

# Comparison of the biological content of air samples collected at ground level and at higher elevation

Kejun Li · Shuofei Dong · Yan Wu · Maosheng Yao

Received: 26 June 2009 / Accepted: 12 March 2010  
© Springer Science+Business Media B.V. 2010

**Abstract** In this study, air samples were collected simultaneously at ground level and from a broadcast tower (open space) 238 m high using two BioSamplers and two portable Biostage impactors. The sampling lasted up to 5 h, from morning to afternoon, and the experiments were independently conducted twice. The samples collected by the BioSamplers were analyzed for bacterial diversity and concentration, endotoxin and (1,3)- $\beta$ -D-glucan content. Denaturing gradient gel electrophoresis (DGGE) fingerprinting, DNA sequencing, real-time quantitative polymerase chain reaction (qPCR), and Limulus amoebocyte lysate (LAL) were used for the analyses. Air samples collected by the portable Biostage impactors were directly incubated at 25°C, and the culturable bacteria and fungi concentrations were manually counted. The results from the culture analysis indicated that the ground air samples had higher (statistically significant) concentrations of culturable bacteria and fungi than those taken at an elevation of 238 m. DGGE profiles of PCR products showed similar bacterial diversity in both ground-level and elevated air samples. *Pseudomonas* was identified by DNA sequencing as the predominant bacterial genus present. The SYBR

qPCR tests revealed that the pooled air samples from both the ground and elevated levels had similar total bacterial concentrations, about 1,000 times their culturable ones. Although the endotoxin and (1,3)- $\beta$ -D-glucan concentrations were observed to vary over the time, no statistically significant differences were observed in the pooled air samples from the ground and elevated levels over the course of the day. The results from this study elucidated the microbial differences in air at different elevations, and added to the body of evidence of atmospheric mixing of biological agents.

**Keywords** Culturability · DGGE · DNA sequencing · qPCR · Bacterial diversity · Elevation · Fungi

## 1 Introduction

Particles of biological origin are a ubiquitous component of the atmospheric aerosol and account for approximately 24% of the total concentration of particles (Matthias-Maser and Jaenicke 1995; Jones and Harrison 2004). Inhalation exposure to these airborne biological particles including bacteria, fungi, virus, endotoxin, and (1,3)- $\beta$ -D-glucan has been shown to cause numerous adverse health effects (Douwes et al. 2003; Walinder et al. 2001; Laumbach and Kipen 2005). Exposure to bacterial endotoxin is demonstrated to be associated with pulmonary symptoms,

---

K. Li · S. Dong · Y. Wu · M. Yao (✉)  
State Key Joint Laboratory of Environmental Simulation  
and Pollution Control, College of Environmental Sciences  
and Engineering, Peking University, Beijing 100871,  
China  
e-mail: Yao@PKU.edu.cn

airway bronchoconstriction, recruitment of neutrophils to the airways, and increased release of proinflammatory cytokines including tumor necrosis factor alpha, interleukin-6, and macrophage inflammatory protein (Thorne 2000). Exposure to fungal (1,3)- $\beta$ -D-glucan can also illicit inflammatory reactions (Czop and Kay 1991) and cause an increase in the number of lymphocytes in the blood when inhaled (Rylander 2005). Although these adverse health effects are mostly encountered in indoor settings, studies indicated that outdoor air is also a major source of indoor air pollutants (Monn 2001; Li et al. 1995; Ren et al. 2001; Pei-Chih et al. 2000; Su et al. 2001; Chew et al. 2003; O'Connor et al. 2004; Lee and Jo 2006).

Numerous studies have been conducted to investigate indoor biological content (Singh and Singh 1996; Cox-Ganser et al. 2009; Rao et al. 2007; Salo et al. 2008), however, only a limited number of studies have been conducted regarding outdoor microbial content, particularly in the upper atmospheric environments. Fulton (1966) studied the micro-population up to an altitude of 3,127 m and found that the micro-population was more stable at higher altitude than that at lower altitude. It was also shown that surface activity could affect the micro-population at an altitude of 690 m (Fulton and Mitchell 1996). In another study, it was indicated that meteorological conditions can have a variety of effects on biological aerosol concentrations (Jones and Harrison 2004). Maki et al. (2008) studied air samples collected at altitudes of 10 and 800 m using DGGE and clone library analysis, and observed similar bacterial composition and particle size distribution at different altitudes. They suggested that atmospheric mixing played a role. There is a growing body of evidence that biological agents can be transported globally (Joo et al. 2002; Ozawa et al. 2001; Sakamoto and Yoshida 2002; Brown and Hovmoeller 2002; Griffin et al. 2006; Wu et al. 2004; Griffin 2007). These transported pollutants could further descend into local environments as a result of atmospheric mixing (Maki et al. 2008), thus causing relevant health effects. It is thus important to learn about the microbial content of air at elevations significantly above ground level.

However, the microbial content of air samples are often poorly described as a result of reliance on the traditional culture method. Application of molecular/biochemistry methods to describe the biological content of air is increasing, because of their low

quantification limits and accuracy (Alvarez et al. 1994; Sawyer et al. 1994; Olsson et al. 1998; Angenent et al. 2005). Recently, DGGE and clone library analysis were used to compare bacterial diversity in the air samples collected from different swine establishments (Nehme et al. 2008).

Different from existing studies, this study was designed to compare microbial content, for example bacteria, fungi, endotoxin, and (1,3)- $\beta$ -D-glucan in the air samples collected simultaneously at ground level (0 m) and at an elevation of 238 m. Molecular and biochemistry based techniques, for example DGGE, gene sequencing, qPCR, and LAL methods were used. The results from this study add to the body of evidence of atmospheric mixing of biological agents.

## 2 Materials and methods

### 2.1 Air sample collection

Figure 1 shows the experimental procedures and the central broadcast tower of 238 m located on the west 3rd ring in Beijing. Initially, a much higher elevation was intended, but the tower was the highest structure found in Beijing for air sampling. The tower has an open-air viewing platform, and the ground sampling site was just outside the broadcast tower. During sample collection, either two portable Biostage impactors (QuickTake 30, SKC) or two BioSamplers (SKC) were operated simultaneously with one on the ground and the other on the top platform of the broadcast tower. The air samples were collected during a four-day campaign in April/May 2008. During the first two days, two BioSamplers were simultaneously operated with 20 ml DI water as the collection liquid for 40 min at 12.5 l/min to collect the air samples for endotoxin, (1,3)- $\beta$ -D-glucan, and bacteria study. The 40-min samples were collected from morning to afternoon with sampling start times 10:30, 11:40, 12:50, and 14:00. In another two-day sampling, two portable Biostage impactors were simultaneously operated at 28.3 l/min for 5 min both on the ground and on the top platform for culturable bacteria and fungi study. Sampling was conducted continuously every 35 min. On Day #1, a total of five groups of samples with three replicates each were obtained with the sampling start times 10:50, 12:00, 13:10, 14:20, and 15:30. On Day #2, a total of six

groups of samples with three replicates of each group were obtained with the sampling start times 9:40, 10:50, 12:00, 13:10, 14:20, and 15:30. The sampling start time for the ground and elevated sampling was communicated via cell phone in this study. The collection liquid from each BioSampler was transferred into a 50-ml pyrogen-free corning tube (Corning, NY, USA) and stored at 4°C for further sample analysis. The air samples collected by the Biostage impactors were directly incubated at 25°C for culturing. Sampling was conducted under clear weather conditions with temperature ranging from 24 to 33°C, relative humidity 22%, and wind velocity 3.4–5.4 m/s both for the ground and elevated sampling.

## 2.2 Sample analysis

The air samples collected by the BioSamplers at different times were individually analyzed both for the ground level and for the 238 m elevation. In addition, samples from either the ground or the elevated levels were also pooled and analyzed for respective averages.

## 2.3 Culturable bacteria and fungi

As shown in Fig. 1, the air samples were incubated for culturable bacteria and fungi growth. Trypticase soy agar (TSA; Becton–Dickinson Microbiology Systems, Sparks, MD, USA) was used to culture bacteria, and malt extract agar (Becton–Dickinson) was used to culture fungi. The number of colonies both for the bacteria and fungi on the agar plates were manually counted and statistically corrected by use of the equation described by Feller (1968). The plate counts for culturable bacteria or fungi were also

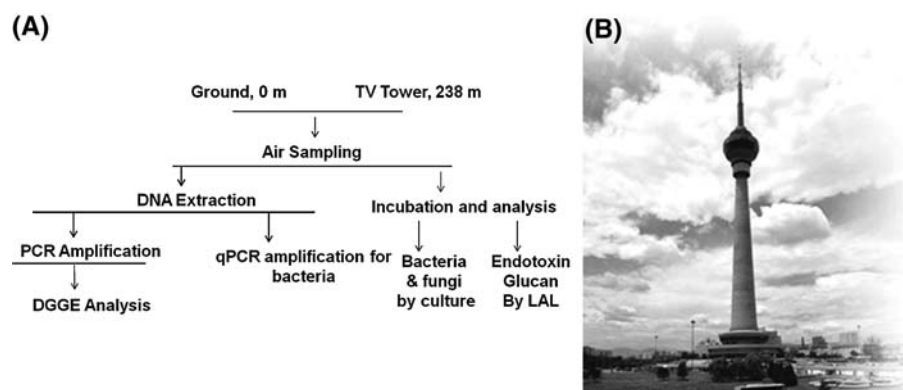
averaged over the sampling periods. The airborne concentrations of culturable bacteria and fungi were converted to standard units (CFU/m<sup>3</sup>).

## 2.4 Total bacterial load and dominant genus

In this study, the total bacterial loads for the air samples collected both at ground level and on the platform were also quantified by use of qPCR as indicated in Fig. 1. Collection liquids (50 µl) of BioSamplers from the ground-level and platform was taken and pooled. Pooled collection liquid (100 µl) was taken out for DNA extraction by use of the Qiagen bacteria DNA extraction kit (Qiagen, Chatsworth, CA, USA). The extracted DNA samples were further resuspended in 50 µl DI water. Bacterial universal forward primer 5'-TCCTACGGGAGGCA GCAGT-3' ( $T_m$ , 59 ± 4°C), reverse primer 5'-GG ACTACCAGGGTATCTAATCCTGTT-3' ( $T_m$ , 58 ± 1°C), and probe (6-FAM)-5'-CGTATTACCGCGGCT GCTGGCAC-3'-(TAMRA) ( $T_m$ , 69 ± 9°C) described by Nadkarni et al. (2002) were used in this study. The amplification and detection of bacterial genes targeting the V3 region of the 16S rRNA gene was performed by qPCR with the ABI-Prism 7300 sequence detection system (Applied Biosystems) using optical grade 96-well plates under the conditions used by Nadkarni et al. (2002). The relative number of bacterial gene copies was calculated for both for the ground level (0 m) and platform (238 m) samples according to the qPCR relative standard curve.

Denaturing gradient gel electrophoresis (DGGE) analysis with subsequent gene sequencing was performed on the pooled air samples ( $n = 8$ ) for both the ground level and elevated samples. The V3 region of the 16S rRNA gene was amplified using

**Fig. 1** **a** Experimental procedures. **b** Broadcast tower used for sampling at 238 m in Beijing



primers P2 (5'-ATTACCGCGGCTGCTGG-3') and P3 (5'-GCclamp-CCTACGGGAGGCAGCAG-3') as described by Muyzer et al. (1993). DGGE was performed with the Bio-Rad DCode mutation-detection system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Approximately 300 ng PCR product was transferred to each well of 8% polyacrylamide gels containing a vertical gradient of denaturant from 30% to 60%. Electrophoresis was performed for 240 min at a constant voltage of 200 V at 60°C. Gels were stained with SYBR-green I solution (10,000× diluted) and photographed (Molecular ImageR Gel Doc XR system; Bio-Rad). Here the bands were scored as either present or absent on the gel and all lanes were compared with each other. Data were recorded and analyzed with sequence detection software (Version 1.4). The bands were excised, reamplified, and sequenced directly. Gene sequencing was performed using the ABI 3730 (Applied Biosystems) by a company (Sangong, Beijing, China) in Beijing, and relevant sequences were submitted to GeneBank with the accession numbers obtained.

Following a similar method, the concentration of the total *Pseudomonas*, the dominant species identified from DGGE and gene-sequencing analysis, was also quantified by use of SYBR qPCR. The representative clone (assigned with *Pseudomonas aeruginosa*) prepared in the lab was used as the standard sample. Primers PSD7F (5'-CAAACTACTGAGCT-AGAGTACG-3') and PSD7R (5'-TAAGATCTCAAGGATCCCAACGGCT-3') were used to quantify the *Pseudomonas* group of organisms (Matsuda et al. 2007).

## 2.5 Endotoxin analysis

For the air samples collected by the BioSampler, endotoxin concentration was quantified. Endotoxin content analysis was conducted using a quantitative kinetic chromogenic limulus amebocyte lysate (LAL) assay (Associates of Cape Cod, East Falmouth, MA, USA). A standard endotoxin dilution series of 0.005, 0.05, 0.5, and 5 endotoxin units (EU)/ml was prepared in lysate reagent water (LRW) for each set of test reagents. For endotoxin measurement, 40 µl standards in duplicate, sample extract dilutions ( $10^{-3}$ ) in triplicate, negative controls (LRW and 0.05% Tween 20), and positive product controls (spiked value 0.25 EU/ml) were pipetted into pre-

designated wells in a 96-well microplate. The microplate was then incubated at 37°C for 15 min. During the incubation, Limulus Amebocyte Lysate agent was reconstituted using Glucashield buffer (Associates of Cape Cod) to remove interference from (1,3)- $\beta$ -D-glucans. After incubation, 40 µl LAL agent were pipetted into each well. The plate was placed inside a spectrophotometer, SpectraMax, pre-mixed for 10 s, and the LAL assay was allowed to proceed for 60 min in the kinetic mode to measure the onset time for color development at 405 nm. Using log-log standard curves of onset time versus standard concentrations ( $r^2 > 99\%$ ), sample concentrations were calculated. The standard curve was generated by software based on the optical densities and concentrations of endotoxin standards (0.005, 0.05, 0.5, and 5 EU/ml) that are supplied by the assay manufacturer. In this study the endotoxin unit (EU) was converted to nanograms (ng) dividing by 10.

## 2.6 (1,3)- $\beta$ -D-Glucan analysis

For the air samples collected by the BioSampler, (1,3)- $\beta$ -D-glucan concentration was quantified. (1,3)- $\beta$ -D-Glucan analysis of the samples was conducted using the quantitative kinetic Glucatell lysate assay, a modified LAL (Associates of Cape Cod). A standard (1,3)- $\beta$ -D-glucan series of 200, 100, 50, 25, and 5 pg/ml was prepared. A 25 µl volume of (1,3)- $\beta$ -D-glucan standards in duplicate, sample extract dilutions in triplicate, negative controls (0.5 M NaOH neutralized by Tris-HCl), and positive product controls (spiked value of 25 pg/ml) were pipetted into pre-designated wells in a 96-well microplate. The microplate was then incubated at 37°C for 15 min. During the incubation, Glucatell lysate agent was reconstituted using 2.8 ml LRW and 2.8 ml Pyrosol buffer (Associates of Cape Cod). After incubation, 100 µl was pipetted into each well of the 96-well plate. The plate was placed inside a spectrophotometer, SpectraMax, pre-mixed for 10 s, and the LAL assay was allowed to proceed for 80 min in the kinetic mode to measure the onset time for color development at 405 nm. Using log-log standard curves of onset time versus standard concentrations ( $r^2 > 99\%$ ), samples concentrations were calculated. The standard curve was generated by software on the basis of the optical densities and the concentrations of glucan standards (200, 100, 50, 25, and 5 pg/ml) that were supplied by the assay manufacturer.

The detection limits for airborne endotoxin and (1,3)- $\beta$ -D-glucan depend on the lowest standards used in this study: endotoxin (0.005 EU/ml) and (1,3)- $\beta$ -D-glucan (5 pg/ml). However, the actual detection limits depend on comparisons between the negative control and the samples. Therefore, the lowest calculated values for the samples based on the standard curve, which might be smaller than the lowest standards but significantly above that of the negative control, should be also considered.

## 2.7 Statistical analysis

The differences for the microbial contents between the ground level (0 m) and elevated (238 m) samples were analyzed by paired *t*-test via the statistical component of SigmaPlot 10. A *p*-value of less than 0.05 indicated a statistically significant difference.

## 3 Results and discussion

Results in this study revealed that no statistically significant differences were found for the levels of endotoxin, (1,3)- $\beta$ -D-glucan, and microbial diversity between the air samples collected at ground level and at an elevation of 238 m. However, the ground-level air samples were observed to have higher culturable bacteria and fungal concentrations than those at an elevation of 238 m.

Figure 2 shows the airborne culturable bacteria concentrations measured for the ground-level and 238-m samples on Day #1 and Day #2. As shown in the figure, irrespective of sampling dates and sampling time (morning to afternoon), the air samples collected at ground-level (0 m) were shown to have significantly higher culturable bacteria concentrations than those obtained at an elevation of 238 m on both Day #1 and Day #2. The airborne culturable bacteria concentrations at an elevation of 238 m ranged from 80 to 600 CFU/m<sup>3</sup> and those for the ground-level sample ranged from 280 to 1,500 CFU/m<sup>3</sup> on both Day #1 and Day #2. The observed culturable concentration differences ranging from 1.3 to 15 times were statistically significant (*p*-value = 0.0047). Lighthart and Shaffer (1995) have shown that sunrise and early evening hours had the highest culturable bacterial concentration averages, whereas the pre-dawn had the lowest. They cited meteorological conditions as an

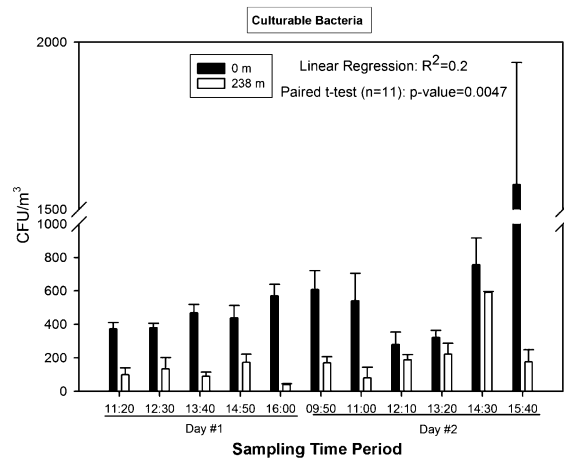


**Fig. 2** Airborne culturable bacterial concentrations measured at 0 and 238 m during different time periods on two days

important regulating effect on airborne bacterial concentrations in the outdoor atmosphere. However, as observed from Fig. 2 in this study, the airborne culturable bacteria concentrations varied greatly over the course of a day, and no clear pattern with regard to time was observed in this study. On average, the ground-level sample had about 5 times higher culturable bacteria concentration than that from an altitude of 238 m. Poor linear regression was observed between culturable bacteria from ground-level and 238 m elevation samples ( $R^2 = 0.2$ ).

Similar findings were obtained when collecting culturable fungi, as shown in Fig. 3. The air samples collected at ground level (0 m) had significantly higher culturable fungal concentrations than those collected at an elevation of 238 m. Fungal concentrations in the air samples collected at ground level were shown to range from 310 to 1,023 CFU/m<sup>3</sup> whereas those in the air samples collected at an elevation of 238 m ranged from 50 to 400 CFU/m<sup>3</sup>. The differences were statistically significant (*p*-value = 0.0003). On average, the ground-level sample had about 3 times higher culturable fungi concentration than that at an elevation of 238 m. The correlation between the culturable fungi at ground level and at an elevation of 238 m ( $R^2 = 0.52$ ) was better than that for culturable bacteria. In this study, the temporal microbial differences were minimized by taking the air samples simultaneously at ground level and at an elevation of 238 m. In addition, all measurements were taken outdoors, thus the culturable bacteria and

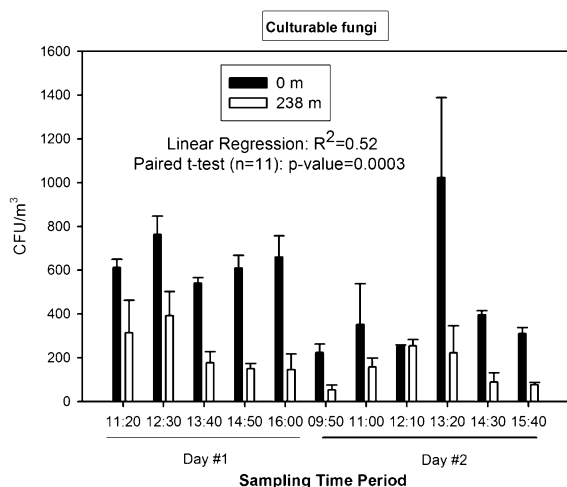


**Fig. 3** The airborne culturable fungal concentrations measured at 0 and 238 m during different time periods on two days

fungi levels reflect those of outdoors at given times at different heights. A study suggested that there was a strong correlation between outdoor and indoor airborne fungi (Dassonville et al. 2008). Other authors also show that outdoor air is the major source of indoor fungi (Li et al. 1995; Ren et al. 2001; Pei-Chih et al. 2000; Su et al. 2001; Chew et al. 2003; O'Connor et al. 2004; Lee and Jo 2006). On the basis of these studies, the work here thus suggested that indoor environments at different heights could have different levels of culturable bacteria or fungi. The results from culturable bacteria and fungi implied that ground-level might be a less stressful environment than the elevation. On the other hand, the fact of higher culturable counts at ground level might also be attributed to the close proximity to soil and vegetation, i.e., the source of bioaerosols upon aerosolization, for the ground air samples.

The bacterial diversity in the air samples collected from the different elevations was studied by 16S rRNA gene-targeted PCR-DGGE analysis. Visual inspection of the DGGE gels showed diverse DNA bands in these environmental samples, as observed in Fig. 4. Similar main DNA migrating patterns in DGGE profiles (bands A, B, C, D, E, F, and G) were observed for the air samples collected at ground level and at an elevation of 238 m, although there are some minor differences seen in the figure. As observed in lanes 2 and 4 in Fig. 4, above band A the ground-level air samples seemed to lack two less visible bands compared with lane 1 and lane 3 for the air

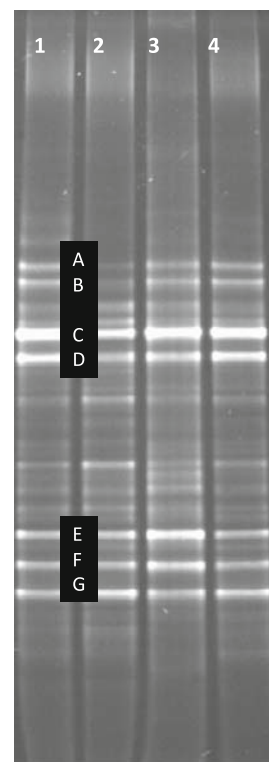


**Fig. 4** DGGE banding profiles of the PCR products of 16S rRNA gene V3 regions amplified from community DNA extracted from airborne bacteria sampled at different elevations. The lanes labeled 1–4 represent four individual mixed samples that were collected on different days and at an elevation of 238 m: 1 238 m (2008/04/31); 2 0 m (2008/04/31); 3 238 m (2008/05/01); 4 0 m (2008/05/01). (A, B, C, D, E, F, and G represent major migrating bands)

samples collected at an elevation of 238 m. DGGE is increasingly being used in bioaerosol studies. In a recent study, similar DGGE profiles were also obtained for the bacterial diversity of air samples collected in eight swine confinement buildings, and suggested the bacterial diversity was similar in the air surrounding these swine confinements (Nehme et al. 2008). In another similar study, the species compositions of the bacterial community in the atmosphere at 800 and 10 m were found to be similar by PCR-DGGE analysis (Maki et al. 2008). However, identification of bacteria by DGGE has a number of potential problems. The DGGE profiles as shown in this study could have many bands which are difficult to purify, and the intraspecies operon heterogeneities can contribute significantly to complex genetic profiles in microbial community analysis (Schmalenberger et al. 2001). It has been suggested that the effect

of operon heterogeneities could be reduced by choosing appropriate variable regions with less intraspecies diversity, e.g., V4 and V5 (Schmalenberger et al. 2001). The results presented here could thus possibly be affected by the relevant limitations of DGGE. Besides, the BioSampler could, in general, run for 30 min without loss of collection liquid and efficiency, and 40 min sampling was used in this study to collect enough air samples. It was recently indicated that there was 0.85 ml collection liquid loss after 40 min sampling for the BioSampler (Yao et al. 2009). This loss should occur to the collection of both ground-level and elevated samples, and thus won't affect the comparison.

SYBR qPCR tests were performed for total bacteria concentrations for the pooled air samples collected at ground level (0 m) and at an elevation of 238 m in two experiments (Day #1 and Day #2). The cycle threshold (Ct) values as shown in Fig. 5a are 22.5 and 23 for the ground-level and 238 m sample, respectively. The negative control was found to have a Ct value of 28.5. The results from the qPCR tests indicated that the air samples collected at ground-level (0 m) had total bacteria concentrations similar to those collected at an elevation of 238 m with a difference of less than 10%, as shown in Fig. 6. In accordance with the method described by Dutil et al. (2007), the total bacteria concentration was calculated by dividing the absolute quantification of the 16S rRNA copy number in each sample by 4.1. Therefore, the ground-level (0 m) and 238 m samples had bacterial concentrations of 1,463 and 1,341 N/I, respectively, more than one thousand times of those culturable concentrations. Nehme et al. (2008) have shown that the total bacterial concentration was about 100–1,000 times higher than the culturable concentration in air samples collected from swine building confinements. Similar magnitude of underestimate resulting from culturing technique was observed by Fabian et al. (2005). Airborne microorganisms in the environments are often stressed by a variety of environmental factors for example desiccation, extreme temperature, oxygen limitation, and exposure to ultraviolet (UV) radiation, thus possibly losing their culturability. In addition, the microorganisms in the atmosphere are diluted and the sampling causes additional stress. In this study, culturability was observed to be less than 0.1% for bacteria. Therefore, the culture-based analysis

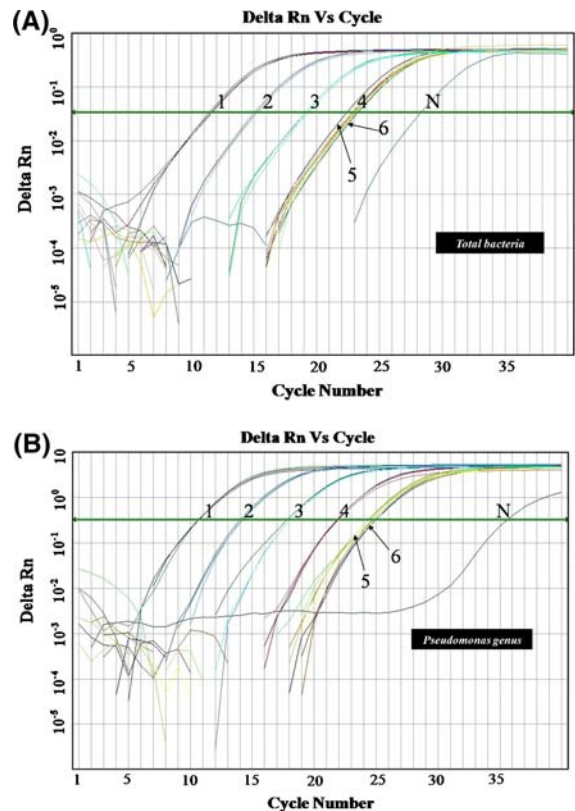


**Fig. 5** Amplification of 16S rRNA genes from the standard and air samples using real-time qPCR: **a** amplification plot of total bacterial load determination; **b** amplification for *Pseudomonas* genus; 1, 2, 3, and 4 represent serially diluted 16S rRNA gene standards ( $9.03 \times 10^{4,5,6,7}$  copies/ $\mu$ l), 5 represents the air sample from ground level, and 6 represents the air sample from the broadcast tower. N stands for the negative control

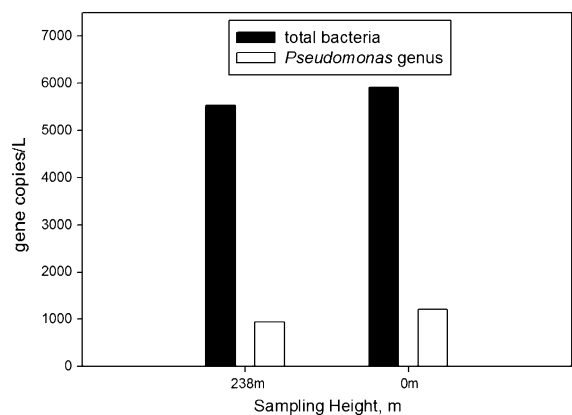


**Fig. 6** The copy number of the 16S rRNA gene of the total bacterial load and of the *Pseudomonas* genus per liter of air from the pooled air samples collected at 0 to 238 m, respectively

significantly underestimates the microbial load in the environment.

DNA sequencing was used when identifying the dominant bacterial strains. As seen in Fig. 4, bands A, B, C, and D with higher intensity were excised, re-amplified, and sequenced directly by Sangong Biotech in Shanghai. For other bands including those with relative lower intensity in the gel, DNA sequencing was not performed in this study. *Pseudomonas* genus was identified from the PCR products (bands A, B, C, and D in Fig. 4) by using the Classifier software in RDP II (the ribosomal database project II). Their accession numbers in the GeneBank are FJ938148, FJ938149, FJ938150, FJ938151, respectively. In Fig. 5b, *Pseudomonas* amplification curves are presented with Ct values close to 25 for the air samples both from ground level and elevation. As indicated in Fig. 6, the air samples from ground level (0 m) were also shown to have *Pseudomonas* genus concentrations similar to those collected at an elevation of 238 m. Its concentrations were quantified as 1,000 and 1,250 gene copies/L for the 238 m and ground-level samples, respectively. Because of the subrepeat variations of rRNA genes ranging from 1 to 15 (Klappenbach et al. 2001; Nadkarni et al. 2002), the conversion of gene copy number to bacteria concentration was not performed for *Pseudomonas*. If gene copy numbers were compared, *Pseudomonas* concentrations tended to be about 1/6 of total bacterial concentrations.

Figures 7 and 8 show the airborne endotoxin and (1,3)- $\beta$ -D-glucan concentrations measured in the air samples collected at ground level (0 m) and at 238 m. As observed from these figures, the endotoxin and (1,3)- $\beta$ -D-glucan levels in the air samples collected at ground level (0 m) and at 238 m varied greatly with sampling time on both Day #1 and Day #2. For endotoxin, the concentration ranged from 0.32 to 5.39 ng/m<sup>3</sup> for the air samples collected at ground level, and from 0.22 to 10.6 ng/m<sup>3</sup> for the air samples collected at an elevation of 238 m, as observed from Fig. 7a. Statistical analysis (paired *t*-test) of the averages, close to 3 ng/m<sup>3</sup>, of 2 days' sampling results as shown in Fig. 7b indicated that the difference in endotoxin concentration between the air samples collected at ground level (0 m) and at 238 m was not statistically significant ( $p = 0.99$ ,  $n = 8$ ). Morgenstern et al. (2005) found little spatial variation in ambient endotoxin concentrations, and no

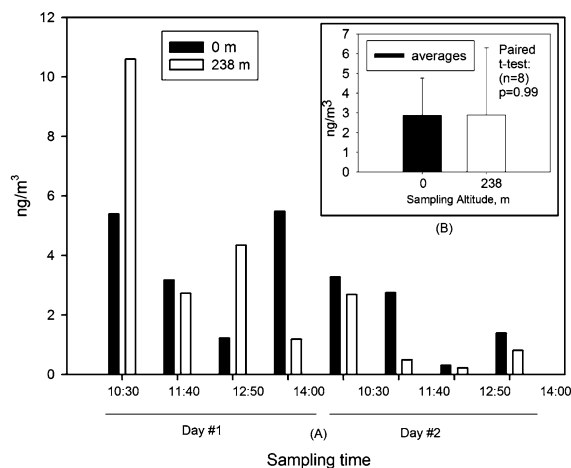


**Fig. 7** Airborne endotoxin concentrations measured at 0 and 238 m during different time periods on two days

difference among geometric mean levels of endotoxin between the city center and the periphery of Munich. Temporal variability of airborne endotoxin was also found not to be significant over weeks in an office building (Hines et al. 2008). For (1,3)- $\beta$ -D-glucan, the concentration level ranged from 0.15 to 0.9 ng/m<sup>3</sup> for air samples collected at ground level, and from 0.1 to 1.64 ng/m<sup>3</sup> for air samples collected at an elevation of 238 m, as observed from Fig. 8a. Statistical analysis (paired *t*-test) of the averages, close to 0.5 ng/m<sup>3</sup>, for 2 days' sampling results as shown in Fig. 8b indicated that the (1,3)- $\beta$ -D-glucan concentration difference in the air samples collected at ground level (0 m) and at 238 m was not statistically significant ( $p = 0.77$ ,  $n = 8$ ) over the course of the day. The similar ambient mean concentrations observed both for the endotoxin and (1,3)- $\beta$ -D-glucan from different elevations might be partially owing to atmospheric mixing. Atmospheric mixing was observed for heights of 800 and 10 m above the ground in another study (Maki et al. 2008). In their study, similar bacterial diversity and particle size distribution were observed at ground level and at 800 m. In their study, they only collected 0.7 m<sup>3</sup> air within 1 h, and in contrast air samples were continuously sampled for 5 h with a total of 30 m<sup>3</sup> of air for each repeat in this study. As calculated from Figs. 7 and 8, endotoxin concentrations were, in general, about 6 times those of (1,3)- $\beta$ -D-glucan, irrespective of elevation.

In this study, sampling was performed using the same sampling tool, the Biostage impactor, at both ground level and at elevation, thus the sampling

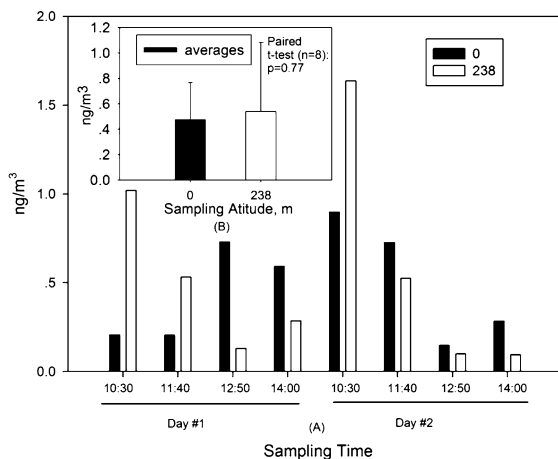


**Fig. 8** Airborne (1,3)-β-D-glucan concentrations measured at 0 and 238 m during different time periods on two days

efficiency and stress remained the same. The total bacterial concentration was similar both for air samples taken from the ground and at an elevation of 238 m. As observed in this study, the air samples from ground level contained higher culturable fungal and bacteria concentrations than those from 238 m, while having similar total microorganism concentrations. Hirvonen et al. (1997) reported that culturable actinomycete (not fungal) spores induced more reactive oxygen species than dead spores in macrophages. In another study, Mitakakis et al. (2001) reported that more *Alternaria allergens* were released from germinated spores. In addition, Green et al. (2003) detected allergens from 11 fungal species before and after germination and found that germination of spores increased the allergen level significantly. It was also suggested that live spores may have greater potential to evoke inflammatory diseases than dead spores when they are deposited in the respiratory tract (Lee et al. 2006). Caro et al. (1999) have demonstrated the simultaneous loss of the culturability and pathogenicity for the bacterium *Salmonella typhimurium*, and a similar finding was observed for *Campylobacter jejuni* (Medema et al. 1992). Therefore, the atmospheric environment close to the ground might represent greater potential risks of exposure to particles of biological origins than those at higher elevations. The results here might, on the other hand, also suggest a possible biological exposure difference among residents who reside in different heights in an urban city with clusters of high-rise buildings.

It is well accepted that fungi concentrations are highly dependent on weather conditions. Lin and Li (2000) indicated that maximum concentrations were observed when the temperature was 25–30°C and relative humidity was 60–70%. Exposure to solar radiation, the presence of ambient air pollutants, and low air temperature and relative humidity can greatly affect the culturability of the microorganisms (Kim 1994; Muilenberg 1995; Mohr 1997). The atmospheric conditions at higher elevation might seem more hostile to bacteria and fungi than those at lower elevation. Solar radiation was found to significantly inactivate the microorganisms (Yukselen et al. 2003; Paez-Rubio and Peccia 2005; Tong and Lighthart 1997). Wegelin et al. (1994) consider UV-A (315–400 nm) to be the most effective component of natural sunlight, because it comprises ca 95% of the higher-energy ultraviolet wavelengths. Similarly, Gameson and Gould (1975) estimated that ca half of the bactericidal effect of sunlight may be attributed to wavelengths lower than 370 nm. Solar radiation is partially depleted and attenuated as it traverses the atmospheric layers, which prevents a substantial portion of it from reaching the earth's surface. This phenomenon results from absorption, scattering, and reflection in the upper atmosphere (stratosphere). The troposphere is an attenuating medium. The solar radiation is reflected and scattered primarily by clouds (moisture and ice particles), particulate matter (dust, smoke, haze, and smog), and various gases. For clear weather conditions, the hourly ratio of photosynthetically active photon flux  $Q_p$  to global solar radiation  $R_s$  ( $Q_p/R_s$ ) was observed to have an increasing trend with altitude, with an average rate of 3.6% per km (Wang et al. 2007). The increasing rate was more noticeable below 900 m (8.8% per km) than above (0.7% per km) (Wang et al. 2007). The increased  $Q_p/R_s$  ratio at higher altitude, e.g., 238 m in this study, might result in increased damage to the culturability of microorganisms in the atmosphere.

This research investigated the biological contents of air samples collected at ground level and at an elevation of 238 m. Different from a previous study (Maki et al. 2008) in which 1-h air samples from two altitudes using 0.45 μm membrane filters were collected, in this research air samples were collected continuously up to 5 h using liquid samplers and agar plates. In addition, the air samples from different elevations were monitored simultaneously, and the

experiments were independently conducted twice in this study. The biological pollutants including endotoxin, (1,3)- $\beta$ -D-glucan, and bacteria were observed to have similar concentration levels in the air samples from ground level and at 238 m. In addition, DGGE analysis indicated there are few differences between the bacterial diversity of pooled air samples collected at ground level and at 238 m. The results presented here have added to the body of evidence that atmospheric mixing is taking place between ground level and the upper atmosphere.

**Acknowledgments** This study was funded by National Science Foundation of China Grant 20877004 and the Peking University “100 Scholar Program” fund.

## References

- Alvarez, A. J., Buttner, M. P., Toranzos, G. A., Dvorsky, E. A., Toro, A., Heikes, T. B., et al. (1994). The use of solid-phase polymerase chain reaction for the enhanced detection of airborne microorganisms. *Applied and Environmental Microbiology*, 60, 374–376.
- Angenent, L. T., Kelley, S. T., Amand, A., Pace, N. R., Mark, T., & Hernandez, M. T. (2005). Molecular identification of potential pathogens in water and air of a hospital therapy pool. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 4860–4865.
- Brown, J. K. M., & Hovmoeller, M. S. (2002). Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science*, 297, 537–541.
- Caro, A., Got, P., Lesne, J., Binard, S., & Baleux, B. (1999). Viability and virulence of experimentally stressed non-culturable *Salmonella typhimurium*. *Applied and Environmental Microbiology*, 65, 3229–3232.
- Chew, G. L., Rogers, C., Burge, H. A., Muilenberg, M. L., & Gold, D. R. (2003). Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics. *Allergy*, 58, 13–20.
- Cox-Ganser, J. M., Rao, C. Y., Park, J. H., Schumpert, J. C., & Kreiss, K. (2009). Asthma and respiratory symptoms in hospital workers related to dampness and biological contaminants. *Indoor Air*, 19, 280–290.
- Czop, J. K., & Kay, J. (1991). Isolation and characterization of beta-glucan receptors on human mononuclear phagocytes. *Journal of Experimental Medicine*, 73, 1511–1520.
- Dassonville, C., Demattei, C., Detaint, B., Barral, S., Bexcapelle, V., & Momas, I. (2008). Assessment and predictors determination of indoor airborne fungal concentrations in Paris newborn babies' homes. *Environmental Research*, 108, 80–85.
- Douwes, J., Thorne, P., Pearce, N., & Heederik, D. (2003). Bioaerosol health effects and exposure assessment: Progress and prospects. *Annals of Occupational Hygiene*, 47, 187–200.
- Dutil, S., Veillette, M., Mériaux, A., Lazure, L., Barbeau, J., & Duchaine, C. (2007). Aerosolization of mycobacteria and legionellae during dental treatment: low exposure despite dental unit contamination. *Environmental Microbiology*, 9, 2836–2843.
- Fabian, M. P., Miller, S. L., Reponen, T., & Hernandez, M. (2005). Ambient bioaerosol indices for indoor air quality assessments in flood reclamation. *Aerosol Science and Technology*, 36, 263–283.
- Feller, W. (1968). *An introduction to probability theory and its applications* (3rd ed., pp. 224–225). New York: Wiley.
- Fulton, J. D. (1966). Microorganisms of the upper atmosphere III. Relationship between altitude and micropopulation. *Applied Microbiology*, 14, 237–240.
- Fulton, J. D., & Mitchell, R. B. (1996). Microorganisms of the upper atmosphere II. Microorganisms in two types of air masses at 690 meters over a city. *Applied Microbiology*, 14, 232–236.
- Gameson, A. L. H., & Gould, D. J. (1975). Effects of solar radiation on the mortality of some terrestrial bacteria in seawater. In A. H. L. Gameson (Ed.), *Discharge of sewage from sea outfalls* (pp. 209–219). Oxford: Pergamon.
- Green, B. J., Mitakakis, T. Z., & Tovey, E. R. (2003). Allergen detection from 11 fungal species before and after germination. *The Journal of Allergy and Clinical Immunology*, 111, 285–289.
- Griffin, D. W. (2007). Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. *Clinical Microbiology Reviews*, 20, 459–477.
- Griffin, D. W., Westphal, D. L., & Gray, M. A. (2006). Airborne microorganisms in the African desert dust corridor over the mid-Atlantic ridge. *Aerobiologia*, 22, 211–226.
- Hines, C. J., Milton, D. K., Larsson, L., Petersen, M. R., Fisk, W. J., & Mendell, M. J. (2008). Characterization and variability of endotoxin and 3-hydroxy fatty acids in an office building during a particle intervention study. *Indoor Air*, 10, 2–12.
- Hirvonen, M. R., Ruotsalainen, M., Savolainen, K., & Nevalainen, A. (1997). Effect of culturability of actinomycete spores on their ability to stimulate production of nitric oxide and reactive oxygen species in RAW264.7 macrophages. *Toxicology*, 124, 105–114.
- Jones, A. M., & Harrison, R. M. (2004). The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. *Science of the Total Environment*, 326, 151–180.
- Joo, Y. S., An, S. H., Kim, O. K., Lubroth, J., & Sur, J. H. (2002). Foot-and-mouth disease eradication efforts in the Republic of Korea. *Canadian Journal of Veterinary Research*, 66, 122–124.
- Kim, J. (1994). Atmospheric environment of bioaerosols. In B. Lighthart & A. J. Mohr (Eds.), *Atmospheric microbial aerosols: theory and applications* (pp. 28–67). New York: Chapman & Hall.
- Klappenbach, J. A., Saxman, P. R., Cole, J. R., & Schmidt, T. M. (2001). rrndb: The ribosomal RNA operon copy number database. *Nucleic Acids Research*, 29, 181–184.
- Laumbach, R. J., & Kipen, H. M. (2005). Bioaerosols and sick building syndrome: Particles, inflammation, and allergy. *Current opinion in Allergy and Clinical Immunology*, 5, 135–139.

- Lee, T., Grinshpun, S. A., Martuzevicius, D., Adhikari, A., Crawford, C. M., & Reponen, T. (2006). Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes. *Atmospheric Environment*, 40, 2902–2910.
- Lee, J. H., & Jo, W. K. (2006). Characteristics of indoor and outdoor bioaerosols at Korean high-rise apartment buildings. *Environmental Research*, 101, 11–17.
- Li, C. S., Hsu, L. Y., Chou, C. C., & Hsieh, K. H. (1995). Fungus allergens inside and outside the residences of atopic and control children. *Archives of Environmental Health*, 50, 38–43.
- Lighthart, B., & Shaffer, B. T. (1995). Airborne bacteria in the atmospheric surface layer: Temporal distribution above a grass seed field. *Applied and Environmental Microbiology*, 61, 1492–1496.
- Lin, W.-H., & Li, C.-S. (2000). Associations of fungal aerosols, air pollutants and meteorological factors. *Aerosol Science and Technology*, 32, 359–368.
- Maki, T., Susuki, S., Kobayashi, F., Kakikawa, M., Yamada, M., Higashi, T., et al. (2008). Phylogenetic diversity and vertical distribution of a halobacterial community in the atmosphere of an Asian dust (KOSA) source region, Dunhuang City. *Air Quality, Atmosphere, & Health*, 1, 81–89.
- Matsuda, K., Tsuji, H., Asahara, T., Kado, Y., & Nomoto, K. (2007). Sensitive quantitative detection of commensal bacteria by rRNA-targeted reverse transcription-PCR. *Applied and Environmental Microbiology*, 73, 32–39.
- Matthias-Maser, S., & Jaenicke, R. (1995). The size distribution of primary biological aerosol particles with radii > 0.2  $\mu\text{m}$  in an urban/rural influenced region. *Atmospheric Research*, 39, 279–286.
- Medema, G. J., Schets, F. M., van de Giessen, A. W., & Havelaar, A. H. (1992). Lack of colonization of 1 day old chicks by viable, non-culturable *Campylobacter jejuni*. *Journal of Applied Bacteriology*, 72, 512–516.
- Mitakakis, T. Z., Barnes, C., & Tovey, E. R. (2001). Spore germination increases allergen release from *Alternaria*. *The Journal of Allergy and Clinical Immunology*, 107, 388–390.
- Mohr, A. J. (1997). Fate and transport of microorganisms in air. In C. J. Hurst, G. R. Knudson, M. J. McInerney, L. D. Stetzenbach, & M. V. Walter (Eds.), *Manual of environmental microbiology*. Washington: ASM Press.
- Monn, C. (2001). Exposure assessment of air pollutants: A review on spatial heterogeneity and indoor/outdoor/personal exposure to suspended particulate matter, nitrogen dioxide and ozone. *Atmospheric Environment*, 35, 1–32.
- Morgenstern, V., Carty, C. L., Gehring, U., Cyrys, J., Wolfgang, B., & Heinrich, J. (2005). Lack of spatial variation of endotoxin in ambient particulate matter across a German metropolitan area. *Atmospheric Environment*, 39, 6931–6941.
- Muilenberg, M. L. (1995). The outdoor aerosol. In H. A. Burge (Ed.), *Bioaerosols* (pp. 163–204). Boca Raton: Lewis.
- Muyzer, G., de Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59, 695–700.
- Nadkarni, M. A., Martin, F. E., Jacques, N. A., & Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*, 148, 257–266.
- Nehme, B., Létourneau, V., Forster, R. J., Veillette, M., & Duchaine, C. (2008). Culture-independent approach of the bacterial bioaerosol diversity in the standard swine confinement buildings, and assessment of the seasonal effect. *Environmental Microbiology*, 10, 665–675.
- O'Connor, G. T., Walter, M., Mitchell, H., Kattan, M., Morgan, W. J., Gruchalla, R. S., et al. (2004). Airborne fungi in the homes of children with asthma in low-income urban communities: the inner-city asthma study. *The Journal of Allergy and Clinical Immunology*, 114, 599–606.
- Olsson, M., Lidman, C., Latouche, S., Björkman, A., Roux, P., Linder, E., et al. (1998). Identification of *Pneumocystis carinii* sp. hominis gene sequences in filtered air in hospital environments. *Journal of Clinical Microbiology*, 36, 1737–1740.
- Ozawa, Y., Ong, B. L., & An, S. H. (2001). Traceback systems used during recent epizootics in Asia. *Revue Scientifique et Technique*, 20, 605–613.
- Paez-Rubio, T., & Peccia, J. (2005). Estimating solar and nonsolar inactivation rates of airborne bacteria. *Journal of Environmental Engineering*, 131, 512–517.
- Pei-Chih, W., Huey-Jen, S., & Chia-Yin, L. (2000). Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. *Science of the Total Environment*, 253, 111–118.
- Rao, C. Y., Riggs, M. A., Chew, G. L., Muilenberg, M. L., Thorne, P. S., Sickie, D. V., et al. (2007). Characterization of airborne molds, endotoxins, and glucans in homes in new orleans after hurricanes Katrina and Rita. *Applied and Environmental Microbiology*, 73, 1630–1634.
- Ren, P., Jankun, T. M., Belanger, K., Bracken, M. B., & Leaderer, B. P. (2001). The relation between fungal propagules in indoor air and home characteristics. *Allergy*, 56, 419–424.
- Rylander, R. (2005). (1  $\rightarrow$  3)- $\beta$ -D-glucan in the environment: a risk assessment. In S.-H. Young & V. Castranova (Eds.), *Toxicology of (1  $\rightarrow$  3)- $\beta$ -D-glucans* (pp. 53–64). Boca Raton: Taylor & Francis.
- Sakamoto, K., & Yoshida, K. (2002). Recent outbreaks of foot and mouth disease in countries of East Asia. *Revue Scientifique et Technique*, 21, 459–463.
- Salo, P. M., Arbes, S. J., Crockett, P. W., Thorne, P. S., Cohn, R. D., & Zeldin, D. C. (2008). Exposure to multiple indoor allergens in US homes and its relationship to asthma. *The Journal of Allergy and Clinical Immunology*, 121, 678.e2–684.e2.
- Sawyer, M. H., Chamberlain, C. J., Wu, Y. N., Aintablian, N., & Wallace, M. R. (1994). Detection of Varicella-Zoster virus DNA in air samples from hospital rooms. *Journal of Infectious Diseases*, 169, 91–94.
- Schmalenberger, A., Schwieger, F., & Tebbe, C. C. (2001). Effect of primers hybridizing to different evolutionarily conserved regions of the small-subunit rRNA gene in PCR based microbial community analyses and genetic profiling. *Applied and Environmental Microbiology*, 67, 3557–3563.

- Singh, A. B., & Singh, A. (1996). Indoor airborne fungi as important occupational sensitizers in poultry workers. *Indoor and Built Environment*, 5, 138–147.
- Su, P., Wu, P., Chen, H., Lee, F., & Lin, L. (2001). Exposure assessment of indoor allergens, endotoxin, and airborne fungi for homes in Southern Taiwan. *Environmental Research*, 85, 135–144.
- Thorne, P. S. (2000). Inhalation toxicology models of endotoxin and bioaerosol-induced inflammation. *Toxicology*, 152, 13–23.
- Tong, Y., & Lighthart, B. (1997). Solar radiation has a lethal effect on natural populations of culturable outdoor atmospheric bacteria. *Atmospheric Environment*, 31, 897–900.
- Walinder, R., Norback, D., Wessen, B., & Venge, P. (2001). Nasal lavage biomarkers: Effects of water damage and microbial growth in an office building. *Archives of Environmental & Occupational Health*, 56, 30–36.
- Wang, Q., Kakubari, Y., Kubota, M., & Tenhunen, J. (2007). Variation on PAR to global solar radiation ratio along altitude gradient in Naeba mountain. *Theoretical and Applied Climatology*, 87, 239–253.
- Wegelin, M., Canonica, S., Mechsner, K., Fleischmann, T., Pesaro, F., & Metzler, A. (1994). Solar water disinfection: scope of the process and analysis of radiation experiments. *Journal of Water Science Research and Technology*, 43, 154–169.
- Wu, P. C., Tsai, J. C., Li, F. C., Lung, S. C., & Su, H. J. (2004). Increased levels of ambient fungal spores in Taiwan are associated with dust events from China. *Atmospheric Environment*, 38, 4879–4886.
- Yao, M., Zhang, H., Dong, S., Zhen, S., & Chen, X. (2009). Comparison of electrostatic collection and liquid impinging methods when collecting airborne house dust allergens, endotoxin and (1,3)- $\beta$ -D-glucans. *Journal of Aerosol Science*, 40, 492–502.
- Yukselen, M. A., Calli, B., Gokyay, O., & Saatci, A. (2003). Inactivation of coliform bacteria in Black Sea waters due to solar radiation. *Environment International*, 29, 45–50.