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Airborne bacteria in institutional and commercial buildings in Korea: Characterization with 16S rRNA gene sequencing and association with environmental conditions

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ABSTRACT

Information on microorganisms in indoor air of various institutional and commercial buildings has significant value in a public health management perspective. However, there is a lack of prior research comparing indoor airborne microbiota across different categories of those buildings. We characterized indoor airborne bacteria in 10 buildings (two for each of five categories: train station, parking garage, mart, public library, and daycare center) during summer and winter. The 16S rRNA gene in the bacterial gDNA extracted from samples was quantified using quantitative real-time polymerase chain reaction and sequenced with the Illumina MiSeq™ platform for characterizing community composition. We collected information on temperature, relative humidity, CO_2 concentration, and particulate matter (PM) concentrations by particle size (<1 µm, 1–2.5 µm, 2.5–10 µm) indoors. We performed a multivariate regression analysis to identify factors influencing bacterial quantity and Permutational Multivariate Analysis of Variance (PERMANOVA) to determine factors affecting cluster dissimilarity. We found that bacterial concentration was significantly (p-values < 0.05) associated with season and CO_2 concentration. The PERMANOVA analyses showed the significant (p-values < 0.05) associations of bacterial cluster dissimilarity with season, building category, and CO_2. Our study indicated that the season, and CO_2 concentrations may be important factors associated with the indoor airborne bacterial concentration and composition. Building category and usage appeared to significantly influence the bacterial community composition but not the concentration. Our study may provide basic data on bacterial community composition and their concentration that are needed for properly managing microbial exposures in occupants or customers of the studied institutional and commercial buildings.

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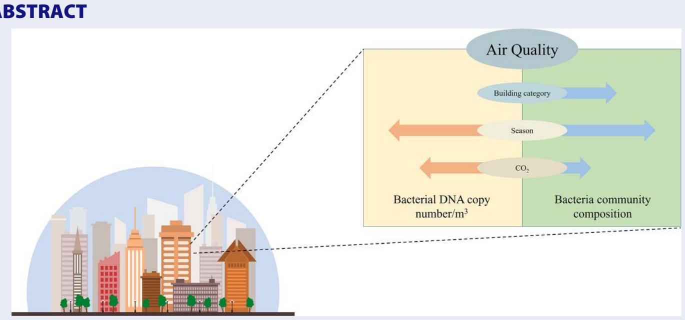

1. Introduction

Exposure to bacteria in indoor environments is inevitable. Indoor bacteria closely interact with occupants' immune systems, which significantly affect occupants' health (Miletto and Lindow 2015; Wei et al. 2022). Airborne indoor bacteria could beneficially or

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adversely affect occupants' potential respiratory illnesses such as asthma or infections (Karvonen et al. 2019; Yang et al. 2017). There are many studies on bacteria in home environments and some in school environments (Kwan et al. 2020; Lee et al. 2021; Madsen et al. 2023; Yang et al. 2022). However, characteristics of indoor bacteria in nonresidential and non-school buildings where the public has access and spend their time are poorly understood. The Indoor Air Quality Control Act (IAQCA) in Republic of Korea regulates indoor air quality in all buildings including "Public-Use Facility (PUF)" that is defined as the institutional and commercial buildings that the public can utilize (Table S1). Indoor microbial environments in PUF are especially important in prevention of microbial infections as well as maintaining healthy microbiota for public. Therefore, we need to better understand airborne bacterial community composition and their concentration in the PUF, and such data will be beneficial to public health officials or those who manage the buildings for healthy indoor environments.

In Korea, the building managements of the PUF have to meet the standards of indoor airborne bacteria according to the IAQCA. However, the airborne bacterial standard in the law (800 colony forming units/m^3) is set based on the traditional impactor air sampling (with a total volume of less than 250 L) and culturing method of which limitations are well acknowledged. These drawbacks include, but not limited to, the lack of standardized methods for sample collection and analysis (Kabir et al. 2020), low culturability of environmental bacteria, need of professional mycologists for identification, and long analytical time (Rinsoz et al. 2008). Previous studies indicated that 99% of microorganisms in the atmosphere are unable to grow on conventional culture media (Kärkkäinen et al. 2010; Toivola, Alm, and Nevalainen 2004). However, recently introduced genetic methods of analysis for indoor airborne bacteria such as next generation DNA sequencing (16S ribosomal RNA (16S rRNA) sequencing) could overcome some of these methodological limitations (Kärkkäinen et al. 2010). The method of 16S rRNA sequencing has also increased taxonomic resolution by confirming microbial communities through metagenomic difference (Luhung et al. 2021).

In this study, we investigated ten institutional and commercial buildings in five categories of PUFs using the quantitative polymerase chain reaction (qPCR) and next generation DNA sequencing method. The objectives of the study are: 1) to characterize airborne bacterial microbiota in each category of building and two seasons (summer and winter); and 2) examine what environmental conditions are associated with the airborne bacterial level and community composition.

2. Methods

2.1. Study buildings and environmental sampling

We investigated ten buildings in five categories (two per each category: train station, mart (market including grocery stores), indoor parking garage, public library, and daycare center) designated by the Korean IAQCA standards for air quality in PUFs (Table S1). Selection of buildings was a convenience sampling by recruiting the buildings of which managements agreed to our study and gave us permission to access their buildings and perform environmental sampling during the coronavirus disease 2019 pandemic. All ten buildings recruited were located within the metropolitan area encompassing cities of Seoul (population: over 10 million) and Suwon (about one million) (Figure S1). Environmental surveys including air sampling at the buildings were conducted for three consecutive days in each building during both summer and winter from August 2021 through August 2022. We collected a total of 60 air samples (10 buildings × 2 seasons × 3 consecutive days) during the surveys.

Samples for airborne bacteria were collected over a period of any 8 h between 9 am and 7 pm every day for three days when the buildings were occupied or being utilized. Prior to sampling, the flow rate for each sampling pump was calibrated using a Primary Pump Calibrator (Model 4146, TSI, Shoreview, USA). The sampling pumps were set up at about 0.7 m above the ground at a location of high traffic area within the building, ensuring minimum mutual interference between the building users and sampling. Each sampling pump (Gillian Air Plus, Sensidyne, St. Petersburg, USA) was connected to a 37 mm closed cassette loaded with polyvinyl chloride (PVC) filters (pore size = 5 µm, SKC Inc., Eighty-Four, PA, USA) to collect air at a flow rate of 4 L/min. Cassettes and filters were sterilized for 1 h inside an ultraviolet disinfection box (HU-4050, Hanshin Medical Co., Incheon, Korea) to inactivate contaminating background DNA. To obtain a sufficient amount of genomic DNA (gDNA) for 16S rRNA gene sequencing, we combined two filter samples collected side-by-side for DNA extraction in each day, resulting in an average of 3841 ± 45 L of air for the pooled daily air samples. One field blank sample was also collected every sampling day, and negative controls were tested.

Test results of field blanks and negative controls were all below the limit of detection. To prevent contamination and moisture infiltration after the sampling, the filter cassettes were sealed using parafilm and stored at −80 °C until sample analysis.

Environmental conditions that may influence bacterial concentrations or community composition were also measured. These included indoor temperature, relative humidity (RH), particulate matters (PM) in particle aerodynamic diameter < 10 µm (PM_{10}), < 2.5 µm ($PM_{2.5}$), and < 1 µm (PM_1), occupant density, and carbon dioxide (CO_2) concentration (Table 1). Indoor temperature and RH were monitored using a smart probe (Testo 605i, Testo, Lenzkirch, Germany) for every 2 min during the sampling period, and daily average temperature and RH were used for data analysis. PM data were monitored using a portable aerosol spectrometer (11-d, GRIMM Aerosol Technik, Ainring, Germany) for every 1 min over the sampling period every day. From these PM measurements, we calculated concentrations for $PM_{2.5\text{-}10}$ (i.e., PM_{10} concentration – $PM_{2.5}$ concentration: coarse PM concentration) and $PM_{1\text{-}2.5}$ ($PM_{2.5}$ – PM_1). CO_2 concentrations were monitored using an IoT-based air quality monitor (Micro Watch: Indoor, Innodigital, Yongin, Korea) for every 1 min during the sampling, and daily average concentrations over the sampling time were used for data analysis. Except for daycare center, the number of hourly users was determined by counting the number of people passing by the sampling station within a radius of 5 m for 10 min for every hour and then multiplying it by 6 (Table S2). Total number of users during the sampling period for each day was then calculated by summing the number of hourly users over 8 h. Then occupant density was calculated by dividing the total number of daily users during the sampling period by the volume of the building space that was obtained from the building managements.

2.2. gDNA extraction and filter processing

The High-Pure PCR Template Preparation Kit (Roche, Basel, Switzerland) was used to extract gDNA from the sampled air filters (Rittenour et al. 2012). In a 2 ml screw-cap microcentrifuge tube, 300 mg of sterilized glass beads (Sigma, St. Louis, MO, USA) with a size range of 212–300 µm were added. Subsequently, two filters that were cut into smaller pieces and 600 µL of CelLytic™ B Cell Lysis solution (Sigma, St. Louis, MO, USA) were added into the tube. Samples were pulverized five times for 30 s at 1484 × g using a homogenizer (Allsheng, Hangzhou, China). After centrifugation at 8000 × g for 1 min, the supernatant was collected and centrifuged again at 8000 × g for 1 min. When the amount of the resulting substance exceeded 200 µl, it was divided into aliquots of 200 µl each. To each aliquot, 200 µl binding buffer and 40 µl proteinase K were added, mixed, and incubated for 10 min at 70 °C. The sample solution consisting of 100 µL of isopropyl alcohol was transferred to a composite of a filter tube and a collection tube, followed by centrifugation for 30 s at 8000 × g. When dividing an equal volume of the same sample, the previous step was repeated to place it in the same filter tube collection tube assembly. The samples were subjected to a washing procedure using an inhibitor removal buffer and wash buffer as per the method

Table 1. Mean (±standard deviation) of environmental measurements by building category, location, and season.

Group	Temp (°C)	RH (%)	PM_{10} (µg/m^3)	$PM_{2.5}$ (µg/m^3)	PM_1 (µg/m^3)	Occupant density (person/m^3)	CO_2 (ppm)
Building category							
Station (n = 12)	23.76 ± 5.44	48.79 ± 16.81	22.40 ± 10.83	17.31 ± 7.83	13.90 ± 6.31	0.17 ± 0.06	513.3 ± 46.25
Mart (n = 12)	20.34 ± 4.51	47.83 ± 11.52	25.25 ± 18.25	21.88 ± 16.29	19.36 ± 14.57	0.23 ± 0.17	629.3 ± 106.9
Parking garage (n = 12)	17.71 ± 12.61	42.80 ± 24.29	29.29 ± 18.84	22.38 ± 18.78	21.18 ± 19.15	0.01 ± 0.01	523.6 ± 102.5
Library (n = 12)	22.81 ± 1.94	39.03 ± 20.93	11.40 ± 4.35	10.12 ± 3.82	9.36 ± 3.73	0.09 ± 0.03	612.2 ± 137.8
Daycare center (n = 12)	21.04 ± 5.79	50.53 ± 17.35	27.05 ± 7.10	19.67 ± 8.75	16.33 ± 8.57	0.22 ± 0.12	855.5 ± 396.4
Location							
Seoul (n = 18)	21.28 ± 6.57	46.90 ± 16.80	24.98 ± 11.62	20.57 ± 10.22	17.97 ± 9.36	0.32 ± 0.08	548.5 ± 80.0
Suwon (n = 42)	21.07 ± 7.32	45.33 ± 19.40	22.26 ± 15.29	17.28 ± 13.75	14.90 ± 12.82	0.07 ± 0.05	660.3 ± 263.6
Season							
Summer (n = 30)	26.50 ± 2.51	62.14 ± 5.52	21.38 ± 12.05	15.66 ± 8.98	12.93 ± 7.49	0.15 ± 0.12	658.8 ± 278.0
Winter (n = 30)	15.77 ± 5.93	29.46 ± 10.77	24.78 ± 16.17	20.88 ± 15.45	18.97 ± 14.74	0.15 ± 0.14	594.7 ± 167.3

prescribed by the kit manufacturer. Finally, 200 µl of elution buffer was added to a new tube to elute gDNA from the sample. The gDNA samples obtained were stored at a temperature of −20 °C until they were analyzed.

2.3. Bacterial DNA sequencing

Targeting the V3 and V4 regions, all sample sequencing libraries were prepared according to Illumina 16S Metagenomic Sequencing Library protocols at a commercial laboratory (Macrogen, Seoul, Korea). The 2 ng of input gDNA was amplified in PCR with 5x reaction buffer, 1 mM of dNTP mix, 500 nM each of the universal F/R PCR primer, and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA, USA). The thermal conditions for the first PCR were as follows: 3 min of the initial denaturation at 95 °C, and 25 cycles 30 sec of denaturation at 95 °C, 30 sec of annealing at 55 °C, and 30 sec of elongation at 72 °C, followed by a 5-min final extension at 72 °C. The first PCR products were purified using AMPure beads (Agencourt Bioscience, Beverly, MA, USA). For gene amplification with the primer sequences in amplicon sequencing, 16S V3–V4 region of the bacterial DNA was used as shown in Table 2. The second PCR was the same as the first PCR condition, except for the number of cycles (10 cycles). The concentration and purity of amplified DNA were assessed using VICTOR Nivo (PerkinElmer, Waltham, MA, USA) and DNA size verified by TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). After sequencing, the Illumina MiSeq raw data were processed using the Cutadapt program (v3.2) (Martin 2011) to generate paired-end FASTQ files for each sample utilizing the index sequences. Errors in the amplicon sequencing process were corrected using the DADA2 (v1.18.0) package in R (v4.0.3) (Callahan et al. 2016). Sequences with an anticipated error rate of 2 or above were excluded, focusing on paired-end reads. After correcting for sequencing errors, the paired-end sequences were combined, and DADA2 was employed to form Amplicon Sequence Variants (ASVs). ASVs less than 350 bp were excluded from the generated set. To compare and analyze the microbial communities, normalization was performed using QIIME (v1.9) (Caporaso et al. 2010). Subsampling was performed based on the read count of the sample with the minimum number of reads among all samples. Each ASV sequence was aligned to the reference DB (NCBI 16S Microbial DB, NCBI_16S – 2022-05-26) using BLAST+ (v2.9.0) (Camacho et al. 2009) to assign taxonomic information to organisms. If the query coverage of the best matching result in the database or the identity of the matched region was less than 85%, taxonomy information was not assigned.

2.4. Quantification of bacterial DNA using qPCR

qPCR analysis was also performed using the primers listed in Table 2 (331 F and 797 R for the 16S rRNA region). We prepared the recommended qPCR reaction mix in 0.2 ml PCR tube (GmBH, Switzerland). The total volume was made up to 20 µl as follows: 4 µl of 5× HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Tartu, Estonia), 1 µl of DNA template, 14.6 µl of nuclease-free water (Qiagen, Hilden, Germany), and 0.2 µl of each forward and reverse primers (0.1 µM of each). For the qPCR system, LineGen 9600 (BIOER, Hangzhou, China) was used with the following thermal conditions: 95 °C for 12 min of initial denaturation, 40 cycles of 95 °C for 15 s of denaturation followed by 50 °C for 20 s of annealing, and then 72 °C extension for 30 s after the cycles. Diluted *Escherichia coli* (*E. coli*) was used to generate a standard curve for qPCR analysis (Lee et al. 2021; Yang et al. 2022). The OD_{600} value before the dilution of the *E. coli* sample used to produce the standard curve was 0.7, and the corresponding DNA concentration measured after extraction of DNA in the *E. coli* standard solution was 55.0 ng/µL. Standard sample of *E. coli* concentration was 1.08×10^7 (Number of copies of DNA template per/µl) that was serially diluted to 10^{-5} times by a factor of 10. PCR efficiency ($R^2 = 0.998$, PCR efficiency = 98.4%) was verified using a standard curve, and accuracy was confirmed using a melting curve. The total bacterial DNA copy number was calculated from the linear regression equation of the standard curve and the cycle threshold (Ct) values of each sample.

Table 2. Primer sequences for amplicon sequencing and qPCR of airborne bacteria.

Method (target region)	Primer	Sequence
Amplicon sequencing	Forward	5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG 3′
(16S rRNA gene V3–V4 region)	Reverse	5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC 3′
qPCR	Forward	5′-TCCTACGGGAGGCAGCAGT-3′
(16S rRNA gene V3–V4 region)	Reverse	5′-GGACTACCAGGGTATCTAATCCTGTT-3′

2.5. Statistical analysis

The data analysis of the 60 air samples at the genus level was performed using Statistical Package for the Social Sciences (SPSS, version 28) and the vegan package in R (v4.2.2). Unverified genus data were excluded from the analyses. Pearson's correlation coefficients were computed among the environmental factors surveyed. Differences in DNA copy number concentration and alpha diversity indices (Shannon-Weaver and Pielou evenness index) of bacteria among the five building categories and between two seasons were analyzed with analysis of variance (ANOVA) and t-tests (Yang et al. 2022). Tukey's honestly significant difference test was used for multiple comparisons among five building categories (Brown 2005). The Bray-Curtis dissimilarity index was calculated using relative abundance of each bacterial genus in each sample. To compare similarity in bacterial community composition within and between groups, we used nonmetric multidimensional scaling (NMDS) and analysis of similarities (ANOSIM) (Pereira et al. 2021). Multivariate linear regression of log-transformed bacterial copy number/m^3 was performed to examine the adjusted effects of environmental conditions on the concentrations of DNA copy number. The final multivariate model included building category, season, sampled city, occupant density, PM_1 and $PM_{2.5\text{-}10}$ concentration, and CO_2 concentration. Temperature, RH, and $PM_{1\text{-}2.5}$ were excluded from the final multivariate model because of high correlations with season and $PM_{2.5\text{-}10}$ concentration, which resolved multicollinearity issues in the model. We used the Permutational Multivariate Analysis of Variance (PERMANOVA) method to examine the adjusted effects of environmental conditions on the similarity in bacterial community composition using the Bray-Curtis dissimilarity index (Anderson 2017). We determined that the p-value < 0.05 was statistically significant.

3. Result and discussion

3.1. Characteristics of environmental variables

The average PM_{10} concentration in the library ($11.40 \pm 4.35\ \mu g/m^3$) was significantly (multiple comparison p-values < 0.05) lower than in the daycare center ($27.05 \pm 7.10\ \mu g/m^3$) and the parking garage ($29.29 \pm 18.84\ \mu g/m^3$) (Table 1). Parking garages had significantly lower occupant density than train stations, marts, and daycare centers (multiple comparison p-values < 0.01) (Table 1). Similarly, the library had significantly lower occupant density than the marts and daycare centers (p-value < 0.05). The average concentration of CO_2 in the daycare center was significantly (p-value < 0.001) higher than the train station, parking garage, and library. The occupant density in three buildings in Seoul (station #1, mart #2, daycare center #2) was significantly (p-value $<$ 0.001) higher than those in Suwon. The average indoor temperature and RH were significantly (p-value < 0.001) higher in summer ($26.50 \pm 2.51\,°C$ and $62.14 \pm 5.52\%$, respectively) than winter ($15.77 \pm 5.93\,°C$ and $29.46 \pm 10.77\%$, respectively). Figure S2 presents the correlations among environmental parameters. Indoor temperature was positively and highly correlated with RH ($R = 0.75$, p-value < 0.001). Conversely, PM_1 showed negative correlation with indoor temperature ($R = -0.39$, p-value < 0.01). RH was weakly but positively correlated with $PM_{2.5-10}$ ($R = 0.32$, p-value < 0.05). The $PM_{2.5-10}$ had a strong positive correlation with $PM_{1-2.5}$ ($R = 0.78$, p-value $<$ 0.001), while $PM_{1-2.5}$ showed a moderate positive correlation with PM_1 ($R = 0.50$, p-value < 0.001). Only $PM_{2.5-10}$ concentration was weakly correlated with CO_2 concentration ($R = 0.30$, p-value < 0.05).

3.2. Number of bacteria, alpha diversity, and bacterial community composition by building category and season

3.2.1. Bacterial concentration and alpha diversity

In the adjusted ANOVA (Figure 1a), we did not observe significant difference in the airborne concentrations of bacterial DNA among the building categories (p-value $= 0.19$). However, Figure 1a shows that in most locations, the geometric mean (GM) number concentration of bacterial DNA copies was significantly (p-value < 0.001) higher in summer (GM $=$ 6922.57 copy number/m^3; geometric standard deviation, GSD $= 1.14$) than winter (GM $= 2755.94$ copy number/m^3; GSD $= 1.08$). Our finding is in line with those from previous studies of office buildings reporting a higher culturable indoor bacteria in summer than winter (Ding et al. 2016; Guo et al. 2021). They also observed higher indoor temperature and RH in summer that might have increased the levels of airborne bacteria, which was similar to what we observed from our study. The only building that showed a similar indoor bacterial concentration between summer and winter was the #1 daycare center. In this daycare center where infants and toddlers (age: $0-2$ years old) were being cared, a fish tank and indoor potted plants were present nearby the air

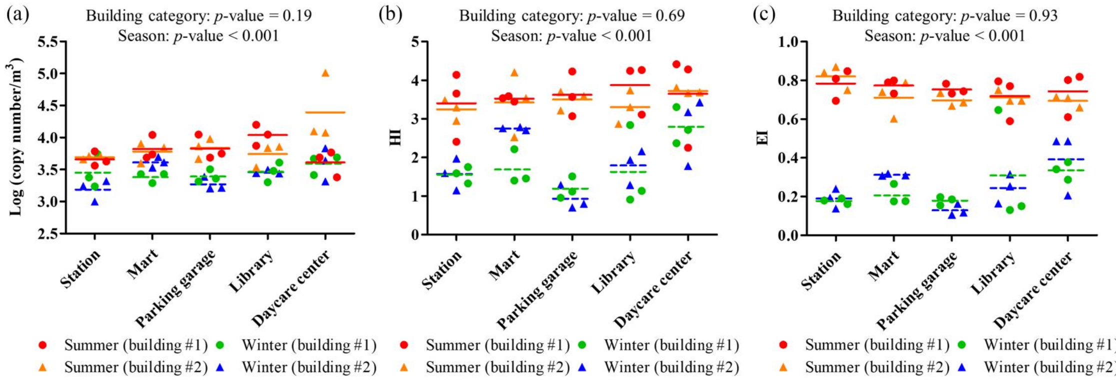

Figure 1. Number concentrations of bacterial DNA copy and alpha diversity indices by building category, building location, and season. (a) Number concentration of bacteria, (b) Shannon-Wiener index (HI), and (c) Pielou's evenness index (EI). The building locations within the same building category were designated by #1 and #2. Bar was the mean of samples.

sampling station. Plant surfaces could be a constant source of airborne bacteria (Miletto and Lindow 2015; Womack, Bohannan, and Green 2010). Aquatic ecosystems had increased levels of microorganisms than in the air (Gołaś, Szmyt, and Glińska-Lewczuk 2022; Kadaifciler and Cotuk 2014), which could influence bacteria in air. Thus, the similarly low levels of bacterial concentration during both seasons in daycare center #1 might be explained by: 1) low level of physical activity in infants and toddlers; 2) mechanical ventilation with limited outdoor air to protect vulnerable infants and toddlers from outdoor air pollutants; and 3) presence of the consistent indoor sources year-round. The highest microbial levels (102,367 copy number/m^3) detected at Daycare Center #2 in summer may be explained by physically active 13 five-year old kids occupying the room under the condition of low natural ventilation rate in summer. Young kids could shed their skin more easily than adults and constantly release bacteria from their skin (Lee et al. 2002). The lowest levels (998 copy number/m^3) at train station #2 that was on the fifth underground floor might have been least affected by direct in-flow of outdoor air that was effectively filtered under high mechanical ventilation rate. Because of the high volume of customers, the train stations usually deliver highly conditioned air with filtration of outdoor air pollutants. We found that the train stations had the lowest average level of CO_2 concentration whereas the daycare centers had the highest level, which might reflect ventilation rate in these types of buildings. There are very limited number of studies of various commercial and institutional buildings like ours that reported airborne bacterial concentrations quantified with the molecular method (qPCR). However, a study that compared concentrations of culturable microorganisms in daycare centers, hospitals, and elderly welfare facilities in Korea, reported the highest microbial levels in the daycare center (Kim and Kim 2007).

The Shannon-Wiener alpha diversity index (HI) was not significantly different (p-value $= 0.68$) among the building types (Figure 1b). The HI indices of all 60 samples in the genus level ranged from 0.32 (in parking garage #2 in winter) to 4.42 (in the daycare center #1 in summer). The highest alpha diversity was observed from the daycare center (HI $= 2.77 \pm 1.17$) while the lowest one from the train station (HI $= 2.03 \pm 1.41$). There was also no significant difference in Pielou's evenness index (EI) among the building types (p-value $= 0.93$) (Figure 1c). The EI index ranged from 0.11 (in parking garage #2 in winter) to 0.87 (in train station #2 in summer). The average EI index was the highest in the daycare center (EI $= 0.54 \pm 0.21$) while it was the lowest in the parking garage (EI $= 0.43 \pm 0.30$). On the other hand, season was a significant factor associated with the bacterial concentration as well as alpha diversity indices in the crude ANOVA. The average alpha diversity was also significantly higher in summer (HI $= 3.53 \pm 0.56$) than winter (HI $= 1.10 \pm 0.68$) (p-value < 0.001) (Figure 1b). Evenness indices describing commonness of bacterial genera in each sample in our study were also significantly (p-value < 0.001) higher in summer (EI $= 0.74 \pm 0.07$) than winter (EI $= 0.25 \pm 0.12$) (Figure 1c). This finding is likely related to significantly higher indoor temperature and RH in summer than winter. Dannemiller also reported that the alpha diversity of bacteria in floor dust increased with the increase in equilibrium relative humidity (Dannemiller, Weschler, and Peccia 2017). A study of school indoor air showed a positive association between indoor temperature and evenness of bacteria (Lee et al. 2021). Airborne bacteria in our study buildings might have also been affected by resuspension of microbiota in floor dust by human activities as suggested by Bhanger et al.'s study finding that over 60% of aerosol particles are generated from the floor (Bhangar et al. 2016). Our study finding of higher bacterial concentration in summer might have resulted from higher bacterial diversity with more similar abundance across the bacterial genera in summer than winter.

3.2.2. Bacterial community composition and relative abundance by building category and season

Figure 2 shows the relative abundances of the top 20 bacterial genera within each building category and sampled season. The top three most abundant bacterial genera in each category were: *Stenotrophomonas*, *Nesterenkonia*, and *Dulcicalothrix* in train station; *Stenotrophomonas*, *Aerosakkonema*, and *Corynebacterium* in mart; *Stenotrophomonas*, *Brevibacillus*, and *Pseudescherichia* in parking garage and library; and *Stenotrophomonas*, *Brevibacillus*, and *Moraxella* in daycare center. The genus *Stenotrophomonas* had the highest relative abundance of >30% in all building categories (Figure 2a). This genus is ubiquitous in environments and commonly found in soil and plants. Within the genus, the species *S. maltophilia* is the only known species to cause human disease as an opportunistic pathogen (Ryan et al. 2009; Wang et al. 2018). *Brevibacillus*, commonly found in parking garage, library, and daycare center in our study, is frequently identified in dust, water, and soil. This genus has been associated with human infections and known as a significant bacterium that promotes plant growth (Panda et al. 2014; Ray, Patel, and Amin 2020). Lee et al.' study of microbiota in toy and furniture surfaces in four daycare classrooms caring for infant to four-year-old children found high abundance of the families *Pseudomonadaceae* and *Oxalobacteraceae* that were possibly from children (Lee, Tin, and Kelley 2007). However, they were not detected in air in our daycare center. The genus *Aerosakkonema* was only observed in the mart in our study, but only a few species within the genus were reported globally as freshwater bacteria (Thu et al. 2012). *Nesterenkonia* found abundantly in the train station is ubiquitous in nature and can even be found in extreme environments, such as saline soils, deserts, and wastewater treatment plants (Chander et al. 2017). Some species of *Nesterenkonia* have been found in fermented seafood (Yoon et al. 2006). *Corynebacterium* was uniformly observed in all facilities in our study and has been widely shown to be pathologically associated with humans and animals. The genus *Pseudescherichia*, Gram-negative bacteria derived from the genus *Escherichia*, was also discovered as a member of human gut bacteria (Alnajar and Gupta 2017; Brenner et al. 1982). Other bacterial genera that may be human pathogens include *Sphingobacterium* (Tronel et al. 2003), *Moraxella* (Murphy and Parameswaran 2009), and *Streptococcus* (Brandt and Spellerberg 2009). The majority of the most frequently identified bacterial genera described above are ubiquitous in our surrounding environments such as plants, water, and soil, indicating that these bacteria might have been introduced into indoor environments through ventilation from outside. The genera *Stenotrophomonas, Corynebacteria, Pseudescherichia, Sphingobacterium, Streptococcus* are also found on human skin and mucous membranes. The genus *Aerosakkonema* associated with food including kimchi (Hong et al. 2021) were the second most abundant in mart in our study.

As shown in Figure 2b, the relative abundance top 20 bacterial genera were quite different between summer and winter. *Brevibacillus*, *Aerosakkonema*, and *Pseudescherichia* were the most abundant top three bacteria in summer samples while *Stenotrophomonas*, *Brevibacillus*, and *Acinetobacter* were the most abundant ones in winter. Diverse bacteria without a predominant genus in summer as described earlier (i.e., significantly higher Shannon-Wiener index and evenness index in summer than winter) were identified in

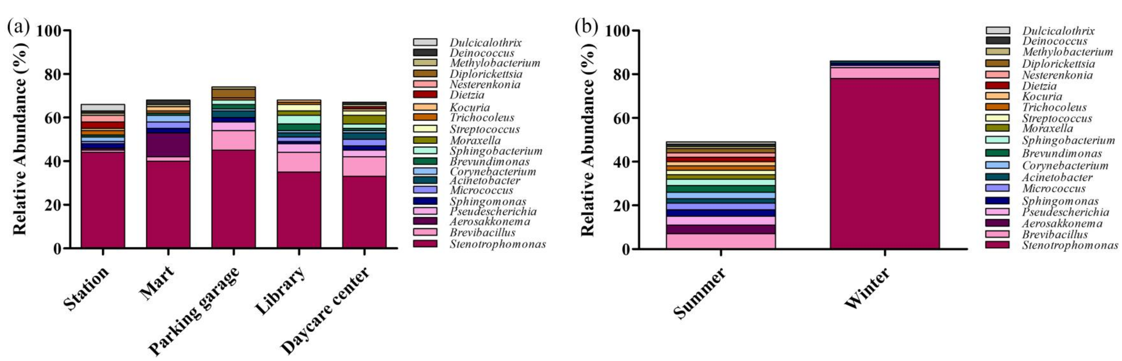

Figure 2. Relative abundance of top 20 bacterial genera by building category (a) and season (b).

all buildings while *Stenotrophomonas* was predominant in winter. The harsh environment in winter likely decreased the growth of common bacteria, and would have caused the dominance of *Stenotrophomonas*, which is highly adaptable in a limited environment (Ryan et al. 2009). A study of indoor air quality in Kunming, China, showed that *Stenotrophomonas* was also the dominant genus among culturable bacteria in five categories of buildings (hospital, office, library, school, and home) in winter (Wang et al. 2022).

From the NMDS plot (Figure 3a), we did not observe the overlap in the 95% confidence ellipses of community composition between daycare center and train station. In addition, there was a little overlap in the 95% confidence ellipses of bacterial composition between the mart and the daycare center, and parking garage and the daycare center while there was a small overlap between the public library and the daycare center. The genera with human skin origin such as *Moraxella* and *Streptococcus* were found only in the library and daycare center. These findings indicated that the daycare center had somewhat similar bacterial community composition to the library, but distinctive composition from those in the train station, the mart, and the parking garage. The community compositions between the mart and the train station also seem somewhat different from each other (Figure 3a). ANOSIM boxplot (Figure 4a) also confirmed the findings of the NMDS plot that there was significant (p-value = 0.04) dissimilarity in bacterial composition among the building categories although the effect size was subtle ($R = 0.072$). ANOSIM also revealed that bacterial composition in the daycare center was the least dissimilar and variable among all building categories (Figure 4a). Our finding that *Stenotrophomonas*, *Brevibacillus*, *Sphingomonas*, *Corynebacterium*, and *Brevundimonas* were found in all facilities and included in the top 20 of the genera (Figure 2a) also supported why there were only small cluster differences among the building categories although the difference was statistically significant.

The effect of season on the bacterial diversity and community composition was most evident in our study (Figures 3b and 4b). Especially, the substantially large R statistics in ANOSIM ($R = 0.912$, p-value = 0.001) indicated that the effect of season on the bacterial community composition was predominantly stronger than any other environmental factors in our study. The ANOSIM plot also indicated that the bacterial composition was more dissimilar in summer than winter. A previous study collected samples in four distinctive seasons documented that summer had a different bacterial community composition from winter but had the similar composition to autumn (Bowers et al. 2012). A longitudinal study of two buildings over a year also reported significant differences in the composition of bacterial species between summer and winter (Rintala et al. 2008).

3.3. Associations of environmental conditions with bacterial concentrations and community composition

Our multivariate linear regression model explained 76.9% (adjusted R^2=0.50) of the total variance in log-transformed DNA copy number concentration. From the model, we found that season and CO_2 were significantly (p-values < 0.015) associated with airborne bacterial concentration (Table 3). The bacterial concentration in winter was significantly lower than summer after adjusting for other environmental conditions. CO_2 concentration was positively associated with airborne bacterial concentration. The indoor CO_2 concentration can be influenced by multiple factors such as ventilation rate, occupancy rate, and occupants' activity level (Lazovic et al. 2016; Settimo

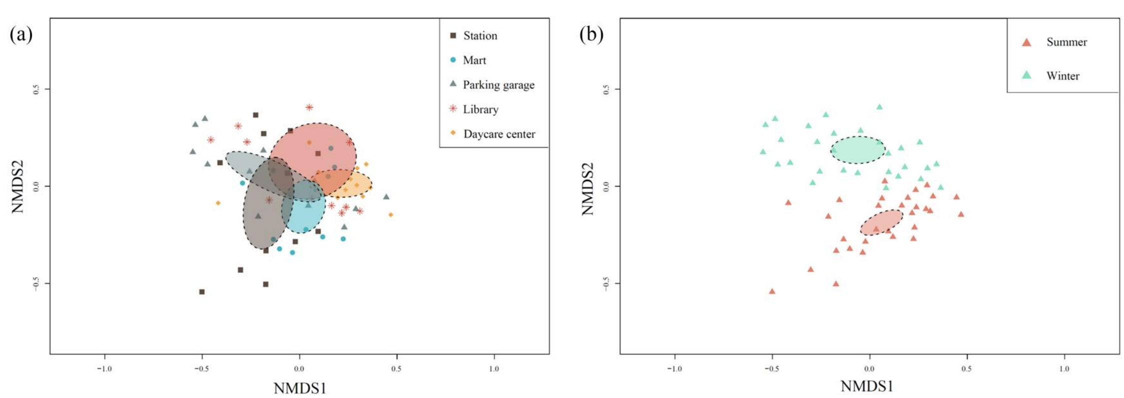

Figure 3. Non-metric multidimensional scaling (NMDS) plot with 95% confidence interval (CI) ellipse using the Bray–Curtis dissimilarity index at the genus level for bacteria by building category (a) and season (b).

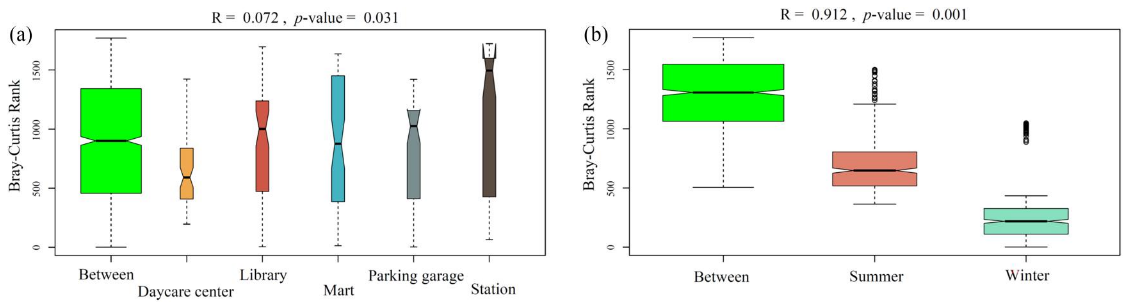

Figure 4. Box plots of analysis of similarity (ANOSIM) at the genus level of bacteria by building category (a) and season (b).

Table 3. Results from univariate and multivariate regression models of log (bacterial DNA copy number/m^3) for environmental factors.

Multi Regression of log (Bacteria copy number/m^3) by environmental factors						
R^2(0.769), Adjusted R^2(0.504), p-value < 0.001***						
	Unadjusted Coefficient		Adjusted Coefficient			
Variable	B	Std. Error	Beta	t-value	p-value	VIF[a]
Seoul (Ref)						
Suwon	−0.036	0.111	−0.053	−0.321	0.750	3.133
Daycare center (Ref)						
Station	−0.147	0.117	−0.192	−1.260	0.214	2.663
Mart	0.010	0.104	0.013	0.093	0.926	2.121
Parking garage	0.041	0.137	0.049	0.296	0.768	3.202
Library	0.081	0.130	0.106	0.627	0.533	3.290
Summer (Ref)						
Winter	−0.312	0.070	−0.502	−4.448	**0.001**	1.462
$PM_{2.5-10}$[b]	0.012	0.010	0.149	1.164	0.250	1.882
PM_1	−0.003	0.003	−0.111	−1.030	0.308	1.326
CO_2	4.E-04	2.E-04	0.337	2.549	**0.014**	2.014
Density	0.172	0.315	0.101	0.545	0.588	3.977

[a]Variance Inflation Factor.
[b]PM_{10} minus $PM_{2.5}$.
Bold numbers: Statistically significant.
Reference: Reference group used for comparison for categorical variables.

et al. 2020) and may be a surrogate measure of a combination of these factors in our study. Kwan et al. reported that human occupancy and resuspension are significant sources of the increase in indoor microbes in residential buildings (Kwan et al. 2020). The human occupancy of rooms increased bacterial abundance by approximately 81 times, compared to non-occupied environments (Hospodsky et al. 2015). In a chamber study, the fluorescent biological aerosol particles emission rate increased by 5–6 times from human walking, with more than 60–70% of the emissions originating from the floor (Bhangar et al. 2016). The coarse PM concentration in our study was positively associated with the bacterial concentration but with no statistical significance. A previous study demonstrated significant positive association of coarse particles with airborne bacterial DNA copy number concentration in school classrooms (Yang et al. 2022) and with airborne cultivable bacterial concentrations in coastal cities (Montero, Dueker, and O'Mullan 2016). Altogether, the positive but weak association of CO_2 concentration with airborne bacterial levels that we found in this study might be associated with human occupants who are one of the strong bacterial sources in indoor environments.

From PERMANOVA including the same variables in the multivariate regression model of bacterial concentration, we found that building category, season, and CO_2 concentration were significantly (both p-values < 0.04) associated with the bacterial community composition (Table 4). Season had the most significant impact, accounting for 44% of the total variation in the microbial dissimilarity, followed by building category (13%), and CO_2 concentration (2%). These findings from the PERMANOVA model also supported the results of NMDS plots and ANOSIM. The effect of the building category on community composition in our study is most likely explained by the different purpose and usage of the building. For example, unique bacterial compositions in daycare center, train station, and mart (Figure 3) could be driven by building environments and users that are quite different from one another. A positive correlation between CO_2 levels and the abundance of

Table 4. Results of permutational multivariate analysis of variance (PERMANOVA) of bacteria community composition (using Bray-Curtis dissimilarity index) associated with environmental factors.

	PERMANOVA model parameters				
Covariate	Degree of freedom	Sum of squares	R^2	F	*p*-value
Season	1	7.5	0.44	55.63	**0.001*****
Building category	4	2.20	0.13	4.05	**0.001*****
City	1	0.29	0.02	2.11	0.081
$PM_{2.5\text{-}10}$	1	0.29	0.02	2.15	0.078
PM_1	1	0.08	0.01	0.60	0.723
CO_2	1	0.34	0.02	2.52	**0.031***
Density	1	0.21	0.01	1.57	0.146
Residual	47	6.37	0.35		
Total	57	17.38	1		

Bold numbers: Statistically significant.

commuter-related bacterial genera such as *Enhydrobacter* and *Micrococcus* has been also reported by a study of Hong Kong subway network using DNA sequencing data (Leung et al. 2014). The authors also suggested this association might be driven by the level of commuter density using the subway system.

One of the limitations of our study is that we had only two buildings for each category in two geographical regions. It was extremely challenging to obtain permission from management to access the buildings for our sampling because of concerns for viral transmission during the COVID-19 pandemic period. Even with this challenge, we were able to recruit ten buildings and collect 60 air samples in summer and winter. However, because of the limited sample size per category in limited regions, caution should be exercised when generalizing the study findings. Even with this limitation, considering that data on indoor microbiota from nonresidential and non-school buildings using culture-independent method are scarce, our study findings provide very useful and basic information on indoor microbiota and associated environmental factors in institutional and commercial buildings. In future studies, a longitudinal sampling for a longer period of time in many institutional and commercial buildings in different regions and collection of more detailed information on building design and characteristics would be the most useful to better elucidate associations of those building environmental factors with microbial compositions. This research could provide valuable information on designing healthier buildings with healthier indoor microbes.

4. Conclusions

This study investigated the bacterial concentration and community composition in air, and their associations with indoor environmental conditions, using qPCR and amplicon sequencing. Our study indicated that season significantly influenced not only higher bacterial concentrations but also community composition with more diverse and larger evenness in summer than winter. Although building category was not associated with bacterial concentration, it substantially affected community composition in each category of buildings. Indoor CO_2 concentration was also significantly associated with both bacterial concentration and composition, which might be linked to occupancy and ventilation rate of the buildings. Our study data provide basic but useful information for managing indoor microbes for public health in studied institutional and commercial buildings. Our study implies that building management and public health officials should understand the effects of season and occupancy and ventilation rate on microbial concentration and community composition to properly observe IAQCA standards and maintain healthy indoor microbial environments in those buildings.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

Disclosure statement

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

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Data availability statement

The raw data that support the findings of this study are available from the corresponding author. Upon reasonable request, we will endeavor to share the requested data in consultation with the funding agency.

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