studies showed that range of nano-sized materials in feces and dust of mattress was under 200 nm (Choi et al., 2015; Kim et al., 2013). However, these might include various ultrafine particles, such as metals and biological and chemical materials. Especially, the biological elements in nanosized airborne particles contained allergens, viruses, microbial EVs, lipopolysaccharides, and other particles (Yang et al., 2020b). Therefore, we amplified the 16S rDNA of microbial EVs to analyze microbiota of nanosized microbial EVs in indoor and outdoor dust.

Fig. 2. Size distribution of nano-sized particles in airborne dust determined by dynamic light scattering analysis. (A) Outdoor airborne dust and (B) indoor airborne dust.
Table 1. Differences in outdoor conditions.

<table>
<thead>
<tr>
<th>Temperature [°C]</th>
<th>Relative humidity [%]</th>
<th>Rainfall [N]</th>
<th>Sunshine hours [hr]</th>
<th>Cloudiness [%]</th>
<th>Wind speed [km h⁻¹]</th>
<th>Haze [N]</th>
<th>Particulate matter PM₁₀ [µg m⁻³]</th>
<th>PM₂.₅ [µg m⁻³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>Mean</td>
<td>Max</td>
<td>N</td>
<td>Volume [mm]</td>
<td>(SD 0.0)</td>
<td>12.7</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td>1st week</td>
<td>14.8</td>
<td>22.1</td>
<td>29.1</td>
<td>37.0</td>
<td>0.0</td>
<td>(SD 7.5)</td>
<td>(SD 0.5)</td>
<td>3.0</td>
</tr>
<tr>
<td>2nd week</td>
<td>17.5</td>
<td>21.7</td>
<td>27.1</td>
<td>58.3</td>
<td>2.0</td>
<td>(SD 0.1)</td>
<td>7.5</td>
<td>(SD 3.9)</td>
</tr>
<tr>
<td>3rd week</td>
<td>19.2</td>
<td>23.1</td>
<td>28.1</td>
<td>67.1</td>
<td>4.0</td>
<td>(SD 0.1)</td>
<td>8.5</td>
<td>(SD 3.3)</td>
</tr>
<tr>
<td>4th week</td>
<td>21.0</td>
<td>26.7</td>
<td>30.4</td>
<td>74.8</td>
<td>7.0</td>
<td>(SD 2.4)</td>
<td>5.0</td>
<td>(SD 2.4)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.018</td>
<td>0.078</td>
<td>&lt; 0.001</td>
<td>0.002</td>
<td>0.003</td>
<td>0.004</td>
<td>0.418</td>
</tr>
</tbody>
</table>

Generally, aerodynamic diameter based-size distribution of airborne culturable bacteria is ranged from 0.65 µm to 12 µm and most of the bacteria particles were found between 7.2 µm and 12 µm ([Clauß, 2015]). Therefore, we defined that microbe is micro-size and microbial EV is nano-size (< 200 nm).

3.2 Outdoor Environment during the Sampling Periods

Table 1 shows the outdoor environmental conditions during the experiment. In the first week (1W), sunshine hours (SH) was significantly higher, and cloudiness was significantly lower in the other weeks (p < 0.05). Furthermore, there were no rainy days. The difference between the maxT and the minT was 14.3°C. In the second week (2W), PM₁₀ and PM₂.₅ were significantly higher than in the other weeks at 66.5 ± 21.0 µg m⁻³ and 40.0 ± 14.6 µg m⁻³, respectively (p < 0.05). Also, there was haze every day. In the third week (3W), four days were rainy, and the maximum RH was significantly higher at 74.8% than in the other weeks (p < 0.05). Each week showed the characteristic of whether conditions: 1W was sunny, 2W was hazy, 3W’s weather were varied, and 4W was rainy.

Environmental factors including temperature and humidity are correlated with not only indoor and outdoor bioaerosols concentrations ([Yang et al., 2015]) but also nano-sized particulate matter (PM) concentrations ([Nguyen et al., 2017]). In addition, haze strongly affect PM ([Eun et al., 2021; Sresawasad et al., 2021]), and we suggested that PM concentration also may be increased by haze. Korea Meteorological Administration defined that haze is a phenomenon in which visibility (< 1 km) and relative humidity (< 75%) when fine and dry particles such as smoke and dust float in the air and the color of the air appears milky ([Jeon, 2017]).

Thus, outdoor environment might be important factor for micro-sized and nano-sized bioaerosols.

3.3 Outdoor Airborne Microbiome according to the Outdoor Environment

In outdoor air, valid reads and OTUs in micro-sized microbe (m-MB) were higher than nano-sized microbial EVs (n-MEV) (Table S1). Microbial EVs reflect microbiome activity ([Choi et al., 2015]), therefore, OTUs of n-MEVs might be affected by the number of active microorganisms. The Chao1 score of m-MBs for 1W and n-MEVs for 4W was higher than in the other weeks (p < 0.05), and both m-MB and n-MEV for 3W were higher than in the other weeks (Table S1). PCA at the genus level yielded distinct clustering between the weeks and ANOSIM showed the significant difference between weeks (Global R = 0.8679, p < 0.01) (Fig. 3(A)).

Comparative analysis of the m-MB composition revealed significant changes between the weeks. At the phylum levels, Cyanobacteria was dominant at 1W (sunny week) and Proteobacteria was dominant at the other weeks (haze week, varied weather conditions week, and rainy week). Proteobacteria was significantly different between all weeks (p < 0.05) (Fig. 3(B), Table S2). At the genus levels, the most dominant genera were unidentified genus in Streptophyta at 1W (sunny week), Enhydrobacter at 2W (haze week), unidentified genus in Enterobacteriaceae at 3W (varied...
Fig. 3. Metagenomic analysis of micro-sized microbes (m-MB) and nano-sized microbial EVs (n-MEV) in outdoor airborne dust (OAD). (A) Principal component analysis was analyzed for beta-diversity. (B) Distribution of dominant taxa were showed using bar graph at the phylum level. Heatmap based on taxon over 1% at any week at the genus level of (C) m-MB and (D) n-MEV.

weather conditions week), and *Pseudomonas* at 4W (rainy week) (Table S2). *unidentified genus in Streptophyta, Enhydrobacter, and unidentified genus in Enterobacteriaceae* were significantly different between all weeks (*p* < 0.05), whereas differences in *Pseudomonas* was not significant. *unidentified genus in Comamonadaceae* and *Sphingobium* at 1W; *Erwinia, unidentified genus in Comamonadaceae, Methylobacterium, Sphingomonas*, and *Sphingobium* at 2W; *unidentified genus in Enterobacteriaceae, Lactobacillus, Proteus, unidentified genus in Lachnospiraceae, and Bifidobacterium* at 3W; and *Cupriavidus* at 4W were significantly altered compared to the other weeks (Fig. 3(C)).

The n-MEVs also showed a significant difference between the weeks. At the phylum level, *Firmicutes* was dominant at 1W (sunny week) and 4W (rainy week), and *Proteobacteria* was dominant at 2W (haze week) and 3W (varied weather conditions week). The abundance of *Bacteroidetes* was significantly higher at 4W than in the other weeks (*p* < 0.05) (Fig. 3(B), Table S3). At the genus levels, the dominant microbial EVs were *Exiguobacterium* at 1W (sunny week), *Methylobacterium* at 2W (haze week), *unidentified genus in Enterobacteriaceae* at 3W (varied weather conditions week), and *Bacteroides* at 4W (rainy week) (Table S3). In addition, there were significant changes in *unidentified genus in Streptophyta, unidentified genus in Ruminococcaceae, unidentified genus in Oxalobacteriaceae, Proteus, Lactobacillus, and Parabacteroides* at 1W; *Pseudomonas, Corynebacterium, Parabacteroides, Propionibacterium, and Acinetobacter* at 2W; *Proteus, Lactobacillus, Enterococcus, and Bifidobacterium* at 3W; and *unidentified genus in Enterobacteriaceae, unidentified genus in Ruminococcaceae, Akkermansia, Bifidobacterium,*
Coprococcus, and Ruminococcus at 4W compared to the other weeks. Bacteroides, Clostridiales, unidentified genus in Lachnospiraceae, and Staphylococcus were significantly changed every week (p < 0.05) (Fig. 3(D)).

In this study, we established that m-MB and n-MEVs were altered by outdoor environmental conditions. The composition and OTUs of the n-MEVs showed many more changes than the m-MB in relation to environmental conditions. Especially, the number of m-MB OTUs decreased in high RH conditions caused by rain (p < 0.05), whereas n-MEV OTUs increased (p < 0.05). Generally, microbial colony-forming units are increased by environmental factors such as temperature and humidity (Zhu et al., 2003). Pseudomonas and Bacteroides were the most abundant m-MB and n-MEV genera respectively, at 4W, which was rainy and had high relative humidity. Pseudomonas aeruginosa in soil was increased at high temperature and high relative humidity (Green et al., 1974), and the survival of Pseudomonas sp. was related to temperature and RH (McEldowney and Fletcher, 1988). The survival of Bacteroides spp. in a moist environment was higher than in a dry environment and higher at a low temperature (4°C) than at a high temperature (35°C) (Hoffmann and Justesen, 1980). We suggest that the growth and death of microorganisms are largely affected by the environment factors and that microbial EVs such as the ectosome and apoptotic body are derived by the growth and death of microbes. In addition, the microorganisms and microbial EVs circulate between the soil, water, and air. Therefore, the microbiota of m-MBs and n-MEVs in air are changed by surrounding environment and environmental conditions such as temperature, humidity, rainfall, and sunshine.

3.4 Microbiota of Biological Particles Derived from Indoor Dust

In indoor, the Chao1 and Shannon index of indoor dust from carpets (IDC) m-MB at 1W was significantly higher than that of the other indoor sample groups, whereas the Chao1 index and Shannon index of n-MEVs was higher in indoor airborne dust (IAD) at 4W and at 3W, respectively (Table S1).

There were differences in the m-MB composition of IAD and IDC. At the phylum levels, Proteobacteria was the most abundant in every sample. Specifically, the proportion of Proteobacteria in IDC at 2W was more than 97.0%. Cyanobacteria in IDC were higher at 1W and Bacteroidetes in IDC were higher at 4W than in the other indoor sample groups (Fig. 4(A), Table S2). At the genus levels of the IDC m-MB, unidentified genus in Streptophyta at 1W, Enhydrobacter at 2W, unidentified genus in Enterobacteriaceae at 3W, and Pseudomonas at 4W were higher than at the other weeks. In the IAD m-MB, Enhydrobacter was higher at 1W and 2W and Cupriavidus was higher at 3W and 4W (Fig. 4(B), Table S2).

Comparative analysis of the n-MEV composition in IAD and IDC showed changes between the weeks. At the phylum levels, Cyanobacteria and Proteobacteria were dominant at 1W, whereas Firmicutes and Proteobacteria were dominant at the other weeks at more than 62.0% in the indoor sample groups (Fig. 4(A), Table S3). At n-MEVs in IDC, the most abundant genera were unidentified genus in Streptophyta at 1W, Methylobacterium at 2W and 3W, and Dosemzia at 4W. On the other hands, at n-MEVs in IAD, proportions of unidentified genus in Streptophyta at 1W, Methylobacterium at 2W, unidentified genus in Enterobacteriaceae at 3W, and Bacteroides at 4W were the highest (Fig. 4(B), Table S3).

In this study, although Methylobacterium, unidentified genus in Enterobacteriaceae, Exiguobacterium, Bacteroides, and unidentified genus in Streptophyta were the dominant m-MB in IAD, a previous study reported that Micrococcus, Paracoccus, Staphylococcus, and Enhydrobacter were dominant (Shin et al., 2015). We suggest that the main factors that likely contributed to the difference between these results were the lack of m-MB and n-MEV separation, the sampling environment, and methodology. Generally, genomic DNA isolated from samples includes m-MB DNA and n-MEV DNA. In human stool sample, total bacterial DNA yield is composed of 72.9% bacteria DNA yield and 27.1% bacterial EV DNA yield (Yang et al., 2019b).

3.5 Correlation of Microbiota between Outdoor and Indoor Dust

There was a positive correlation between m-MB and n-MEV microbiota, as well as between outdoor airborne dust (OAD), IAD, and IDC. The correlations between weeks were positive despite differences in environmental conditions such as temperature, weather, and PM levels. However,
m-MB and n-MEV microbiota were not significantly correlated with week when more than 2 weeks have passed. At 2W, all correlation coefficients of microbes and microbial EVs between the sampling sites were higher than 0.80 and 0.96 ($p < 0.001$), respectively. Especially, outdoor microbiota was related to indoor microbiota. The m-MB microbiota in OAD was more correlated with IDC (mean $r = 0.804$, $p < 0.001$) than with IAD (mean $r = 0.367$, $p < 0.05$), whereas the correlation between n-MEVs in IAD and OAD with an $r = 0.742$ ($p < 0.01$) was higher than that between IDC and OAD at $r = 0.442$ ($p < 0.05$). In addition, the correlation between m-MB in IAD and IDC was lower than that of n-MEVs. Indoor microbiota was related to the indoor microbiota of the following week, whereas outdoor microbiota was not correlated with that of the next week. The correlation of m-MB in IAD was high between 1W and 2W ($r = 0.979$, $p < 0.001$) and between 3W and 4W ($r = 0.838$, $p < 0.001$), whereas the correlation between 2W and 3W was low at $r = 0.051$ ($p > 0.05$). In IAD n-MEVs, high correlation was seen between 3W and 4W at $r = 0.775$ ($p < 0.001$), whereas the correlation coefficient was $r = 0.335$ ($p > 0.05$) between 2W and 3W and $r = 0.212$ ($p > 0.05$) between 1W and 2W (Figs. 5(A) and 5(B)).

Because of infiltration through windows, cracks, ventilation systems, and other places, indoor airborne particles including PM and bioaerosols were highly correlated with outdoor airborne particles (Yang et al., 2015). In addition, nano-sized particles penetrate better than micro-sized particles (Long et al., 2001). In this study, m-MB in OAD were more correlated with IDC than with...
IAD, whereas n-MEVs in OAD were more related with IAD than IDC, perhaps because the m-MBs were bigger and heavier than n-MEVs and therefore, most of the m-MB sank. However, most microbial EVs float in the atmosphere. Airborne particles are affected by particle size. The bigger the particle size, the higher the deposition loss and resuspension rate (Thatcher and Layton, 1995). Also, nano-sized particles can float in the air with lower air velocity, however, to be settled in the air, micro-sized particles needed a higher velocity than nano-sized particles (Chen and Zaho, 2011). In outdoor air, the correlation coefficients of m-MBs and n-MEVs according to the outdoor environments were not significant. In IAD, the correlation of m-MB between one week and the next was higher than those for n-MEVs. We maintained indoor environments regardless of the outdoor environment using heating, ventilation, and air conditioning (HVAC) systems. We suggest that the indoor airborne m-MBs were more affected by the indoor environment than by the outdoor environment, whereas indoor airborne n-MEVs showed the opposite effect.

3.6 Limitations

Although several researchers have studied bioaerosols and the correlation between outdoor and indoor air, this study was the first to use n-MEV metagenomics of bioaerosols and dust in carpet. However, there were some limitations of this study. First limitation was the diversity of the sampling areas because the samples were collected from only one office building in rural office blocks for pilot study instead of collecting from more sampling areas and different urban areas. Second, we think that air volume or dust weight were not necessary, and therefore we collected the airborne samples using passive setting plates for a week. However, these results had a limitation as a sampling method to represent airborne dust because some bioaerosols floats in the air, not fall by in regard of particle size and there were differences in the meteorology conditions each day. In addition, there were previous studies for airborne sampling, such as personal sampling for inhalable microorganisms and endotoxin (Rui et al., 2020). We think that standard sampling method for bioaerosols including nano-sized metagenomic analysis is necessary. Therefore, we have a plan of further study about sampling method for bioaerosols metagenomic analysis including nano-sized particles. Finally, in this study, comparisons were conducted only to analyze differences of taxa abundance (proportion). These results showed that whether distribution of microbiota or dominant taxa is changed or not. Also, dominant taxa can strongly affect a human health. Therefore, these results suggested that taxa proportion is important to control and monitor bioaerosols for human health in a specific environment. However, these
results had a limitation for quantitative analysis. We perform the further study which should
include quantitative analysis data such as concentrations.

Future studies should include samples collected from more diverse areas and every day to
achieve more thorough and accurate bioaerosols and dust metagenomic analysis. In addition, we
should consider seasonal conditions and events, such as rainy season and yellow dust.

4 CONCLUSIONS

The results of this study highlight the m-MB and n-MEV composition of OAD, IAD, and IDC.
Dominant taxon of airborne microorganisms was different according to outside environment.
Bioaerosols were correlated with environmental factors and weather, such as sunny, rainy, and
hazy. Risk of bioaerosols can be different according to dominant taxa, and therefore we suggested
that further study for the risk of dominant taxa according to environments is necessary. In addition,
this study showed differences between micro-sized and nano-sized biological particle-derived dust.
The differences in the composition and correlation between m-MB and n-MEVs were significant.
Outdoor bioaerosols had more influence in IAD in case of nano-sized, whereas in case of micro-
sized had more influence in IDC. Additionally, in IAD, the correlation of m-MB between one week
and the next was higher than those for n-MEVs. Thus, indoor bioaerosols can be affected by the
different source according to bioaerosol size. However, studies and management for bioaerosols
have been performed without separation between micro- and nano-size. Therefore, we suggested
that separation of bioaerosol sizes and the flow of m-MB and n-MEVs, including infiltration rates
and retention time in the atmosphere, should be considered for managing airborne microorganisms
and to predict indoor air quality. Nano-sized airborne microorganisms also can be an important
marker for assessment and management of air quality.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version at https://doi.org/
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