



Contents lists available at ScienceDirect

## Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)

# Identification of airborne bacterial and fungal community structures in an urban area by T-RFLP analysis and quantitative real-time PCR

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## ARTICLE INFO

## Article history:

Received 30 December 2008

Received in revised form 6 August 2009

Accepted 23 October 2009

Available online xxxx

## Keyword:

Bioaerosols

Molecular analysis

Bacteria

Fungi

TSP

## ABSTRACT

This study explores the characteristics of bacterial and fungal communities of total suspended particles (TSP) in the atmosphere by using various molecular methods. TSP samples were collected on a glass fiber filter at an urban location in the middle of the Korean Peninsula (Seoul) between middle autumn and early winter in 2007. From the aerosol samples, DNA could be extracted and DNA sequences were determined for bacteria and fungi. Terminal restriction length polymorphism (T-RFLP) analysis was applied to analyze the community structure of them. To estimate the concentration of DNA originating from bacterial and fungal communities, we used the quantitative real-time polymerase chain reaction (Q-PCR). Sequence analyses were also used to determine the identity of biological organisms. The number of bacteria and fungi in the air were between  $5.19 \times 10^1$  and  $4.31 \times 10^3$  cells  $m^{-3}$  and from  $9.56 \times 10^1$  to  $4.22 \times 10^4$  cells  $m^{-3}$ , respectively and bacterium/fungus ratios ranged from 0.09 to 0.76 across the seven sampling dates. Most of the bacterial sequences found in our TSP samples were from *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*. The fungal sequences were characteristic for *Ascomycota*, *Basidiomycota*, and *Glomeromycota* which are known to actively discharge spores into the atmosphere. The plant sequences could be also detected. We found that large shifts in the community structure of bacteria and fungi were present in our TSP samples collected on different dates. The results demonstrated that in our TSP samples collected at the urban site; (1) there were very diverse bacterial and fungal groups including potential pathogens and allergens and (2) there were temporal shifts in both bacterial and fungal communities in terms of both diversity and abundances across an inter-seasonal period.

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## 1. Introduction

Aerosols in the air have a size distribution of approximately  $5.0 \times 10^{-4}$ – $5.0 \times 10^2 \mu m$  in the diameter, and the diameter of collected suspended particles ranges with  $1.0 \times 10^{-1}$ – $1.0 \times 10^2 \mu m$ , typically (Jaenicke, 2005). As aerosols are inhaled, they may cause damage to not only the respiratory tract but also the cardiovascular system. The major factors that determine the degree of damage are size and chemical composition (Laden et al., 2000).

In addition to soil and water environments, atmosphere is one of the biospheres harboring microorganisms, which include bacteria, fungi, and viruses. Most of them are present and transmitted in the form of biogenic aerosols, which originated from oceans, soil, wastewater treatment plants, domestic farms, agricultural factories, waste landfills, animal manure, or respiratory tracts. In the outdoor environment, it seems that microorganisms may not be able to survive for a long time due to the circulation of air impacted by wind

and the exposure to UV by sunlight. However, some of them might cause or enhance plant, animal, and human diseases such as pneumonia and allergies, while most of atmospheric microorganisms are harmless to humans (Salem and Gardner, 1994).

Therefore, the study on airborne microorganisms is important in many respects such as ecology and public health. However, there are only a few studies on microorganisms in the atmosphere, particularly in the outdoor environment. For example, Shinn et al. (2000) reported that microbes were transported across the Atlantic by desert dust storms and influenced the reef ecosystem in the Caribbean basin. Bauer et al. (2002) reported that microorganisms could colonize remote environments by way of bacterial fluxes. Moreover, until recently, conventional microbial analytic approaches such as microscopy and cultivation methods have been generally used for the analysis of airborne microorganisms. Molecular techniques are widely used for analysis of microorganisms in the environment since they have high sensitivity and are time-saving. Recently, these molecular analyses were applied to areas of airborne microbiology (Boreson et al., 2004; Hughes et al., 2004; Maron et al., 2005; Kuske et al., 2006; Brodie et al., 2007), which revealed that the diversity of airborne microorganisms was higher than that from traditional methods and

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varied spatially and temporally. However, these studies had been focused on only one specific group of organisms (bacteria or fungi) or on the community structure (diversity) of organisms.

In the present study, we conducted the analysis of both bacterial and fungal community structures in TSP samples using the T-RFLP method as a fingerprinting method and sequence analysis of some distinct OTUs (operational taxonomic units) to identify the type of organisms, specifically. Additionally, we performed quantitative real-time PCR to estimate the density of bacteria and fungi in TSP samples, which had little been applied in earlier studies. Finally, through these analyses, we investigated the inter-seasonal variability in airborne bacterial and fungal communities.

## 2. Materials and methods

### 2.1. TSP sample collection

Seoul is the capital of Korea with 10 million inhabitants and 2.5 million vehicles in 2007. Samples were collected on a 3 m high platform on the roof of Asan Hall in Ewha Womans University, a five-story building of 15 m height at Seoul, Korea. It is adjacent to a road on the West, Mt. Ansan on the North, and stands on a hill commanding the campus. Sampling was carried out for 24 h at every sixth day with no rain from October 11th to December 1st, 2007. A total of 7 samples were obtained. Detailed descriptions on the sampling location and procedure were given in Lee and Kim (2007). In brief, a high volume TSP sampler (Tisch Environmental, TE-1000) was operated at a calibrated flow rate of  $0.225 \text{ m}^3 \text{ min}^{-1}$  to obtain the total sample volume of greater than  $300 \text{ m}^3$  over the 24 h period. Particles were collected on the glass fiber filters (Whatman, G653). After collection of air samples, we seized each filter with a size of  $50.0 \times 50.0 \text{ mm}$  and each seized filter harbored approximately 0.4 g of suspended particulates. For alleviating the bias due to the use of a small part of the filter, we prepared a duplicate seized filter and used it for the next analysis. Until analysis, the sample filters were stored in a refrigerator at  $-20^\circ\text{C}$ . Analysis was carried out within a month of the first aerosol sample.

### 2.2. Molecular analyses of bacterial and fungal communities in TSP samples

DNA was extracted from the seized filter using the MoBio Ultraclean™ Soil DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA) according to the manufacturer's instructions. To check for sample contamination, a parallel DNA extraction procedure was performed with the unused filter which was used as "blank" for further analysis. DNA samples were amplified by PCR using the fluorescently labeled forward primer 27F (5'-[6FAM]-AGAGTTT-GATCCTGGCTCAG-3') and the unlabeled reverse primer 927R (5'-CCGTCAATTCCTTTTTRAGTTT-3'), which target bacterial 16S rRNA genes (Lane, 1991). For fungal communities, PCR was performed using the fluorescently labeled forward primer ITS1F (5'-[Hex]-CTTGGTCATTTA-GAGGAAGTAA-3') and the unlabeled reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), which target the fungal internal transcribed spacer (ITS) region of the rRNA gene (Gardes and Bruns, 1993; White et al., 1990). Reaction mixtures (50  $\mu\text{l}$  reactions) contained TSP sample DNA (100 ng), *Taq* DNA polymerase (2.5 U), PCR buffer (1 $\times$ ; 10 mM KCl, 10 mM  $(\text{NH}_4)_2\text{SO}_4$ , 20 mM Tris-HCl, 2 mM  $\text{MgSO}_4$ , 0.1% Triton X-100, pH 8.8), deoxynucleoside triphosphates (0.2 mM each), forward and reverse primers (0.2  $\mu\text{M}$  each) and bovine serum albumin (8  $\mu\text{g}$ ).

DNA of each sample was amplified using an MJ Research thermal cycler PTC 100 (MJ Research, Waltham, MA) and the following program was applied for analyzing both bacterial and fungal communities: 5 min  $94^\circ\text{C}$ , followed by 35 cycles of  $94^\circ\text{C}$  for 1 min,  $50^\circ\text{C}$  for 1 min and  $72^\circ\text{C}$  for 1 min and 30 s, and a final extension step at  $72^\circ\text{C}$  for 10 min. For each sample, the PCR products of two reactions were pooled and

purified using a MoBio PCR purification kit (MoBio) according to the manufacturer's instructions.

For T-RFLP analysis, approximately 300 ng of purified PCR product was added to a reaction mixture (final vol. 25  $\mu\text{l}$ ) containing 10 U of restriction endonuclease HhaI (Promega, Madison, WI) and incubated at  $37^\circ\text{C}$  for 4 h. Digests were desalted and aliquots (1  $\mu\text{l}$ ) were used for T-RFLP analysis. Terminal fragment-size analysis was performed using ABI 377 DNA Analyzers (Applied Biosystems) in conjunction with the GeneScan software (Applied Biosystems).

### 2.3. Statistical analyses of T-RFLP data

Terminal reaction fragments (T-RFs) were quantified by peak area integration using a minimum peak height threshold of 50 relative fluorescent units. We excluded T-RFs below size 35 and calculated the proportion of each T-RF in each sample. T-RFs having proportion below 0.1% were excluded for subsequent analyses. We analyzed the T-RFLP profile using multivariate statistical analyses, and nonmetric multidimensional scaling (NMS) method with PC-ORD version 5.0 software (MjM Software, Oregon). The NMS method has been known to be adequate to investigate the similarities among samples based on the information of species composition (McCune and Grace, 2002).

### 2.4. Real-time PCR for 16S rRNA gene and ITS region

To estimate the bacterial and fungal biomass, we used the quantitative real-time PCR (Q-PCR) method. Q-PCR was performed using an I-Cycler™ (Version 3.0a, Bio-Rad, Hercules, CA) and SYBR Green as the detection system (Bio-Rad) in a reaction mixture of 30  $\mu\text{l}$  with the specific primer set for each group (for bacteria; 27F-927R and for fungi ITS1-ITS4 primer pair was used, respectively). The amplification followed a three step PCR for both bacterial 16S rRNA gene and fungal ITS regions: 40 cycles with denaturation at  $94^\circ\text{C}$  for 25 s, primer annealing at  $50^\circ\text{C}$  for 25 s, and extension at  $72^\circ\text{C}$  for 25 s. Two independent real-time PCR assays were performed on each of the two replicate TSP sample DNA extracts. A standard curve was created using 10-fold dilution series of plasmids containing the bacterial 16S rRNA gene and fungal ITS region from environmental samples.

### 2.5. 16S rRNA gene and ITS region cloning, sequencing, and phylogenetic analyses

16S rRNA and ITS region fragments generated from the PCR of TSP sample DNA were cloned using a pGEM-T Easy Vector system (Promega) according to the manufacturer's instructions. PCR products were pooled from all the TSP samples. Clones were screened using a colony-PCR method with specific primers for the bacteria and fungi described above. 43 and 52 clones were sequenced for analysis of bacterial and fungal communities, respectively. Plasmids were prepared using Wizards miniprep (Promega) according to the manufacturer's instructions. Capillary sequencing was performed on ABI 3730 XL DNA Analyzers. Resulting sequences were queried against GenBank using BLAST. Nucleotide sequences of 16S rRNA gene and ITS region were aligned using Clustal\_X, and trees were constructed using the neighbor-joining algorithm (PHYLP) with bootstrap analysis of 1000 replicates.

### 2.6. Nucleotide sequence accession numbers

The GenBank accession numbers of sequences from this study are FJ213458 to FJ213498 for bacterial 16S rRNA gene fragments and FJ213499 to FJ213550 for fungal ITS region fragments.

**Table 1**

Diversity of eubacterial and fungal communities in the air samples calculated from the data of T-RFLP analysis.

|             | Eubacteria |             |             | Fungi    |             |             |
|-------------|------------|-------------|-------------|----------|-------------|-------------|
|             | <i>S</i>   | <i>E</i>    | <i>H</i>    | <i>S</i> | <i>E</i>    | <i>H</i>    |
| Oct-11-2007 | 96 ± 4     | 0.88 ± 0.01 | 4.02 ± 0.04 | 71 ± 2   | 0.73 ± 0.00 | 3.10 ± 0.02 |
| Oct-18-2007 | 95 ± 13    | 0.84 ± 0.02 | 3.83 ± 0.18 | 92 ± 1   | 0.83 ± 0.01 | 3.76 ± 0.01 |
| Oct-25-2007 | 70 ± 9     | 0.87 ± 0.04 | 3.66 ± 0.06 | 74 ± 1   | 0.79 ± 0.08 | 3.39 ± 0.34 |
| Nov-01-2007 | 102 ± 2    | 0.86 ± 0.02 | 3.99 ± 0.12 | 60 ± 16  | 0.75 ± 0.02 | 3.03 ± 0.14 |
| Nov-08-2007 | 51 ± 15    | 0.83 ± 0.07 | 3.19 ± 0.03 | 46 ± 3   | 0.63 ± 0.02 | 2.42 ± 0.01 |
| Nov-23-2007 | 80 ± 1     | 0.85 ± 0.01 | 3.70 ± 0.02 | 39 ± 4   | 0.71 ± 0.20 | 2.60 ± 0.80 |
| Dec-01-2007 | 48 ± 2     | 0.77 ± 0.01 | 2.96 ± 0.01 | 70 ± 9   | 0.70 ± 0.01 | 2.97 ± 0.14 |

*S* = richness = number of species (T-RFs) in samples.

*E* = evenness =  $H/\ln S$ .

*H* = diversity =  $-\sum (P_i \cdot \ln(P_i))$  = Shannon's diversity index.

## 2.7. Backward trajectory analysis

To further understand the temporal variability in bacterial and fungal communities, backward trajectory analysis was performed for the sampling days using the HYSPLIT 4 (Hybrid Single-Particle Lagrangian Integrated Trajectory) model (Draxler and Rolph, 2008; Rolph, 2008). Back trajectories were calculated using the vertical velocity method for 72 h at the three starting heights of 500, 1500, and 3000 m, respectively. The trajectory starting time was set at 9 pm in LST. GDS meteorological data were used for the analysis.

## 3. Results and discussion

### 3.1. DNA extraction and PCR analyses with TSP samples

To apply a molecular approach into the investigation of biological organisms in the environment including atmosphere, it is necessary to establish the appropriate DNA extraction method. In this study, we

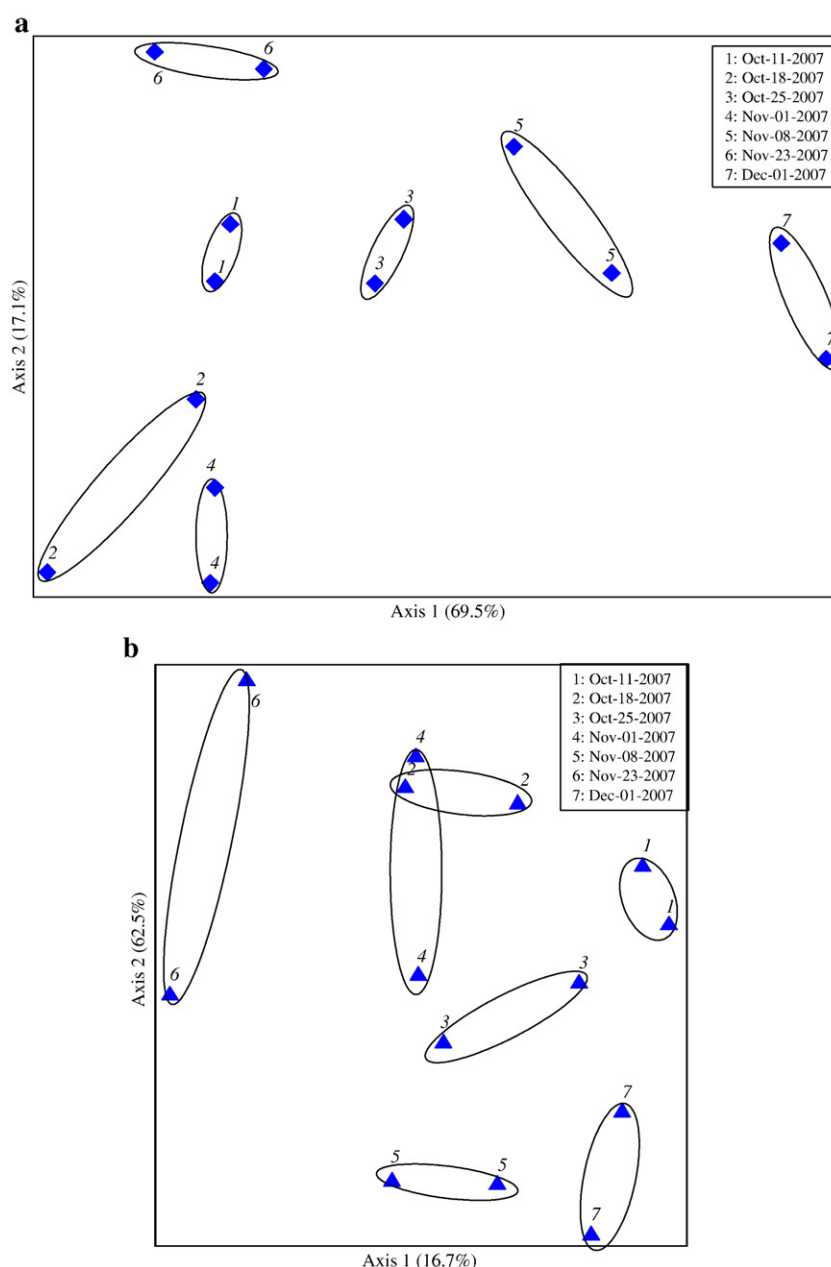


**Fig. 1.** NMS ordination of microbial community structure in the TSP samples. (a) Bacterial community and (b) fungal community.

applied the soil DNA extraction protocol into the extraction of DNA from the atmosphere, assuming that filters harboring TSP have similar characteristics with soils. With the DNA extract from each of the samples, three PCRs have been performed to amplify DNA from bacteria and fungi. PCR products were obtained for both categories and no PCR products were obtained with control samples, which indicated that the DNA extraction method used in this study was appropriate for molecular monitoring of airborne microorganisms.

### 3.2. Diversity and temporal variability of bacterial and fungal communities in TSP samples

The airborne bacteria and fungi collected in this study were phylogenetically diverse. Across all samples, we obtained the unique 158 T-RF peaks (OTUs) for bacteria and 122 for fungi, respectively. This result was comparable to those of other studies which showed a highly diversified microbial community in air samples (Maron et al., 2005; Radosevich et al., 2002). The number of T-RF peaks of the T-RFLP profile of microbial community in TSP samples ranged from 36 to 108 for bacterial and from 35 to 93 for fungi, respectively (Table 1). The Shannon diversity of microbial community ranged from 2.95 to 4.01 for bacteria and from 1.80 to 3.77 for fungi, respectively (Table 1).

These values are lower than those ( $H=4.38$ ) of other studies for airborne bacteria (Maron et al., 2005) and those ( $H=4.33$ ) reported in the soil environment (Hill et al., 2003), indicating that our air samples were dominated by some specific microbial groups. Another explanation for such a discrepancy is that we used the molecular fingerprinting data such as T-RFLP profiles for calculating the diversity indices, which may overlook the detailed taxonomic groups of bacteria in samples and lead to the underestimation of species diversity. This is the same for fungal community structure indicating that the species diversity of fungal community in our TSP samples might be higher than that estimated from this study.

The species richness and diversity indices for microbial community varied among samples for both bacteria and fungi. In particular, the diversity of microbial community was lower in the TSP samples from November 8th than those before November 8th. There were noticeable shifts in the bacterial and fungal community structures across the seven TSP samples (Fig. 1). The NMS ordination results showed the shifts in the airborne bacterial and fungal community structures across the sampling dates. In particular, the bacterial community in the air samples collected on Nov. 8th, 2007 was distinct from those found in the other samples.

For fungal community, we also observed the temporal variability of airborne fungi, which suggests that fungi could be changed with time of two-month period.

Interestingly, the structure of microbial community in our TSP samples shifted during the period at November 8th and December 1st for both bacteria and fungi (Fig. 1). This pattern was observed in diversity index results showing that the bacterial and fungal diversities in TSP samples at late-sampling dates were lower than those at early-sampling dates (Table 1). The NMS results revealed that the microbial community structure in some TSP samples were different among the sub-samples suggesting a high variability of microbial community in TSP samples (Fig. 1). These results were similar to those of Fierer et al (2008), which reported a short-term temporal variability of airborne bacteria.

In Fig. 2, the backward trajectory analysis results are shown. Backward trajectory analysis has been widely used to identify probable air parcel transport patterns (Park et al., 2004; Lee and Kim, 2007). In Korea, during late autumn and early winter, typical backward trajectories are from northwest China based on cluster analysis (Park et al., 2004). However, during the sampling dates in this study, the backward trajectories are from all directions as shown in Fig. 2. Also, no relationship could be found between the shift of the structure of microbial community and air parcel trajectory. Thus, it is likely that the shift of the microbial community was caused by seasonal change of the community.

### 3.3. Types and abundances of bacteria and fungi in TSP samples

To analyze bacterial and fungal communities in TSP samples more specifically, we inferred the identity of each T-RF by *in silico* digestion of bacterial 16S rRNA gene databases and the fungal ITS region of rRNA gene databases in GenBank (Fig. 3). We also sequenced some clones for both bacterial and fungal genes from all samples (Figs. 4 and 5).

In this study, overall, we observed that the TSP samples were dominated by alpha *Proteobacteria* (23.4%), gamma *Proteobacteria* (15.9%), *Firmicutes* (15.6%), *Actinobacteria* (12.7%), beta *Proteobacteria* (10.7%) and *Bacteroidetes* (7.6%) by *in silico* digestion of T-RFLP profiles. Fierer et al. (2008) reported that bacteria affiliated with *Bacteroidetes* were common in many of the air samples. Also in this study, we observed that *Bacteroidetes* were detected across all samples.

However, the proportion of each group per sample was different among the samples. Similar to the result of NMS ordination, the samples collected at later-times were distinguished from those at early-times

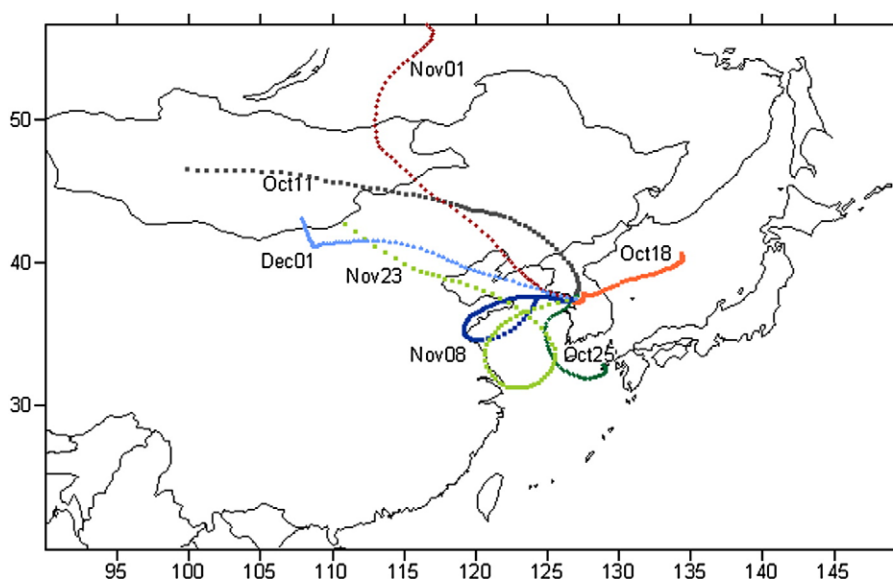


Fig. 2. Backward trajectory analysis results arriving at 1500 m in Seoul for the sampling day.

(Fig. 3). This result was comparable to that of Maron et al. (2005) reporting that the most represented bacterial groups were the *Proteobacteria*, the *Firmicutes*, and the *Actinobacteria*. However, in that study they found no bacteria affiliated with the *Bacteroidetes*, but we found that there were many numbers of bacteria classified as *Bacteroidetes*. This observation was confirmed by a sequence analysis revealing the presence of clones affiliated with the *Bacteroidetes* (Fig. 5).

The results of sequence analysis for bacterial community also showed the presence of diverse bacterial species in our samples, which is similar to those of T-RFLP analysis (Fig. 4). Of 43 sequences analyzed, we obtained 41 bacterial sequences, which consisted of 19 *Proteobacteria*, 7 *Actinobacteria*, 5 *Firmicutes*, 3 *Bacteroidetes*, and other taxa including unidentified sequences. Two sequences were determined as one originated from plant

(*Platanus*) and plant pathogen (*Phytophthora*), which may be due to the proximity of our sampling site from a forest.

Regarding the *Bacteria* detected in our TSP samples, *Proteobacteria* is one of the largest groups in the domain *Bacteria*. They include many pathogens as well as saprophytic or symbiotic bacteria in soil habitat. *Actinobacteria* and *Firmicutes* are also commonly found in soil and most of them are Gram positive bacteria, many of which form spores for aerial transport. *Bacteroidetes* are known to be distributed in soil, sediments, sea water and in the guts of animals and some of them such as genus *Bacteroides* are opportunistic pathogens.

As with the airborne bacteria, the airborne fungi collected in this study were also diverse. Four phyla for fungal domain were found in our samples, which included *Ascomycota*, *Basidiomycota*, *Glomeromycota*, and

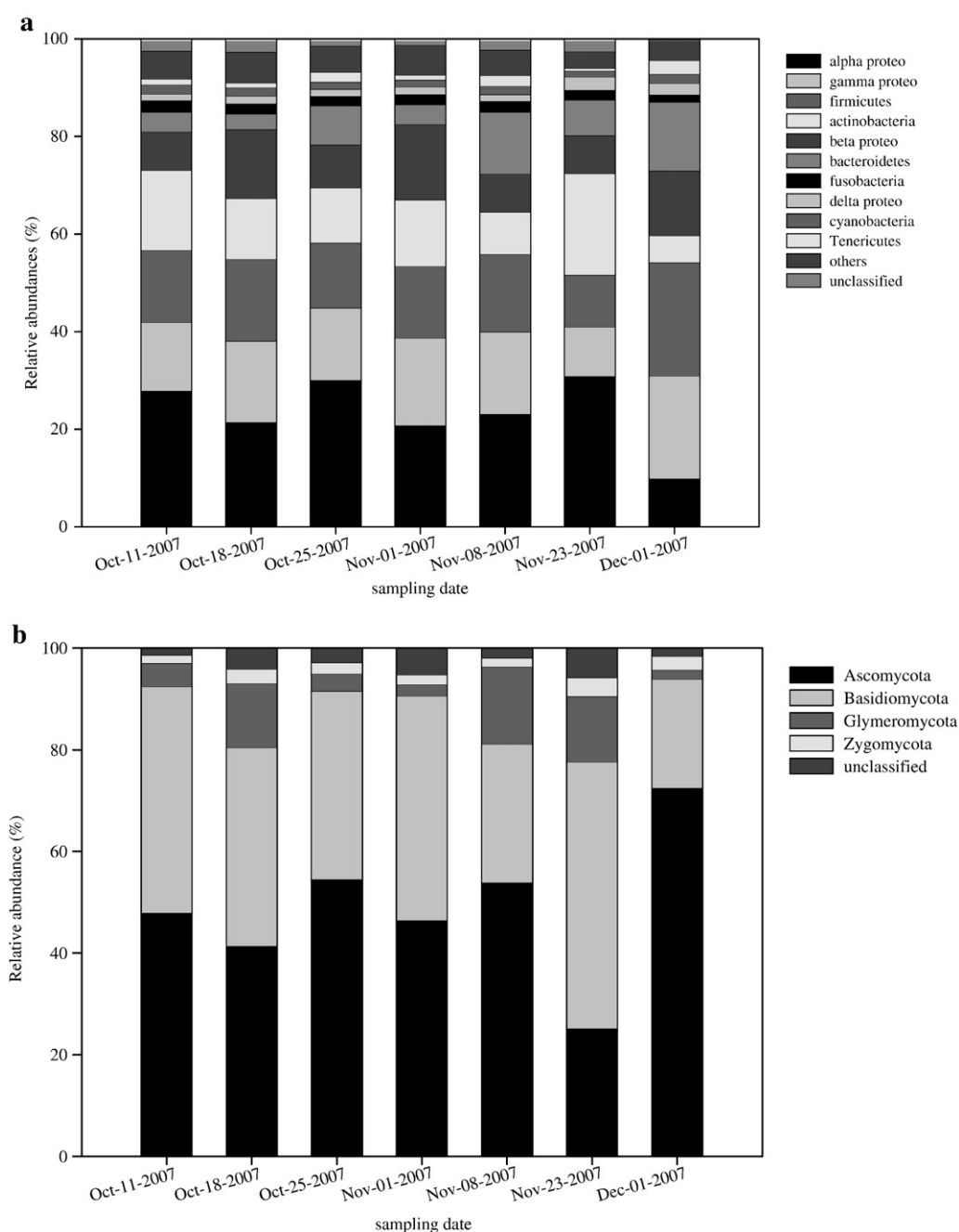


**Fig. 3.** Relative abundances of bacteria and fungi in the TSP samples estimated by *in silico* digestion of bacterial 16S rRNA gene and fungal rRNA gene databases. All data were derived from the T-RFLP profiles for (a) bacteria and (b) fungi.

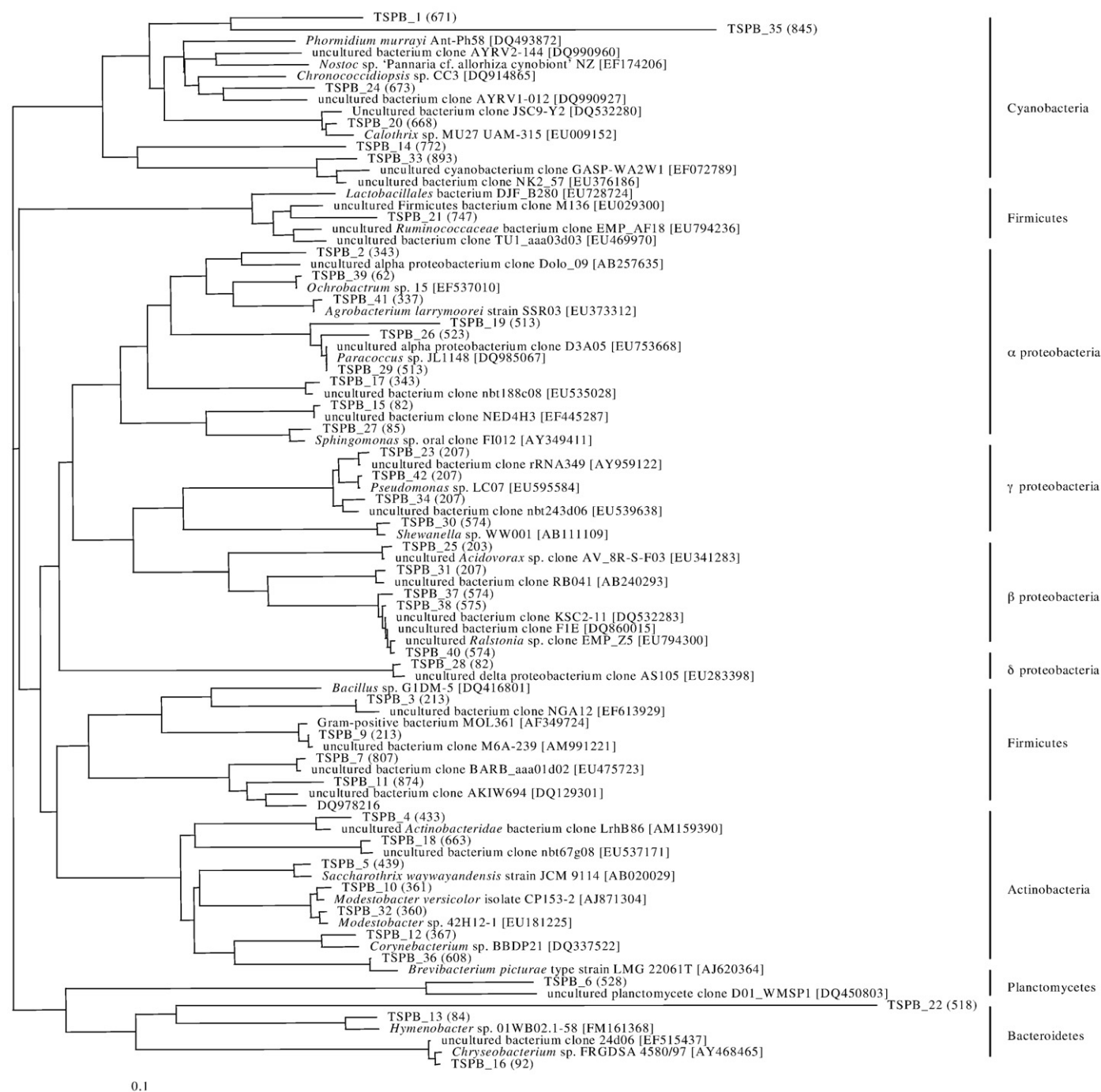


**Fig. 4.** Neighbor-joining tree showing the phylogenetic relationship of airborne bacterial clone based on partial 16S rRNA gene sequences ( $\approx 900$  bp) with 16S rRNA reference gene sequences available in GenBank. A bar represents 0.1 substitutions per nucleotide position. The number in the parenthesis indicates theoretical T-RF size of that sequence.

*Zygomycota*. By *in silico* digestion of T-RFLP profiles for fungal community, most T-RFs in our samples were determined to correspond with fungal species affiliated with *Ascomycota* (48.8%) and *Basidiomycota* (38.0%). Interestingly, the T-RFs corresponding to fungal species belong to *Glomeromycota*, known as the arbuscular mycorrhizal fungi (AMF), were also detected with a quite high proportion (7.5%). In three samples (at Oct-18-2007, Nov-08-2007, and Nov-23-2007), the proportion of *Glomeromycota* exceeded 10.0%. This may be due to the characteristics of the sampling site. Although the sampling site was located in an urban area, there was a forest located around the sampling site.

Most of fungal sequences in the air samples collected by Fierer et al. (2008) were classified as *Ascomycota* and Angenent et al. (2005) observed

that the predominant fungal rRNA gene sequences in aerosol samples were from the phyla *Basidiomycota* and *Ascomycota*. Although we did not identify all the T-RF peaks but only sequenced some of the clones, we obtained an approximately similar proportion of sequences from *Basidiomycota* and *Ascomycota*, which suggests that our samples were relatively similar to those of Angenent et al. (2005).

For fungal community, phylogenetic analysis yielded 20 *Ascomycota*, 30 *Basidiomycota*, and 2 unclassified sequences. Although the result of sequence analysis was more or less different from that of the T-RFLP analysis, the overall figures of fungal community in our samples were found to be similar through both approaches. In other words, approximately half of the fungal types found in our samples affiliated with the

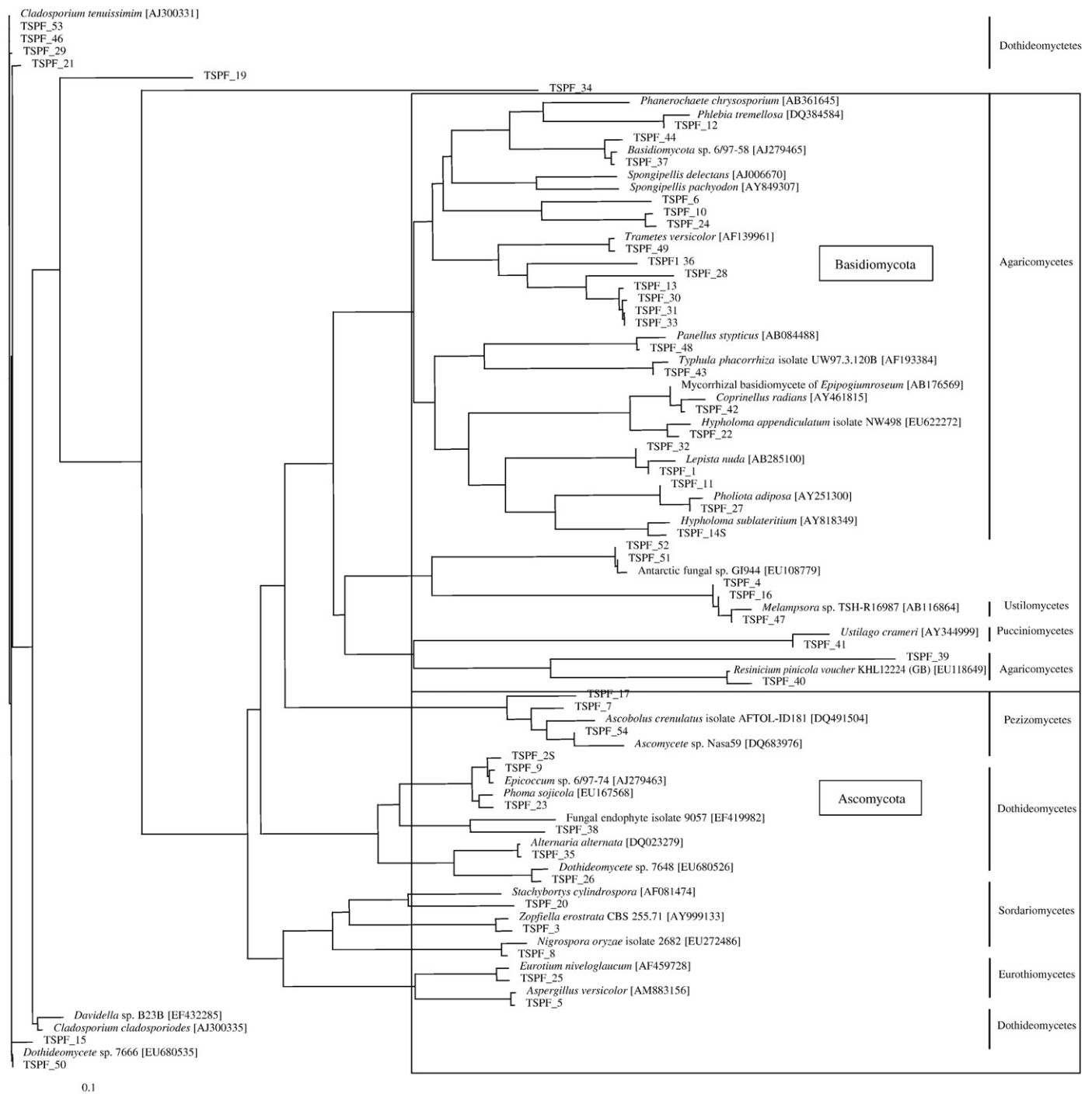


**Fig. 5.** Neighbor-joining tree showing the phylogenetic relationship of airborne fungal clone based on partial rRNA gene sequences ( $\approx 700$  bp) with rRNA reference gene sequences available in GenBank. A bar represents 0.1 substitutions per nucleotide position. The number in the parenthesis indicates theoretical T-RF size of that sequence.

phylum of *Ascomycota* or *Basidiomycota*. In this study, the most abundant types of fungi were determined to be *Cladosporium* and *Alternaria*, which corresponds to the results of other studies reporting that *Cladosporium* and *Alternaria* were the most common airborne fungi (Burge, 2002; Shelton et al., 2002; Zeng et al., 2006; Tsai et al., 2007).

By using real-time PCR, we estimated the number of bacteria and fungi in air samples, assuming an average rRNA gene copy number of 4 per bacteria and 200 per fungal or oomycete genome, respectively (van Doorn et al., 2007; Klappenbach et al., 2001).

Through this process, the number of bacteria and fungi in air samples were determined to range from  $5.19 \times 10^1$  to  $4.31 \times 10^3$  cells  $m^{-3}$  and from  $9.56 \times 10^1$  to  $4.22 \times 10^4$  cells  $m^{-3}$ , respectively (Fig. 6). The

bacterial number in our results was lower than that of Maron et al. (2005) reporting that acridine orange direct count (AODC) of bacterial cells in the air samples were  $1.1 \times 10^5$  and  $6.3 \times 10^4$  cells  $m^{-3}$ . Instead, our results were similar to bacterial densities generally reported in air samples that ranged from  $10^2$  to  $10^3$  CFU  $m^{-3}$  (Lighthart, 1997; Shaffer and Lighthart, 1997). For fungi in our samples, the results for fungal number was comparable to other studies, which reported that the concentration of culturable fungi and fungal spores in outdoor air samples around an office building in the US were 680 CFU  $m^{-3}$  and 6540 spore  $m^{-3}$  (Tsai et al., 2007). Our results showing the amount of microbes in our air samples may reflect the number of all microbes including uncultivated groups which are overlooked by traditional

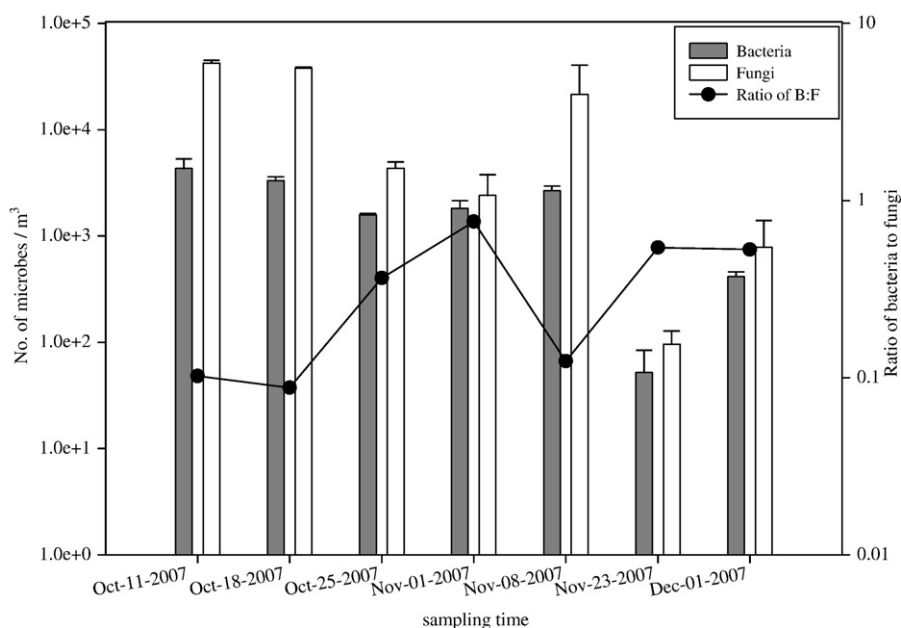


Fig. 6. Number of bacteria and fungi in the TSP samples estimated by real-time Q-PCR.

cultivation techniques. The discrepancy between our results and that of Maron et al. (2005) may be due to the difference of sampling time or location. Maron et al. (2005) monitored the air samples collected during the spring season in a rural environment, while our air samples mostly consisted of autumn and winter season samples from an urban environment. Another explanation for that discrepancy is that there is a possibility for our result to underestimate the amount of microbes in our air samples because we used plasmid DNA as a standard for real-time PCR and the gene copy number of each microbe is various.

We observed noticeable inter-seasonal variability in bacterial and fungal community structures in the TSP samples at our sampling site. In addition, in terms of abundances, bacterial and fungal numbers in TSP samples were different with sampling dates, which contrasted to other studies, which reported no distinct temporal variation of microbial abundance (Angenent et al., 2005; Wilson et al., 2002; Fierer et al., 2008). This discrepancy may be due to the difference of sampling locations, monitoring methods, or periods. In particular, the monitoring period spanning the winter season in our study was thought to be the largest reason for this difference. The result for abundance of bacteria and fungi showed that the number of both bacteria and fungi in late-time samples was lower than those in early-time ones.

Bacteria/fungi ratios ranged from 0.09 to 0.76 across the seven sampling dates (Fig. 6). It has been generally known that the fungal spore is more resistant to environmental stress than bacterial cell and can survive atmospheric transport (Griffin, 2004). The large shift of bacteria/fungi ratios is also similar to another study that reported the bacterial/fungal ratios to range from 8 to 0.08 (Fierer et al., 2008). The number of bacteria and fungi estimated in this study represents total bacteria and fungi irrespective of their viability. As such, it is thought that, if possible, more advanced molecular tools such as for detecting mRNA are adequate for estimating the viable number of airborne bacteria and fungi.

#### 4. Conclusions

Ambient TSP samples were collected at Seoul between middle autumn and early winter in 2007 with a 6-day cycle. The results demonstrated that in our TSP samples collected at an urban site; (1) there were very diverse bacterial and fungal groups including potential pathogens and allergens; (2) there were temporal shifts in both bacterial and fungal communities in terms of both diversity and abundances across an inter-

seasonal period; and (3) air might play an important role in terms of microbial ecology. To our knowledge, this is the first report that investigates the inter-seasonal variability of airborne bacterial and fungal community structures in terms of both diversity and abundances using various molecular approaches. These results suggest that the monitoring of airborne microorganisms have to be conducted with more advanced tools such as molecular approaches and continuous monitoring in order to obtain an overall picture of the microbial community typically found at that site.

#### Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the National Research Lab. Program (No. R0A-2006-000-10221-0) and the Center for Aquatic Ecosystem Restoration (CAER) of Eco-STAR project (No. 08-III-12) from Ministry of Environment (MOE), Korea. The authors gratefully acknowledge the NOAA Air Resources Laboratory (ARL) for the provision of the HYSPLIT transport and dispersion model.

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