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Identifying Airborne Fungi in Seoul, Korea Using Metagenomics

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Fungal spores are widespread and common in the atmosphere. In this study, we use a metagenomic approach to study the fungal diversity in six total air samples collected from April to May 2012 in Seoul, Korea. This springtime period is important in Korea because of the peak in fungal spore concentration and Asian dust storms, although the year of this study (2012) was unique in that were no major Asian dust events. Clustering sequences for operational taxonomic unit (OTU) identification recovered 1,266 unique OTUs in the combined dataset, with between 223-396 OTUs present in individual samples. OTUs from three fungal phyla were identified. For Ascomycota, *Davidiella* (anamorph: *Cladosporium*) was the most common genus in all samples, often accounting for more than 50% of all sequences in a sample. Other common Ascomycota genera identified were *Alternaria*, *Didymella*, *Khuskia*, *Geosmitha*, *Penicillium*, and *Aspergillus*. While several Basidiomycota genera were observed, Chytridiomycota OTUs were only present in one sample. Consistency was observed within sampling days, but there was a large shift in species composition from Ascomycota dominant to Basidiomycota dominant in the middle of the sampling period. This marked change may have been caused by meteorological events. A potential set of 40 allergy-inducing genera were identified, accounting for a large proportion of the diversity present (22.5–77.2%). Our study identifies high fungal diversity and potentially high levels of fungal allergens in springtime air of Korea, and provides a good baseline for future comparisons with Asian dust storms.

Keywords: allergen, fungi, metagenomics, springtime

Introduction

An important stage in the life cycle of fungi is the spore, the dispersal agent of the organism that is either discharged into the air or becomes airborne from rain splash (Deacon, 2006). Due to this airborne stage, fungal spores are widespread and common in the atmosphere. Although prevalence is highly variable and affected by factors such as habitat type and climate (Li and Kendrick, 1994; Fang *et al.*, 2005; Kauserud *et al.*, 2005; Simon-Nobbe *et al.*, 2008), fungal spores can account for 4–11% of fine particle mass (≤ 2.5 μm) in the air (Womiloju *et al.*, 2003). Sometimes, airborne fungi and their parts negatively affect humans by causing allergies (Simon-Nobbe *et al.*, 2008). Allergies (i.e. type I hypersensitivity) are common during the spring season, often coinciding with peaks in plant pollen and fungal spores in a variety of ecoregions including temperate (Wu *et al.*, 1999; Kim and Kim, 2012) and tropical zones (Vermani *et al.*, 2010). To date, more than 100 fungal genera have been identified as causing allergies (Horner *et al.*, 1995; Simon-Nobbe *et al.*, 2008).

Several studies of outdoor environments identified the same set of dominant fungal genera (*Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium*) from different localities around the world (Song, 1965; Burge, 1985; Singh *et al.*, 1995; Rosas *et al.*, 1997; Oh *et al.*, 2000; Su *et al.*, 2001; Shelton *et al.*, 2002; Wu *et al.*, 2004; Fang *et al.*, 2005). Most of these studies depended on spore morphology and/or culture dependent methods for identification. As a majority of fungi are not readily cultured (Pace, 1997; Rappé and Giovannoni, 2003; Fröhlich-Nowoisky *et al.*, 2009) or easily identified by spores alone (Burge, 1985), these results are expected to be an underestimation of airborne fungal diversity (Wu *et al.*, 2004). Metagenomics, a new field in biology, uses new DNA sequencing technologies (e.g., pyrosequencing) to directly sequence and identify environmental microbes (Riesenfeld *et al.*, 2004). Such methods are expected to vastly improve our ability to identify and study the full spectrum of fungal diversity in the air.

In this study, we use 454-pyrosequencing technology and a metagenomic approach to identify springtime fungal diversity in the air. We document the species composition and temporal shift of fungal diversity over a two-month span (April-May) at a single site in Seoul, Korea. Springtime in Korea is a critical period because it is one of yearly peaks of fungal spore concentration (Kim and Kim, 2012) and Asian dust storm events (Yeo and Kim, 2002). By elucidating airborne fungal diversity, we will gain insight into its role in ecology and human health in Korea.

Materials and Methods

Sample collection

Air samples were collected on the roof (approximately 20–30 m above the ground) of the 3rd Engineering building of Yonsei University in Seoul, Korea (37°33′42″ N, 126°56′07″ E). This location, far from any industrial activity, was selected to be a representative location of urban areas in Seoul.

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A total of six air samples were collected from April to May 2012. Two concurrent samples were taken on three collecting dates to check for within-date consistency: April 21 (A21), April 28 (A28), and May 30 (M30). Meteorological data were collected for these three dates and the day previous to collection from monthly weather report publications of the Korean Meteorological Administration. Parameters recorded included mean temperature, relative humidity, precipitation, mean wind speed, max wind speed, max wind direction, mean cloud amount, and horizontal radiation. The weather station in Seoul (37°34′ N, 126°57′ E) is less than 2 km away from the collection site at Yonsei University, so the weather conditions are expected to be the same.

Air samples were collected using a total suspended particulate (TSP) high volume air sampler (model no.5000; E & Instrument, Korea). Approximately 30 L of air/min were collected on 8×12 inch, 0.2 μm pore size track-etched poretics polyester membrane filters (GE, USA) for a 24-h period on days with no rain. The sterile filters were autoclaved prior to sampling. After sampling, filters were brought to the laboratory and cut into 100 × 100 mm pieces using sterile scissors and transferred to a sterile petri dish. Each piece of filter harbored approximately 0.2–1.0 g of suspended particles. Samples were stored in a -20°C freezer until DNA extraction.

Laboratory methods

Genomic DNA was extracted from filters according to a method described by Radosevich *et al.* (2002). Amplification of the large subunit (LSU) of nuclear ribosomal DNA was performed using the primers LSU-26F and LSU-657R as described by Hur *et al.* (2012). Primers were barcoded to distinguish each sample prior to sequencing. Maxime PCR Premix (iNtRON Biotechnology, Korea) was used to each PCR reaction with 1 μl of DNA in a total volume of 20 μl. To recover a sufficient amount of purified PCR product from the purification steps and minimize sampling bias, three 20 μl reactions were combined prior to PCR purification.

Amplification was performed on a C1000 Thermal Cycler (Bio-Rad, USA) as follows: 94°C for 1 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 40 sec, and a final extension step at 72°C for 5 min. After amplification, PCR products were purified once using HighPure™ PCR product Purification Kit (Roche, Germany). The DNA concentrations of purified PCR products were measured using Picogreen (Invitrogen, USA). The quality of pooled products was checked using a Bioanalyzer 2001 (Agilent, USA), then short fragments were removed using AMPure Beads (Agencourt, USA). Pyrosequencing was performed at ChunLab Inc. (http://www.chunlab.com/) using a Roche/454 GS Junior platform (Roche, USA).

Sequence analysis

All processing of sequence data was performed using the software package Mothur v.1.29.2 (Schloss *et al.*, 2009). Names of specific commands used are put in italics. Sequences from each of the six samples were de-noised (*shhh.flows*) and trimmed separately (*trim.seqs*; maximum differences in the primer=2, maximum homopolymers=8, minimum length=200). The number of sequence reads per sample was normalized via subsampling (*sub.sample*). All sequences were combined while retaining information on group membership (*make.group* and *merge.files*) and aligned to a representative LSU sequence in GenBank (AF363648-*Hannaella*) using *align.seqs*. Next, sequencing errors were reduced by merging sequences that were within 1 bp of a more abundant sequence using the *pre.cluster* command. Chimeras were identified and removed (*chimera. uchime*) using the UCHIME program (Edgar *et al.*, 2011). For defining operational taxonomic units (OTUs), a distance matrix was generated and sequences were clustered using the furthest-neighbor method and a 0.03 cutoff value (*cluster*). As we have used a strict filtering protocol to minimize error, we retain singletons. This allows us to maintain power in estimating how well we have sampled the community (i.e., rarefaction curve), as well as keep rare, but potentially important OTUs of the community.

OTU identity to the genus level was determined by using a naïve Bayesian classifier (Wang *et al.*, 2007) for fungal LSU (Liu *et al.*, 2012), available from the Ribosomal Database Project (RDP) website (http://rdp.cme.msu.edu/classifier/classifier.jsp). A bootstrap score for each assignment accompanies taxonomic classification by RDP, and following the recommendation of RDP for sequences shorter than 250 bp, we retain the assignment if the bootstrap score is ≥ 50. The exception to this rule is for OTUs identified as Chytridiomycota; since a majority of Chytridiomycota sequences were excluded from RDP (Liu *et al.*, 2012), we retain higher-level classification of Chytridiomycota despite having a bootstrap value of <50. Especially for Ascomycota, fungal species often have two different scientific names based on whether they are telomorphic (sexual) or anamorphic (asexual). For this paper, we use the checklist of Hyde *et al.* (2011) and a consistent rule of combining synonymous taxa and retaining the telomorphic name. For example, in this paper, anamorphic *Cladosporium* is referred to by its telomorphic name, *Davidiella*. In the case of anamorphic genera that correspond to several possible telomorphic taxa, we retain the anamorphic name appended with an "(a)" to indicate the uncertainty, as in "*Aspergillus* (a)".

Alpha and beta diversity analyses were all performed in Mothur. Alpha diversity of the individual samples was visualized using rarefaction and Inverse Simpson curves (*rarefaction.single* and *collect.single*, respectively). For beta diversity analyses, a heatmap (*heatmap.sim*) was constructed using the theta index (the Yue & Clayton community overlap measure; Yue and Clayton, 2005). Lastly, a non-metric multidimensional scaling (NMDS) ordination graph was plotted (*nmds*). A scree plot comparing stress values was used to decide on the appropriate number of axes in the NMDS plot. An AMOVA (*amova*) was performed to determine whether the spatial separation between points in the NMDS plot is statistically significant. By measuring the correlation of the relative abundance of the OTU with the axes, the OTUs responsible for the shifts across the axes were identified using Spearman's rank correlation coefficient (*corr.axes*).

Table 1. Meteorological conditions of sampling dates and the day previous to sampling

Parameter	Sampling date					
	A20	A21	A27	A28	M29	M30
Mean temp (°C)	18.4	11.4	15.2	17	21.6	20.7
Rel. Humidity (%)	39	73	32	42	41	44
Precipitation (mm)	0	0	0	0	0	0
Mean wind speed (m/sec)	3.3	7.1	4.1	3.1	2.9	2.4
Max wind speed (m/sec)	7.0	10.5	8.0	6.5	4.5	6.2
Max wind direction	**ENE**	**ENE**	**SW**	**SW**	**ENE**	**ENE**
Mean cloud amount	**6.1**	**9.9**	**1.8**	**1.8**	**6.8**	**9.1**
Horizontal radiation (MJ/m^2)	18.54	**2.56**	21.43	**19.21**	13.48	**8.81**

* Bold values represent potential conditions causing changes in fungal diversity in this study.

Results

Sample collection

Meteorological conditions are listed in Table 1. Noteworthy parameters that differed between A21/M30 and A28 sampling dates are max wind direction (MWD), mean cloud amount (MCA), and horizontal radiation (HR). MWD and MCA differed for both the day of and the day previous to collecting, with MWD from the east-northeast and relatively high MCA (6.1–9.9) for A21/M30, and MWD from the southwest and relatively low MCA (1.8). Additionally, HR differed between the days of collection, with HR relatively low (2.56–8.81) for A21/M30 and relatively high for A28 (19.21).

Dataset characteristics

After initial de-noising, trimming, and filtering of data, only high-quality sequences remained. The total number of sequences ranged from 3,984–7,810 for the samples (Table 2). All samples were sub-sampled to the lowest number (3,984 reads in A28-2), to allow for subsequent diversity measure comparisons. A second round of filtering, aligning, and removing chimeric sequences further reduced the number of sequence reads per sample (Table 2). The final length of each sequence was approximately 170 bp, covering the D2 region of LSU.

Table 2. Descriptive characteristics of samples, sequences, and OTUs

Characteristics	Sample ID					
	A21-1	A21-2	A28-1	A28-2	M30-1	M30-2
High-quality sequences	6773	7810	4631	3984	7654	6291
Post-subsample & filter	3693	3498	3374	3621	3192	3463
OTUs	396	359	238	319	250	223
Unique OTUs[a]	84 (21%)	90 (25%)	92 (39%)	120 (38%)	62 (25%)	66 (30%)
Total genera	103	92	851	79	96	75
Ascomycota	57	47	15	23	39	35
Basidiomycota	45	45	36	48	48	40
Chytridiomycota	0	0	0	0	7	0
Unknown	1	0	0	8	2	0

[a] Unique OTUs are displayed as: number (% of OTUs in sample)

OTU identification

Clustering sequences for OTU identification recovered 1,266 unique OTUs in the combined dataset, with between 223–396 OTUs present in individual samples (Table 2). A

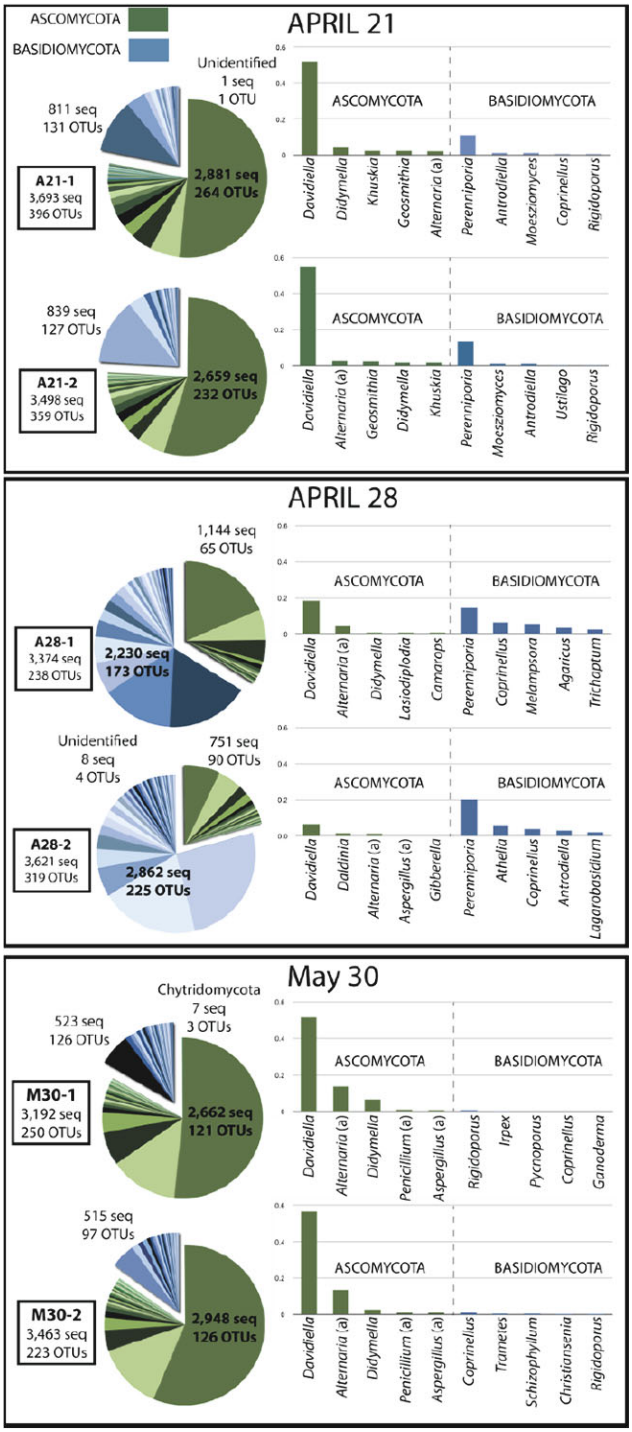

Fig. 1. Summary of the number of sequences and OTUs from each air sample. Pie charts illustrate the proportion of OTUs in each fungal phylum. Bar graphs indicate the top 5 most common genera in Ascomycota and Basidiomycota.

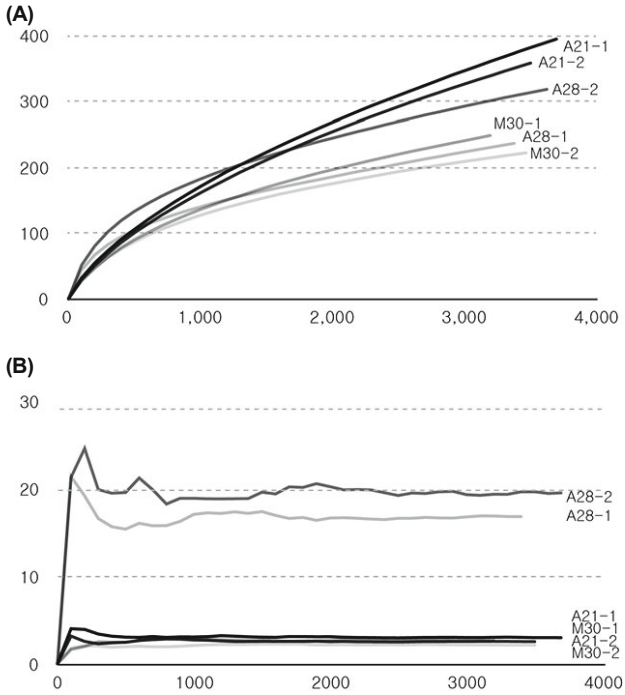

Fig. 2. Alpha diversity measures. (A) Rarefaction curve, (B) and Inverse Simpson Index.

proportion of these OTUs (21–39%) were unique to individual samples (Table 2). OTUs from three fungal phyla (Ascomycota, Basidiomycota, Chytridiomycota) were identified (Table 2 and Fig. 1). For Ascomycota, *Davidiella* (anamorph: *Cladosporium*) was the most common genus in all samples, often accounting for more than 50% of all sequences in a sample. Other common Ascomycota genera identified were *Alternaria* (a), *Didymella*, *Khuskia*, *Geosmitha*, *Penicillium* (a), and *Aspergillus* (a). For Basidiomycota, the most common genus was *Perenniporia* in 4 of 6 samples, with *Moesziomyces*, *Antrodiella*, *Melampsora*, *Agaricus*, *Trichaptum*, *Athelia*, *Lagarobasidium*, *Rigidoporus*, and *Coprinellus* also common (Fig. 1). Chytridiomycota OTUs were only present in one sample (M30-1).

Alpha & beta diversity analyses

To measure the alpha diversity in each sample, rarefaction and Inverse Simpson Index (1/λ) were plotted (Fig. 2). For the rarefaction curve, plots have not reached full saturation, but have begun to flatten. The Inverse Simpson graphs have reached saturation, with both samples from A21 and M30 being more similar to each other (1/λ=3.1–3.9), while samples from A28 were different (1/λ=17.8–20.5).

For beta diversity measures (between and within date sampling), a heatmap of membership and structure similarity and a NMDS plot were made (Fig. 3). There was consistency of the within-date sampling, as samples from the same date are more similar to each other than other samples, seen in the brighter red cells in the heatmap and clustering in the NMDS plot (Fig. 3). Although the variation in A28 samples was relatively high, these two samples were still more similar to each other than to other samples. When comparing between-date sampling, we see that the OTU membership and structure of A21 and M30 samples are very similar, relative to A28 samples, again seen in the heatmap and NMDS plot (Fig. 3). The heatmap shows that four samples (A21-1, A21-2, M30-1, and M30-2) are highly similar in membership and structure. For NMDS, the scree plot (not shown) indicated no improvement beyond the third dimension, so three axes were used. The NMDS plot shows strong clustering and similarity between the A21 and M30 samples (Fig. 3B). AMOVA analyses found that the groups (A21, A28, M30) were statistically different from each other ($F[2,3]=10.15$, $P<0.001$).

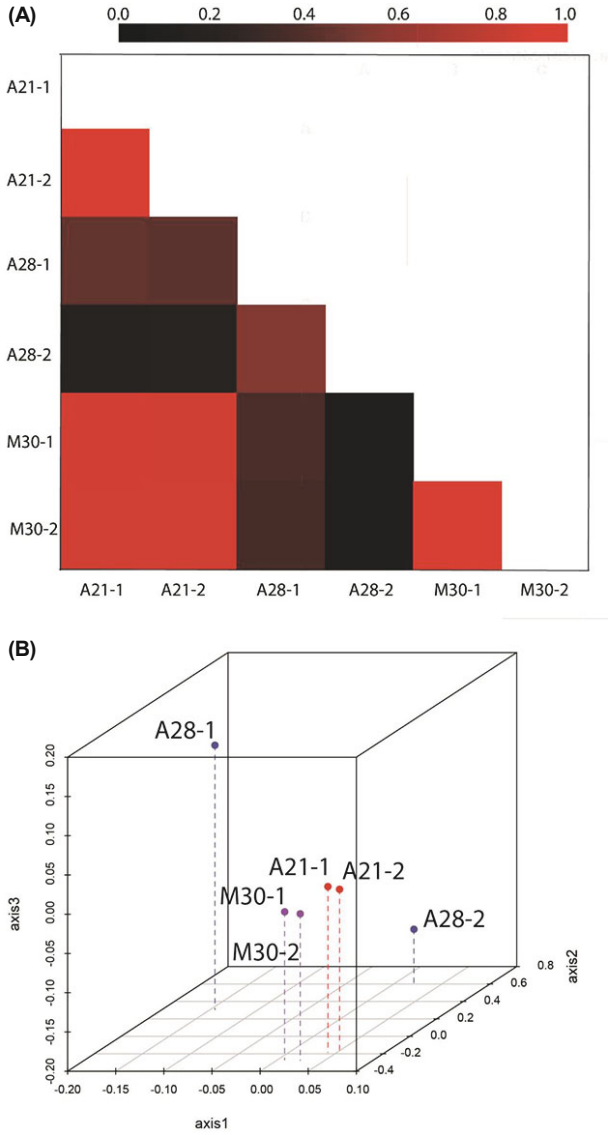

Fig. 3. Beta diversity comparisons of six air samples. Sampling was done in duplicate for April 21 (A21), April 28 (A28), and May 30 (M30) (A) heatmap, (B) Non-metric multidimensional scaling (NMDS) plot. Note the scale difference for each of the axes in the NMDS plot.

Discussion

The number of sequences, OTUs, and unique OTUs recovered was similar for each of the individual samples (Table 2). To understand the alpha diversity of each sample, we use rarefaction and Inverse Simpson Index curves. Rarefaction curves describe the growth of a number of species discovered as a function of the number of samples. For all of our samples, curves have begun to flatten, which is typical in metagenomic studies (Tringe *et al.*, 2005; Fierer *et al.*, 2007), indicating that representative, unbiased sampling of fungal diversity has been recovered (Fig. 2). Our second measure of alpha diversity, the Inverse Simpson curve, shows saturation (Fig. 2). The Inverse Simpson Index curve provides information on both species richness and species evenness, and reveals the largest difference between our samples. All

Table 3. List of the potential fungal allergens present in this study. Duplicate sampling for each date was combined as they were similar (Seq.=number of sequences)

Fungal name	Total OTUs	A21		A28		A30		Alternative name
		Seq.	OTUs	Seq.	OTUs	Seq.	OTUs	
Ascomycota	**220**	**4759**	**145**	**1329**	**54**	**5029**	**89**	
Alternaria[a]	5	171	4	205	1	906	3	
Aspergillus[a]	18	105	12	40	3	50	10	
Capnodiales	1	0	0	0	0	1	1	
Chaetomium	4	8	4	0	0	0	0	
Columnosphaeria	1	0	0	2	1	1	1	*Aureobasidium*[a]
Daldinia	6	11	2	70	5	0	0	
Davidiella	44	3826	36	885	16	3600	17	*Cladosporium*[a]
Didymella	2	229	2	27	1	303	2	
Emericella	2	0	0	0	0	6	2	*Aspergillus*[a]
Epicoccum	2	2	2	0	0	0	0	
Eurotium	20	100	13	19	8	30	5	
Gibberella	17	28	8	25	5	19	8	*Fusarium*[a]
Hydropisphaera	2	3	2	0	0	0	0	*Acremonium*[a]
Hypocrea	17	56	14	11	2	17	5	*Trichoderma*[a]
Khuskia	17	153	15	0	0	14	4	*Nigrospora*[a]
Leptosphaeria	1	0	0	2	1	0	0	
Microascus	8	8	6	2	1	1	1	*Scopulariopsis*[a]
Nectria	4	0	0	16	4	0	0	*Acremonium*[a]
Neurospora	7	7	2	15	2	5	4	
Paecilomyces[a]	4	5	4	0	0	0	0	
Penicillium[a]	29	25	13	9	3	73	24	
Petriella	2	1	1	0	0	2	1	*Scopulariopsis*[a]
Rodentomyces	5	21	5	0	0	0	0	*Trichothecium*[a]
Westerdykella	1	0	0	1	1	0	0	*Phoma*[a]
Xylaria	1	0	0	0	0	1	1	
Basidiomycota	**49**	**79**	**23**	**243**	**18**	**108**	**19**	
Agaricus	2	0	0	115	1	3	2	
Coprinus	2	0	0	0	0	3	2	
Ganoderma	4	26	3	17	2	25	1	
Geastrum	5	3	3	16	2	0	0	
Hypholoma	1	4	1	18	1	0	0	
Inonotus	4	1	1	7	3	0	0	
Laetiporus	2	0	0	14	2	0	0	
Lycoperdon	1	5	1	0	0	0	0	
Malassezia	3	1	1	20	3	0	0	
Pleurotus	1	0	0	10	1	2	1	
Psilocybe	2	1	1	0	0	1	1	
Puccinia	3	0	0	0	0	18	3	
Schizophyllum	5	1	1	0	0	38	5	
Ustilago	6	20	5	0	0	12	2	
Wallemia	8	17	6	26	3	6	2	
Total	**269**	**4838**	**168**	**1572**	**72**	**5137**	**108**	
% sample	**21.5%**	**67.3%**	**27.0%**	**22.5%**	**14.4%**	**77.2%**	**27.5%**	

[a] Asexual or anamorphic name; % sample: proportion of each sample

samples from A21/M30 have relatively similar scores compared to samples from A28 (Fig. 2). With a similar number in OTUs across all samples (Table 2), the difference can be attributed to changes in species evenness. For A21/ M30, the most prevalent OTU (*Davidiella*) represents >50% of all sequences in the samples. In contrast, the most common OTU from A28 (*Davidiella* or *Perenniporia*) constitutes <20% of all sequences (Fig. 1). We see that fungal diversity and composition is similar within sampling dates, as seen in the heatmap and NMDS plot (Fig. 3).

There are both seasonal (Ebner *et al.*, 1989; Li and Kendrick, 1994; Rosas *et al.*, 1997; Fang *et al.*, 2005; Lee *et al.*, 2010) and short-term (Adhikari *et al.*, 2004; Fang *et al.*, 2005) changes in airborne fungal diversity. For example, temperature (Fernández *et al.*, 1998) and humidity (Levetin, 1989, 1990; Wu *et al.*, 2004), which change at both seasonal and short-term scales, are positively correlated with fungal spore density. On a smaller scale, sporadic phenomena such as soil disturbances or sporulation events also have an influence on fungal diversity in the air (Lighthart and Stetzenbach, 1994; Madelin, 1994; Fierer *et al.*, 2007). Interestingly, in our study, there is more similarity in fungal diversity in the beginning (A21) and end (M30) of spring compared to our sample in the middle (A28) (Fig. 1). For A21/M30, Ascomycota dominates in terms of the number of OTUs, sequences, and identified genera, with a single genus (*Davidiella*) accounting for more than 50% of sequences (Table 2 and Fig. 1). Contrary for A28, not only is fungal diversity more even (Fig. 2B), Basidiomycota genera represented a majority of the OTU diversity (Fig. 1). This change can be the result of a decrease in Ascomycota, an increase in Basidiomycota, or a combination of both. Our analyses do not give us the power to differentiate between these different scenarios, but rather allows us to see a trend in how proportions of particular OTUs change. In particular, *Davidiella* drops from ~50% to <20% of sequences (Fig. 1). Spearman's rank correlation coefficient analyses highlight the species that are significantly correlated with shifts across the three axes of the NMDS plot, and identify decreases in *Davidiella* and *Didymella* (Ascomycota), along with slight increases in *Perenniporia*, *Athelia*, and *Melampsora* (Basidiomycota).

Previous studies have demonstrated the importance of meteorological factors like wind speed, radiation, and precipitation affecting spore density in the air (Oh, 1998; Burge and Rogers, 2000; Deacon, 2006). Three meteorological variables were different between A21/M30 and A28 [max wind direction, mean cloud amount and horizontal radiation (Table 1)] and may be related to the shift from Ascomycota to Basidiomycota dominance seen in this study (Table 3). Our results are different from previous findings on airborne fungal spore levels of *Davidiella* and *Alternaria*, the two most common genera in our study. Hjelmroos (1993) found that *Davidiella* levels were higher with increased daily mean temperature and increased precipitation, while *Alternaria* (a) tended to increase with daily precipitation, wind velocity, and total cloud cover. These trends were not seen in our study. Instead, our data point towards max wind direction (i.e., different environmental source) and mean cloud amount as potential factors influencing aerial fungi diversity. With the current dataset, it is difficult to confirm the causal relationship of these climate conditions and fungal diversity variation, and this relationship should be investigated in more detail.

Past aerial fungal diversity studies have been performed in Korea using spore morphology and culture based method (Song, 1965; Oh and Lee, 1997; Oh, 1998; Oh *et al.*, 1998, 2000; Kim *et al.*, 2000; Kim and Kim, 2012). These studies, as well as ours, found three common fungal genera in air samples of Korea–*Cladosporium* (*Davidiella* in our study), *Alternaria*, and *Phoma* (*Epicoccum*, *Leptosphaeria*, and *Westerdykella* in our study). Although the major composition of aerial fungi was congruent using traditional and metagenomic approaches, a pyrosequencing approach revealed much higher species diversity. Traditional approaches were limited to detection and identification of common genera that have distinct morphological characteristics or can easily be cultivated, which was approximately 20 species. Many spores remained unidentifiable, and as a result a large component of aerial fugal diversity remained undiscovered. Conversely, metagenomic approaches utilizing next-generation sequencing are powerful in profiling both major and minor components of aerial fungal community. We utilize LSU sequences and identify more than 1,200 OTUs in 103 genera (Table 2). This trend of uncovering greater species diversity has been seen in other aerial fungal studies across the globe (Ebner *et al.*, 1989; Fernández *et al.*, 1998; Gioulekas *et al.*, 2004; Wu *et al.*, 2004, 2007; Fang *et al.*, 2005; Fröhlich-Nowoisky *et al.*, 2009). We predict with longer reads and more refined databases, identification of fungal taxa will become more accurate and resolution will reach the species/ strain level, and an even greater fungal diversity will be realized in the future.

Aerial fungal diversity is regularly studied because fungal spores can cause human allergies and illnesses (D'amato and Spieksma, 1995; Garrett *et al.*, 1998; Chew *et al.*, 2000; Gioulekas *et al.*, 2004). Allergies are induced by a large number of fungal genera, the majority of which are in Ascomycota. To date, over 80 mold (anamorph Ascomycota) genera have been identified as causing allergies in humans (Horner *et al.*, 1995; Simon-Nobbe *et al.*, 2008). Of the 25 potentially allergy-inducing Ascomycota genera found in our study, the three most common were *Davidiella*, *Alternaria* (a), and *Didymella*. *Davidiella* (anamorph: *Cladosporium*) and *Alternaria* (a), both saprophytes, are two of the most commonly found fungal allergens in the environment (Hjelmroos, 1993; Achatz *et al.*, 1995). *Didymella* (anamorph: *Stagonosporopsis*) is known to be a human allergen as well as a plant pathogen, and spore levels have been tied to proximity to and seasonality of agricultural activity (Frankland and Gregory, 1973). Such allergens are expected to be high in urban areas adjacent to farmland. Although a large, urban city, there are mountains and agricultural areas surrounding Seoul. Depending on the point sources and wind direction and strength, these nearby farms may contribute to the fungal allergens present in the air of Seoul.

Since cultivating Basidiomycota is less efficient relative to Ascomycota, allergy research for Basidiomycota is lagging. To date, only ~50 of the over 20,000 Basidiomycota species have been tested, with 25 being identified as allergenic (Burge *et al.*, 1982; Horner *et al.*, 1995; Simon-Nobbe *et al.*, 2008). Levels of basidiospores in the air have been shown to

peak in the spring and autumn in conjunction with the increased presence of fruiting bodies (Levetin, 1990). Despite the limited number of Basidiomycota allergens identified, 15 genera were identified in this study to be potential allergens, with *Agaricus* being the dominant genus. The genus *Agraricus* contains both edible and poisonous species and includes two commonly cultivated mushrooms for human consumption, *A. bisporus* and *A. campestris.* Again, depending on the strength and direction of wind, the adjacent mountains to Seoul may be point sources for airborne basidiospore allergens. Our identification of potential fungal allergens should be considered preliminary, as a majority of fungi have not been tested for their allergenicity (especially in Basidiomycota) and molecular identification of OTUs to species/strain was limited due to the small LSU type-strain database. Future studies focusing on epidemiology and sequencing additional markers (e.g., internal transcribed spacer) will be needed to identify specific allergenic fungi in the air.

Springtime marks the period in Korea when there are Asian dust storms (Cho, 1980; Kim *et al.*, 1986; Chun *et al.*, 2001a, 2001b; Kim, 2008). These dust storms have been associated with increased cardiopulmonary emergency visits (Chan *et al.*, 2008; Kang *et al.*, 2012) and increased respiratory symptoms of subjects with asthma (Park *et al.*, 2005). The year of this study (2012) was unique in that there were no major dust events. Although we cannot see the effect of Asian dust storms on aerial fungal diversity, our study provides baseline information on fungal allergens in Korea for future comparison.

In conclusion, fungi in the air are plentiful and diverse. In this study we found a high diversity of aerial fungi during the springtime in Korea. Consistency was observed within sampling days, but a large shift in species composition (Ascomycota to Basidiomycota) was seen between days. In regards to public health, we identified a set of potential allergenic fungi whose concentration in the air may be influenced by meteorological conditions and the adjacent mountain and agricultural landscape adjacent to Seoul. Although there were no Asian dust storms the year of this study, continued environmental sampling is underway to compare the effect of dust storm events on microbial diversity and allergen levels.

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