

# Applicability of a modified MCE filter method with Button Inhalable Sampler for monitoring personal bioaerosol inhalation exposure

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**Abstract** In this study, a “modified” mixed cellulose ester (MCE) filter culturing method (directly placing filter on agar plate for culturing without extraction) was investigated in enumerating airborne culturable bacterial and fungal aerosol concentration and diversity both in different environments. A Button Inhalable Sampler loaded with a MCE filter was operated at a flow rate of 5 L/min to collect indoor and outdoor air samples using different sampling times: 10, 20, and 30 min in three different time periods of the day. As a comparison, a BioStage impactor, regarded as the gold standard, was operated in parallel at a flow rate of 28.3 L/min for all tests. The air samples collected by the Button Inhalable Sampler were directly placed on agar plates for culturing, and those collected by the BioStage impactor were incubated directly at 26 °C. The colony forming units (CFUs) were manually counted and the culturable concentrations were calculated both for bacterial and fungal aerosols. The bacterial CFUs developed were further washed off and subjected to polymerase chain reaction–denaturing gradient gel electrophoresis (DGGE) for diversity analysis. For fungal CFUs, microscopy method was applied to studying the culturable fungal diversity obtained using different

methods. Experimental results showed that the performance of two investigated methods varied with sampling environments and microbial types (culturable bacterial and fungal aerosols). For bacterial aerosol sampling, both methods were shown to perform equally well, and in contrast the “modified” MCE filter method was demonstrated to enumerate more culturable fungal aerosols than the BioStage impactor. In general, the microbial species richness (number of gel bands) was observed to increase with increasing collection time. For both methods, the DGGE gel patterns were observed to vary with sampling time and environment despite of similar number of gel bands. In addition, an increase in sampling time from 20 to 30 min was found not to substantially alter the species richness. Regardless of the sampling methods, more species richness was observed in the outdoor environment than the indoor environment. This study described a new personal bioaerosol exposure assessment protocol, and it was demonstrated applicable in monitoring the personal bioaerosol exposure in replace of an Andersen-type impactor.

**Keywords** “Modified” MCE filter culturing method · Button-inhalable sampler · BioStage impactor · Culturable aerosol diversity · Bacterial and fungal aerosols · Polymerase chain reaction (PCR) · Denaturing gradient gel electrophoresis (DGGE) · Similarity dendrogram

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## Introduction

Bioaerosols, including airborne bacteria, fungi, viruses, and their derivatives, are ubiquitous both in indoor and outdoor environments. It is commonly accepted that exposure to these biological aerosols can result in numerous adverse health effects (Cox and Wathes 1995), including lung impairments (Douwes et al. 2003; Trout et al. 2001), asthma

exacerbation (Murray et al. 2004; Bundy et al. 2009), and infectious diseases (Ayres et al. 2009; Guan et al. 2003; Perez-Padilla et al. 2009). In quantifying the biological exposure, bioaerosol sampling as the first step for the task plays an important role. Among the available sampling techniques, filtration and impaction based methods are widely used.

Andersen-type impactor such as BioStage (SKC) is generally used as a standard for bioaerosol sampling. The six-stage Andersen sampler was first introduced in 1958 (Andersen 1958), and thereafter some modification and evaluation studies of the sampler were carried out (Flesch et al. 1967; Jones et al. 1985; Mayl 1964; Solomon 1970). In 1964, the Andersen sampler was recommended for bioaerosols sampling during an international symposium on aerobiology (Brachman et al. 1964). Use of impaction-based method is often associated with counting colony forming units (CFUs) after the sampling (Carnelley et al. 1887; Yao and Mainelis 2006, 2007; Zhen et al. 2009). When airborne microorganisms concentrations are higher, positive hole correction should be used to statistically modify the observed CFUs (Feller 1968). Impaction-based method utilizes the particle inertia, which is realized with a high impaction velocity, e.g., 24 m/s for the BioStage impactor when operated at a flow rate of 28.3 L/min (SKC). Studies have shown that such high impaction velocity could cause particle bounce, desiccation and embedding of microorganisms into the agar plates (Stewart et al. 1995; Willeke and Baron 1993; Zhen et al. 2009). Nonetheless, impaction-based method is simple and easy to operate. Among reported applications, a BioStage impactor was used to sample the reaerosolized *Bacillus anthracis* spores in the contaminated senator's office (Weis et al. 2002). In their study, the agar plates were incubated, and the colony forming units (CFUs) were counted and further subjected to polymerase chain reaction (PCR) to confirm the presence of the species (Weis et al. 2002).

In the meantime, filtration-based method is also widely used in bioaerosol sampling (Burton et al. 2005; Guan and Yao 2010; Xu and Yao 2011; Wu and Yao 2010). Studies indicated that filter sampling could result in desiccation effects (Agranovski et al. 2004; Burton et al. 2005; Wang et al. 2001; Xu and Yao 2011). However, lower impaction stress is encountered for filter sampling due to its relatively lower sampling flow rate compared to those of impaction based methods. Application of filter for bioaerosol sampling usually proceeds with the extraction procedure for culturing and PCR (Burton et al. 2005; Droogenbroeck et al. 2009; Hospodsky et al. 2010; Mastorides et al. 1999; Stark et al. 1998). However, the extraction procedure could cause culturability loss for the bioaerosols sampled and introduced environmental pollutants to the final solution which affects PCR amplification (Alvarez et al. 1995; Wilson 1997).

Recently, we have also utilized culturing PCR approach to investigate the culturable bacterial and fungal aerosol diversity obtained by different sampling tools including the BioStage impactor (SKC Inc, Eighty Four, PA, USA) and the direct mixed cellulose ester (MCE) filter culturing method which places the MCE filter with air sample directly onto agar plate (Xu and Yao 2011). In other studies, similar methods were also adopted for culturing microorganisms (Bernardo et al. 2002; Lighthart et al. 2000; Yao et al. 2005). In another study, button inhalable sampler in conjunction with gelatin filter (can be dissolved in water) was shown to be less efficient than the BioStage impactor in measuring environmental bioaerosols due to the strong desiccation effects of the filter material (Yao and Mainelis 2007). In our recent study, we have shown that the direct MCE filter culturing method performed reasonably well when compared to the BioStage impactor in sampling airborne bacteria and fungi for 15 min sampling time (Xu and Yao 2011). Filter sampling has the portability advantage, especially for assessing personal bioaerosol exposure. However, the performance of such filter sampling and culturing method is not thoroughly compared to a standard bioaerosol sampler such as BioStage impactor under different sampling times, environments, and microbial types; past studies are only limited to a single sampling time (Xu and Yao 2011; Bernardo et al. 2002). An increasing number of studies show that the sampling time plays a very important role in enumeration of airborne biological community (Abdulmir et al. 2010; Durand et al. 2002; Folmsbee et al. 2000; Mainelis and Tabayoyong 2010; Wang et al. 2001; Woodward et al. 2004; Zhen et al. 2009).

The BioStage impactor is primarily used for environmental air sampling, while the Button Inhalable Sampler is appropriate for personal sampling. In many studies, the results obtained by a BioStage impactor were used for estimating human exposure to the bioaerosols in various environments. However, the results from environmental sampling are not representative for personal exposure, and it is not convenient (too big and require external power source) to use the BioStage impactor for personal bioaerosol exposure monitoring. Accordingly, this study was designed to investigate the applicability of a Button Inhalable Sampler with a “modified” MCE filter culturing method, i.e., without extraction, in assessing personal bioaerosol exposure under different sampling times and environments when compared to the standard sampler, BioStage Impactor. The collected air samples by the filter and the BioStage impactor were directly cultured at room temperature, and CFUs were counted, further removed for PCR–denaturing gradient gel electrophoresis (DGGE) analysis of the culturable aerosol diversity. Fungal aerosol species collected by different methods were also identified using the microscopy technique. The results obtained here can lend information to

the development of robust environmental and personal biological aerosol quantification tools.

## Materials and methods

### Bioaerosols sampling and cultivation

In this study, a Button Inhalable Sampler (SKC, Inc.) in conjunction with MCE filters (Millipore, Bedford, MA, USA) composed of cellulose acetate and cellulose nitrate with a pore size of 0.45  $\mu\text{m}$  was used for the bioaerosol sampling. Here, a gelatin filter was not used due to its pronounced desiccation effects especially during a prolonged sampling time period despite of its water solubility. During the sampling, the sampler was operated at a flow rate of 5 L/min for three different sampling times: 10, 20, and 30 min to collect air samples over three different time periods (9:00 AM, 10:00 AM, and 14:00 PM). The air samples were collected for quantifying airborne bacteria and fungi both in an indoor (an office environment on Peking University campus) and an outdoor environment (outside of a two-story building) nearby the 4th Ring Road of Beijing. As a comparison, a BioStage impactor was also operated in parallel to collect the air samples for three different times: 10, 20, and 30 min at a flow rate of 28.3 L/min over the same time periods. The sampling heads of both samplers were placed upside 1.3 m above the ground. For each test condition (indoor/outdoor, bacterial/fungal aerosols, different sampling times), three independent air samples were taken.

During indoor air sampling, the range of temperature was 18.1–18.9 °C and the relative humidity was 36–38 %; for outdoor air sampling, the range of temperature was 19.1–19.9 °C and the relative humidity was 34–38 %. All sampling flow rates were calibrated using a Mini-Buck calibrator (AP Buck Inc., Orlando, FL, USA) in this study. The usual filter collection method would be to extract and elute bioaerosol particles from the filter after the sampling. Here, we did not perform the extraction after the bioaerosol collection instead, we placed the filter along with the bioaerosol samples collected directly onto the agar plate for enumerating bioaerosols at 26 °C. This “modified” MCE filter protocol minimizes the potential microbial stress associated with the extraction step. For BioStage impactor plates, they were also incubated directly at 26 °C. The bacterial aerosols were grown on trypticase soy agar (Becton, Dickson and Company, Sparks, MD, USA) plates for 2–3 days, and fungal aerosols were grown on Malt Extract Agar (Becton, Dickson and Company) for 3–5 days. The CFUs were manually counted after the cultivation. Culturable bacterial and fungal aerosol concentrations were then calculated as CFU per cubic meter. For BioStage

impactor, CFU counts were also manually corrected based on the positive holes correction method (Feller 1968). For all sampling experiments, agar plates and MCE filters were brought to the sampling sites without actual environmental sampling as blank controls. The Button Inhalable Sampler was washed using ethanol and disinfected using UV after each set of experiments.

### Culturable bioaerosol diversity analysis

The PCR–DGGE analysis was applied to analyzing the culturable bacterial aerosol communities in the air samples collected both by the Button Inhalable Sampler with MCE filter and the BioStage impactor in different environments. For the air samples collected in this study, bacterial aerosol CFUs after culturing from three replicates were washed off the agar plates using deionized (DI) water and pooled together to a total liquid volume of 10 ml in a 50-ml Corning® Premium Quality Centrifuge Tubes (Corning Inc., Acton, MA, USA). The microbial suspension was first centrifuged for 1 min at the speed of 7,000 rpm (Centrifuge 5804R, Eppendorf, Hauppauge, NY, USA). The supernatant of the suspension was then poured out and another centrifugation step was performed to remove possible chemical inhabitants after washing the pellets with 5 mL DI water.

After the purification, 1 ml of the bacterial suspension for each bacterial sample was taken out for DNA extraction by a bacteria DNA extraction kit (Tiangen Co., Beijing) according to the manufacturer’s instruction. The extracted DNA samples were further suspended into 50  $\mu\text{L}$  DI water. The V3 region of the 16S rRNA gene was amplified using primers P2 (5′-ATTACCGCGGCTGCTGG-3′) and P3 (5′-GCClamp-CCTACGGGAGGCAGCAG-3′) through PCR as described by Muyzer et al. (Muyzer et al. 1993). PCR reaction mixture (total volume was 25  $\mu\text{L}$ ) included 2  $\mu\text{L}$  DNA template, 1  $\mu\text{L}$  primer P2 (10  $\mu\text{M}$ ), 1  $\mu\text{L}$  primer P3 (10  $\mu\text{M}$ ), 12.5  $\mu\text{L}$  2 $\times$  Master Mix (10 $\times$  Taq Buffer, dNTP Mixture, Taq (2.5 U/ $\mu\text{L}$ )) (Tiangen Co., Beijing), and 12.5  $\mu\text{L}$  dd H<sub>2</sub>O. The cycle conditions were 94 °C for 3 min, 30 cycles of [94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min], and 72 °C for 5 min. DI water (free of DNA and RNA) and *Bacillus subtilis* var niger was used as the negative and positive controls, respectively, in the PCR experiments. DGGE was performed with the Bio-Rad DCode mutation-detection system (Bio-Rad, Hercules, CA, USA) according to the manufacturer’s instructions. In details, approximately 20  $\mu\text{L}$  PCR products was transferred to each well of 8 % polyacrylamide gels containing a vertical gradient of denaturant from 30 to 65 %. The electrophoresis was performed for 300 min at a constant voltage of 180 V at 60 °C. After the electrophoresis, gels were stained with GelRed solution (10,000 $\times$  diluted with DI water; Biotium, Hayward, CA, USA) and photographed

(Molecular Imager Gel Doc XR system, Bio-Rad) under ultraviolet lamp at the wavelength of 254 nm. The DGGE bands for the samples obtained by each of the methods investigated were further compared using dendrograms obtained using the BioRad built-in software functions “band detection” and “unweighted pair group method with arithmetic mean (UPGAMA)” clustering, and the similarity of the culturable microbial aerosol diversities was analyzed using the scale bar produced by the methods.

For culturable fungal aerosols, microscopy method was used for fungal genera identification. Similar to bacterial study, fungal colonies were first washed off from the agar plates, and about 10  $\mu\text{L}$  of fungal suspension was pipetted onto the glass slide. One hundred times magnification was achieved using the immersion oil using Olympus CX 41 (Minneapolis, MN, USA) to record the images of the fungi recognized. The fungal genera were identified through visual comparison of the images taken and existing fungal morphologies according to a reference book written by Harold et al. (1980).

### Statistical analysis

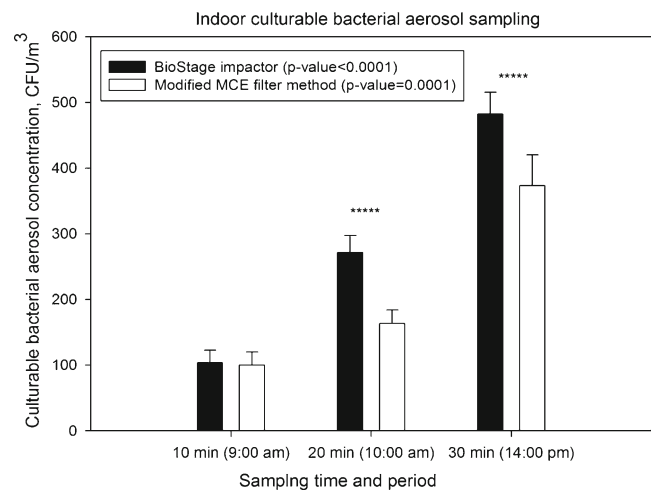
The differences in the culturable bacterial and fungal aerosol concentrations obtained by the direct MCE filter culturing method and the BioStage impactor were analyzed by a paired *t* test via the statistical component of SigmaPlot 10 (Systat Software, Inc.) and also analysis of variance. A *p* value of less than 0.05 indicates a statistically significant difference at a confidence level of 95 %.

### Results and discussion

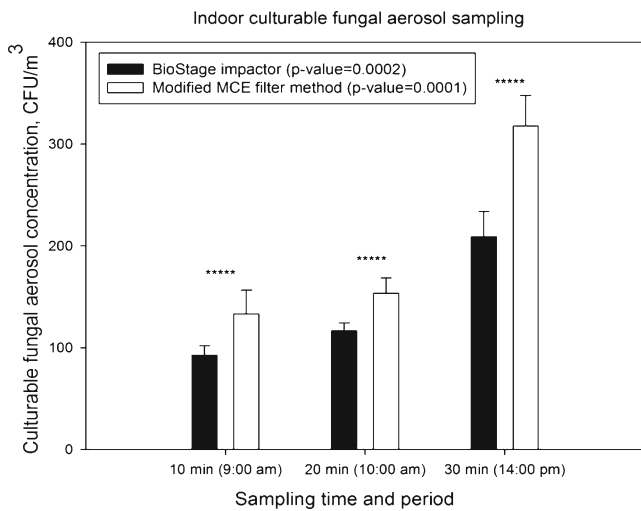
Experimental results revealed that the performances of both methods depended on the sampling time, environment, and microbial types (bacterial and fungal aerosols). Figure 1 shows the indoor culturable bacterial aerosol concentrations obtained by the “modified” MCE filter culturing method and the BioStage impactor. As observed in Fig. 1, the indoor CFU concentrations for bacterial aerosols measured by the BioStage impactor were significantly higher than those by the “modified” MCE filter culturing method for 20 and 30 min (*p* value=0.0279, 0.030 (*t* test)). However for 10-min sampling, no statistically significant differences were detected between two methods (*p* value=0.108), which might be due to the less desiccation effect produced by the filter sampling for a shorter sampling time. In a previous study, Bernardo et al. (2002) found that the BioStage impactor obtained significantly higher (~23 % more) total culturable bioaerosol concentrations than the cellulose nitrate membrane filter using a similar method in swine bars.

In their study, different sampling times were used: 1 min for the Andersen sampler and 3 min for the filter. In addition to the different environment, different samplers and filter types were used in our study. In this study, temporal variations in bioaerosol concentrations were also observed. As seen from Fig. 1, it seems that airborne culturable bacterial concentration increased from morning to afternoon ranging from 100 to 500  $\text{CFU}/\text{m}^3$  (*p* values=0.0001 and 0.0001). Our previous study also found that airborne culturable bioaerosol concentrations appeared to be higher in the afternoon than in the morning (Li et al. 2010). The MCE filter method and the BioStage impactor can result in different type of stress. The impaction stress (function of impaction velocity) of the BioStage impactor was indicated to be around 240 times that caused by the MCE filter given the operating parameters used in this study. According to our previous study (Zhen et al. 2009), both methods resulted in similar degree of desiccation (per unit of air received per unit of sampling area) given the operating parameters used in this study. For the BioStage impactor, however, there was more moisture on the agar plate than the MCE filter, which thus provides a layer of protection from the desiccation for the microorganisms especially for longer sampling time. Therefore, the sampling time and impaction velocity play a role in the performances of both samplers when collecting bioaerosols.

In contrast, for indoor airborne fungal aerosol sampling as shown in Fig. 2, the “modified” MCE filter culturing method was observed to obtain significantly higher culturable concentrations regardless of the sampling times tested (*p* value=0.045 (*t* test), 0.0293, and 0.0484). The difference



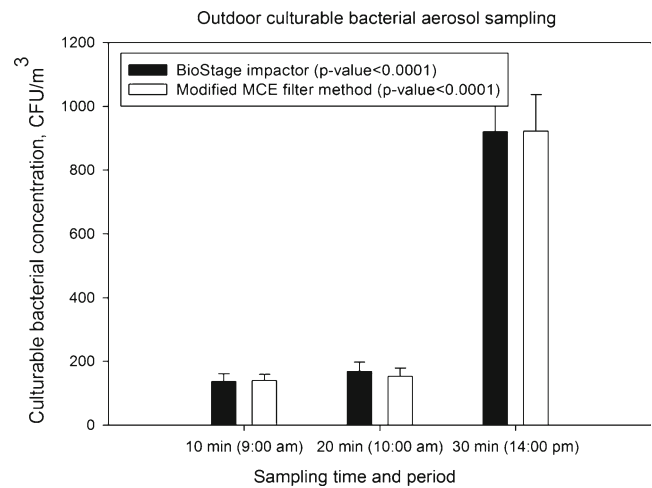
**Fig. 1** Comparison of indoor culturable bacterial aerosol concentrations obtained by the “modified” MCE filter method and the BioStage impactor at different sampling times (10, 20, and 30 min) over three different time periods; the sampling flow rates for the BioStage impactor and the modified MCE filter method were 28.3 and 5 L/min, respectively; the times in the brackets indicated the starting times of sampling for each set; data points represent the averages of three repeats; \*\*\*\*statistically significant difference between two methods



**Fig. 2** Comparison of indoor culturable fungal aerosol concentrations obtained by the “modified” MCE filter method and the BioStage impactor at different sampling times (10, 20, and 30 min) over three different time periods; the sampling flow rates for the BioStage impactor and the modified MCE filter method were 28.3 and 5 L/min, respectively; the times in the brackets indicated the starting times of sampling for each set; *data points* represent the averages of three repeats, and *error bar* stands for the standard deviation; \*\*\*\*statistically significant difference between two methods

in 10-min sampling for indoor bacteria and fungi might be owing to the fact that fungal species appear to be more desiccation resistant than indoor bacterial aerosols. Stewart et al. (1995) have shown that impaction stress could cause bacterial injuries, resulting in culturability loss. Here, fungal species might be more susceptible to the impaction stress due to their larger sizes compared to bacterial species; accordingly, the fungal aerosol concentrations obtained by the BioStage impactor in general appeared to be lower than those obtained using the “modified” MCE filter culturing method regardless of the sampling environment and sampling time. Similar to indoor bacterial sampling, the indoor fungal aerosol concentrations (relatively lower in respective sampling time periods) were shown to increase from morning to afternoon ranging from 100 to 300 CFU/m<sup>3</sup> (*p* values=0.0001 and 0.0002). It was suggested that geographical location, human activity, growth cycle of organisms, and meteorological factors have influence on the diurnal distribution of airborne bacteria and fungi in the atmosphere (Hameed et al. 2009).

Figures 3 and 4 show the results from the outdoor bacterial and fungal aerosol samplings, respectively. Different from indoor bacterial sampling, for outdoor bacteria aerosols no statistically significant differences were detected as observed in Fig. 3, between the “modified” MCE filter culturing method and the BioStage impactor regardless of the sampling times tested (all *p* values>0.05). As shown in Fig. 3, in the morning, low culturable bacterial aerosol concentrations were observed to be around 200 CFU/m<sup>3</sup>

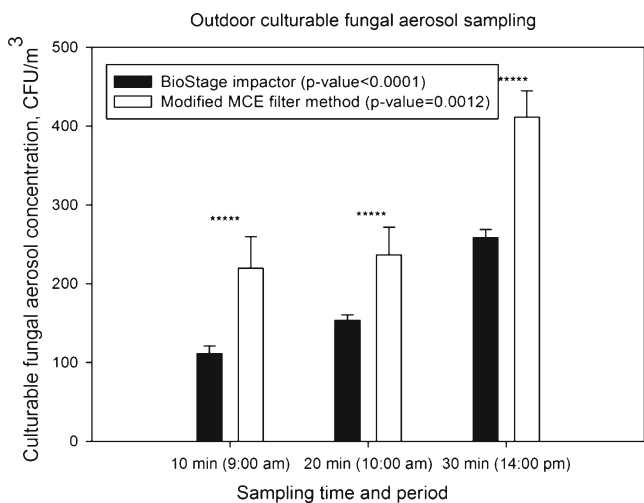


**Fig. 3** Comparison of outdoor culturable bacterial aerosol concentrations obtained by the “modified” MCE filter method and the BioStage impactor at different sampling times (10, 20, and 30 min) over three different time periods; the sampling flow rates for the BioStage impactor and the modified MCE filter method were 28.3 and 5 L/min, respectively; the times in the brackets indicated the starting times of sampling for each set; *data points* represent the averages of three repeats, and *error bar* stands for the standard deviation

while in the afternoon, the concentration reached as much as 1,000 CFU/m<sup>3</sup>. Compared to the indoor bacterial species, the outdoor bacterial species might be less susceptible to the sampling stress especially the impaction stress such that both methods produced similar levels of concentrations as observed in Fig. 3 in this study. Similar to indoor fungal aerosol sampling, the “modified” MCE filter culturing method was shown to obtain significantly higher outdoor culturable fungal concentrations than the BioStage impactor regardless of the sampling times tested over different time periods as observed in Fig. 4 (*p* value=0.037, 0.0512, and 0.0266).

The results obtained here agreed well with those observed in our recent study in which only a 15-min sampling time was tested for the BioStage impactor and the “modified” MCE filter culturing method (Xu and Yao 2011). The results for fungal aerosol samplings shown in Figs. 2 and 4 suggest that the “modified” MCE filter culturing method is a viable sampling protocol for fungal aerosols. Use of such method could provide an improved estimate for fungal aerosol exposure, especially for personal type. While for bacterial aerosol sampling, the described method performs reasonably well compared to the BioStage impactor, while having the portability advantage.

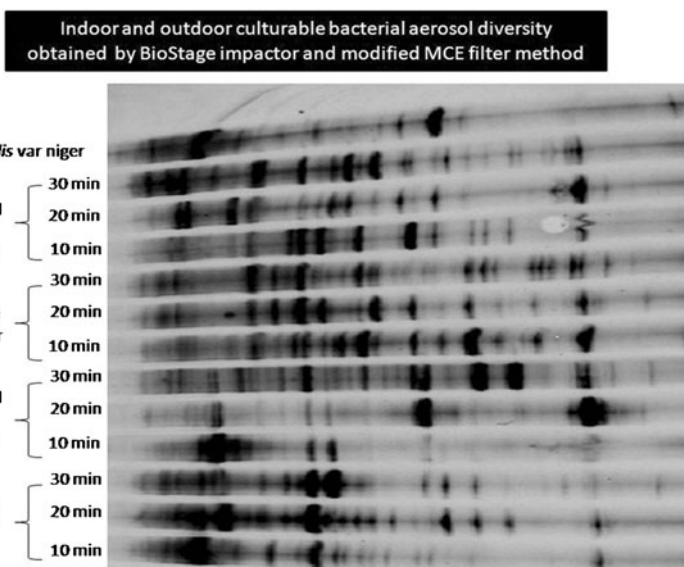
Figure 5 shows the similarity dendrograms of the indoor and outdoor culturable bacterial aerosol diversity obtained by the “modified” MCE filter culturing method and the BioStage impactor. In general, the culturable diversity (gel patterns) was observed to strongly depend on the sampling method, sampling time, and environment. Outdoor



**Fig. 4** Comparison of outdoor culturable fungal aerosol concentrations obtained by the “modified” MCE filter method and the BioStage impactor at different sampling times (10, 20, and 30 min) over three different time periods; the sampling flow rates for the BioStage impactor and the modified MCE filter method were 28.3 and 5 L/min, respectively; the times in the brackets indicated the starting times of sampling for each set; data points represent the averages of three repeats, and error bar stands for the standard deviation; \*\*\*\*statistically significant difference between two methods

culturable bacterial aerosols were observed to have more species richness (number of gel bands) than those indoors regardless of the sampling methods and sampling times tested. This observation is in line with the results shown in Figs. 1 and 3 where outdoor bacteria appeared to be more stress resistant, especially for the desiccation effect. For outdoor sampling, the gel patterns were observed different for different sampling times (10, 20, and 30 min) by the “modified” MCE filter culturing method. For 30-min sampling, the air samples obtained by the “modified” MCE filter method appeared to have more culturable species richness

**Fig. 5** DGGE profiles of culturable bacteria and fungal aerosols collected by the “modified” MCE filter method and the BioStage impactor at different sampling times (10, 20, and 30 min) for both indoor and outdoor environments; the sampling flow rates for the BioStage impactor and the “modified” MCE filter method were 28.3 and 5 L/min, respectively; gel patterns represent those from the CFU mixture of three independent air samples

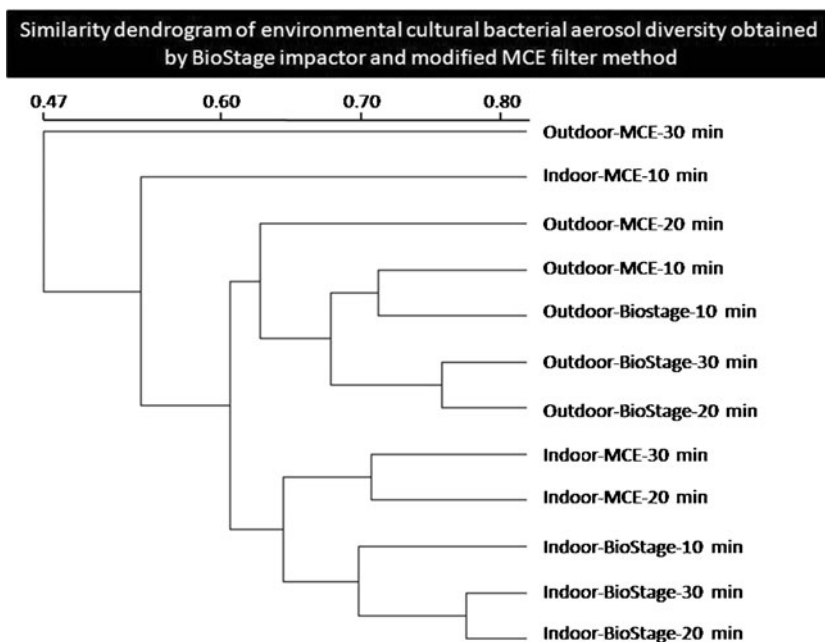


than 10- and 20-min samplings. It was also observed in Fig. 5 that some bands were present in 20- and 30-min air samples, but absent from that of 10-min sampling.

For the BioStage impactor sampling in outdoor environment, specific identified species varied with the sampling time. Cluster analysis shown in Fig. 6 indicated that for 20- and 30-min samplings, the BioStage impactor obtained similar species richness about 75 % for outdoor bacterial aerosol samples. For the “modified” MCE filter culturing method, the species richness similarity was observed lower for outdoor bacterial aerosol samples for these two sampling times. It seems that the MCE filter method for 10-min sampling had higher species similarity with that obtained by the BioStage impactor for 10 min for outdoor bacterial aerosol samples. Increasing collection time would lead to the collection of more microorganisms, which correspondingly increased the probability of the collection of less frequently occurring species or enhanced bacterial aerosol detection. On the other hand, an increase in sampling time would introduce a stronger desiccation effect on the microorganisms already collected. Accordingly, there is an optimal sampling time at which an enough air sample can be obtained while minimizing the desiccation effects.

For indoor sampling, increasing sampling time was observed to lead to more bacterial species richness for both methods as observed in Fig. 5. Cluster analysis shown in Fig. 6 indicated that for 20- and 30-min samplings, the BioStage impactor obtained similar species richness close to 80 % for indoor bacterial aerosol samples. Similar finding was observed for the “modified” MCE filter culturing method for indoor aerosol samples, but with slightly lower similarity (70 %) as shown in Fig. 6. The results obtained here for the culturable bacterial aerosol diversity both in indoor and outdoor environments agreed well with those obtained using similar methods in our previous study in which only 15-min sampling time however was tested (Xu and Yao 2011).

**Fig. 6** Similarity dendrograms for the DGGE profiles shown in Fig. 5 of culturable bacterial aerosol diversity obtained by the “modified” MCE filter method and the BioStage impactor; the *scale bar* represents the similarity of culturable diversity obtained by two sampling methods



In this study, indoor and outdoor dominant culturable fungal aerosols obtained by different methods were also identified using an optical microscopy method. In general, as shown in Table 1, increasing sampling time resulted in more fungal genera identified regardless of sampling methods and environments. However, specific fungal species varied with sampling

methods, sampling time, and sampling environment. This might be due to the composition of fungal species and the variation of their susceptibility to the sampling stress, especially from the impactation. Among the fungal genera identified, *Alternaria* and *Cladosporium* as well as Hyphal fragments were found in most aerosol samples collected.

**Table 1** Dominant culturable fungal aerosols obtained by the modified MCE filter and the BioStage impactor both in indoor and outdoor environments

Bioaerosol sampling and culturing	Indoor dominant culturable fungal aerosols		
	Sampling time		
	10 Min	20 Min	30 Min
Direct MCE filter culturing method	<i>Hyphal fragments</i>	<i>Alternaria</i>	<i>Alternaria</i>
	<i>Saccharomyces</i>	<i>Hyphal fragments</i>	<i>Cladosporium</i>
	<i>Scopulariopsis</i>	<i>Stemphylium</i>	<i>Saccharomyces</i>
	<i>Hyphal fragments</i>		<i>Hyphal fragments</i>
BioStage impactor	<i>Alternaria</i>	<i>Hyphal fragments</i>	<i>Cladosporium</i>
	<i>Cladosporium</i>	<i>Saccharomyces</i>	<i>Hyphal fragments</i>
	<i>Hyphal fragments</i>	<i>Scopulariopsis</i>	<i>Nigrospora</i>
	<i>Saccharomyces</i>		<i>Scopulariopsis</i>
Direct MCE filter culturing method	<i>Alternaria</i>	<i>Alternaria</i>	<i>Alternaria</i>
	<i>Cladosporium</i>	<i>Cladosporium</i>	<i>Cladosporium</i>
	<i>Hyphal fragments</i>	<i>Stemphylium</i>	<i>Epicoccum</i>
	<i>Scopulariopsis</i>	<i>Hyphal fragments</i>	<i>Hyphal fragments</i>
BioStage impactor			<i>Nigrospora</i>
	<i>Aspergillus</i>	<i>Ascospores</i>	<i>Alternaria</i>
	<i>Cladosporium</i>	<i>Aspergillus</i>	<i>Aspergillus</i>
	<i>Hyphal fragments</i>	<i>Hyphal fragments</i>	<i>Chaetomium</i>
			<i>Cladosporium</i>
			<i>Hyphal fragments</i>
		<i>Stemphylium</i>	

In our study, two bioaerosol samplers (Button Inhalable Sampler and BioStage impactor) were operated in parallel. Therefore, if there are any changes in airborne microbial community during the sampling, they would apply to both samplers. In addition, we have tested the samplers in three different time periods of the day, thus the bioaerosol fluctuation was taken into account. Such comparisons under different conditions would minimize the influence of changes in airborne microbial community on the results obtained here.

For the existing sampling techniques, they have optimal sampling flow rates, thus it is difficult to achieve isokinetic sampling without the tradeoff of the sampler's performance. For example, for the Andersen type sampler, the standard sampling flow rate is 28.3 L/min, which corresponds to an inlet flow of ~0.9 m/s (aerosol inlet diameter is about 2.6 cm) and a jet velocity of 24 m/s. These parameters are set for Andersen-type samplers, but the characteristics, e.g., wind speed and atmospheric movement speed in vertical direction, could be higher or lower than 0.9 m/s in different environments. For isokinetic sampling, the flow rate needs to be adjusted; however, this would affect the sampler performance. For Button Inhalable Sampler, it is designed to be less sensitive to the wind direction with a face velocity of ~0.16 m/s. Again, the representativeness of its samples collected depends on the wind velocity. In our study, we wanted to minimize the desiccation effects while collecting more bioaerosol particles so that we increased the sampling flow rate of 5 L/min. Such an increase resulted in about 25 % increase for the face velocity, i.e., from 0.16 m/s (SKC) to 0.2 m/s. For environmental air, it typically has a wind velocity of 100–300 cm/s, therefore increasing the sampling flow rate of the Button Inhalable Sampler to 5 L/min will not significantly affect inhalation sampling convention. However, for viable bioaerosol particles, the representativeness of the samples also depends on the degree of the sampling stress caused by the samplers.

Here, we adopted a culturing method with the MCE filter; accordingly, only culturable microbial concentration and diversity were studied in this study. To study the total airborne microorganisms (both culturable and nonculturable), different collection medium instead of agar, e.g., DI water, should be used. In this study, PCR–DGGE method was applied to describing the airborne culturable bacterial community diversity. For PCR amplification, a variety of influencing factors including sample preparation, DNA extraction, inhibitors, differential amplification, formation of PCR artifacts as well as DNA contamination have been thoroughly discussed somewhere (Wilson 1997; Wintzingerode et al. 1997). Accordingly, the results here might be negatively impacted by the limitations of the methods used. In this study, culture–PCR based method was used, which did not detect those nonculturable species collected by the methods. However, PCR could also amplify

those dead cells if total air sample including culturable and nonculturable cells were used otherwise. However, the concentration of those nonculturable cells if washed off from the agar plates was relatively low compared to those from the colonies. Due to the PCR amplification limitations, those nonculturable cells collected with low concentration were not likely to be amplified. Likewise, use of DGGE method also could result in diversity bias due to the intra-species operon heterogeneities. However, such limitations, if any, should apply to both the “modified” MCE filter culturing method and the BioStage impactor. Accordingly, the microbial diversity comparisons made here are valid.

## Conclusions

In this study, a “modified” MCE filter method (directly placing filter on agar plate for culturing) with the Button Inhalable Sampler was compared with the BioStage impactor in enumerating airborne culturable bacterial and fungal aerosol concentration and diversity under different operating conditions. The results revealed that the “modified” MCE filter culturing method performed better for fungal aerosols and reasonably well for bacterial aerosols when compared to the BioStage impactor. For both the “modified” MCE filter culturing method and the BioStage impactor, the DGGE gel patterns were shown to vary with sampling time and environment despite of their similar number of gel bands. In the future, specific species can be identified by gene sequencing to investigate their individual responses to the sampling stress induced by different sampling methods and tools. This study described a new personal bioaerosol exposure assessment protocol and it was demonstrated applicable in monitoring the personal bioaerosol exposure in replace of an Andersen-type impactor.

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## References

- Abdulmir AS, Yoke TS, Nordin N, Abu Bakar F (2010) Detection and quantification of probiotic bacteria using optimized DNA extraction, traditional and real-time PCR methods in complex microbial communities. *Afr J Biotechnol* 9:1481–1492
- Agranovski IE, Safatov AS, Borodulin AI, Pyankov OV, Petrishchenko VA, Sergeev AN, Agafonov AP, Ignatiev GM, Sergeev AA, Agranovski V (2004) Inactivation of viruses in bubbling processes utilized for personal bioaerosol monitoring. *Appl Environ Microbiol* 70:6963–6967
- Alvarez AJ, Buttner MP, Stetzenbach LD (1995) PCR for bioaerosol monitoring—sensitivity and environmental interference. *Appl Environ Microbiol* 61:3639–3644



- Andersen AA (1958) New sampler for the collection, sizing, and enumeration of viable airborne particles. *J Bacteriol* 76:471–484
- Ayres JG, Forsberg B, Annesi-Maesano I, Dey R, Ebi KL, Helms PJ, Medina-Ramon M, Windt M, Forastiere F (2009) Climate change and respiratory disease: European Respiratory Society position statement. *Eur Respir J* 34:295–302
- Bernardo ZP, Urban JE, Maghirang RG, Jerez SB, Goodband RD (2002) Assessment of bioaerosols in swine barns by filtration and impaction. *Curr Microbiol* 44:136–140
- Brachman PS, Erlich R, Eichenwald HF, Cabelli VJ, Kethley TW, Madein SH, Maltman JR, Middlebrook G, Morton JD, Silver IH, Wolfe EK (1964) Standard sampler for assay of airborne microorganisms. *Phil Trans Biol Sci* 144:1295
- Bundy KW, Gent JF, Beckett W, Bracken MB, Belanger K, Triche E, Leaderer BP (2009) Household airborne *Penicillium* associated with peak expiratory flow variability in asthmatic children. *Ann Allergy Asthma Immunol* 103:26–30
- Burton NC, Adhikari A, Grinshpun SA, Hornung R, Reponen T (2005) The effect of filter material on bioaerosol collection of *Bacillus subtilis* spores used as a *Bacillus anthracis* simulant. *J Environ Monit* 7:475–480
- Carmelley T, Haldane JS, Anderson AM (1887) The carbonic acid, organic matter, and micro-organisms in air, more especially of dwellings and schools. *Phil Trans Biol Sci* 178:61–111
- Cox CS, Wathes CM (1995) Bioaerosols handbook. Lewis, Boca Raton
- Douwes J, Thorne P, Pearce N, Heederik D (2003) Bioaerosol health effects and exposure assessment: progress and prospects. *Ann Occup Hyg* 3:187–200
- Droogenbroeck VC, Risseghem VM, Braeckman L, Vanrompay D (2009) Evaluation of bioaerosol sampling techniques for the detection of *Chlamydomypha psittaci* in contaminated air. *Vet Microbiol* 135:31–37
- Durand KTH, Muilenberg ML, Burge HA, Siexas NS (2002) Effect of sampling time on the culturability of airborne fungi and bacteria sampled by filtration. *Ann Occup Hyg* 46:113–118
- Feller W (1968) An introduction to probability theory and its applications, 3rd edn. Wiley, New York
- Flesch JP, Norris CH, Nugent AE Jr (1967) Calibrating particulate air samplers with monodisperse aerosols: application to the Andersen cascade impactor. *Am Ind Hyg Assoc J* 28:507–516
- Folmsbee M, Strevett K, Stafford K, Evenson C (2000) The effect of sampling time on the total efficiency of the Andersen microbial sampler: a field study. *J Aerosol Sci* 31:263–271
- Guan T, Yao M (2010) Use of carbon-nanotube filter in removing bioaerosols. *Journal of Aerosol Science* 41:611–620
- Guan Y, Zheng BJ, He YQX, Liu L, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JSM, Poon LLM (2003) Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China. *Science* 302:276–278
- Hameed AAA, Khoder MI, Yuosra S, Osman AM, Ghanem S (2009) Diurnal distribution of airborne bacteria and fungi in the atmosphere of Helwan area, Egypt. *Sci Total Environ* 407:6217–6222
- Harold CB, Constantine JA, Theodore D (1980) Morphology of plants and fungi. Harper & Row, New York City
- Hospodsky D, Yamamoto N, Peccia J (2010) Accuracy, precision, and method detection limits of quantitative PCR for airborne bacteria and fungi. *Appl Environ Microbiol* 76(21):7004–7012
- Jones W, Moring K, Morey P, Sorenson W (1985) Evaluation of the Andersen viable impactor for single stage sampling. *Am Ind Hyg Assoc J* 46:294–298
- Li K, Dong S, Wu Y, Yao M (2010) Comparisons of biological contents in the air samples collected from the ground and an altitude of 238 m. *Aerobiologia* 26:233–244
- Lighthart B, Prier K, Loper GM, Bromenshenk J (2000) Bees scavenge airborne bacteria. *Microb Ecol* 39:314–321
- Mainelis G, Tabayoyong M (2010) The effect of sampling time on the overall performance of portable microbial impactors. *Aerosol Sci Tech* 44:75–82
- Mastorides SM, Oehler RL, Greene JN, Sinnott JT, Kranik M, Sandin RL (1999) The detection of airborne *Mycobacterium tuberculosis* using micropore membrane air sampling and polymerase chain reaction. *Chest* 115:19–25
- Mayl KR (1964) Calibration of a modified Andersen bacterial aerosol sampler. *Appl Environ Microbiol* 12:37–43
- Murray CS, Simpson A, Custovic A (2004) Allergens, viruses, and asthma exacerbations. *Proc Am Thorac Soc* 1:99–104
- Muyzer G, Dewaal EC, Uitterlinden AG (1993) Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16s ribosomal-RNA. *Appl Environ Microbiol* 59:695–700
- Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, Hernandez M, Quiñones-Falconi F, Bautista E, Ramirez-Venegas A, Rojas-Serrano J, Ormsby CE, Corrales A, Higuera A, Mondragon E, Cordova-Villalobos JA, INER Working Group on Influenza (2009) Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. *N Engl J Med* 361:680–689
- Solomon WR (1970) A simplified application of the Andersen sampler to the study of airborne fungus particles. *J Allergy Clin Immunol* 45:1–13
- Stark KDC, Nicolet J, Frey J (1998) Detection of *Mycoplasma hypopneumoniae* by air sampling with a nested PCR assay. *Appl Environ Microbiol* 64:543–548
- Stewart SL, Grinshpun SA, Willeke K, Terzieva S, Ulevicijus V, Donnelly J (1995) Effect of impact stress on microbial recovery on an agar surface. *Appl Environ Microbiol* 61:1232–1239
- Trout D, Bernstein J, Martinez K, Biagini R, Wallingford K (2001) Bioaerosol lung damage in a worker with repeated exposure to fungi in a water-damaged building. *Environ Health Perspect* 109:641–644
- Wang Z, Reponen T, Grinshpun SA, Gomy RL, Willeke K (2001) Effect of sampling time and air humidity on the bioefficiency of filter samplers for bioaerosol collection. *Journal of Aerosol Science* 32:661–674
- Weis CP, Intrepido AJ, Miller AK, Cowin PG, Durmo MA, Gebhardt JS, Bull R (2002) Secondary aerosolization of viable *Bacillus anthracis* spores in a contaminated US Senate Office. *JAMA* 288:2853–2858
- Willeke K, Baron PA (1993) Aerosol measurement: principle, techniques and applications. Van Nostrand Reinhold, New York
- Wilson IG (1997) Inhibition and facilitation of nucleic acid amplification. *Appl Environ Microbiol* 63:3741–3751
- Wintzingerode VF, Gobel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* 21:213–229
- Woodward CL, Park SY, Jackson DR, Li XI, Birkhold SG, Pillai SD, Ricke SC (2004) Optimization and comparison of bacterial load and sampling time for bioaerosol detection systems in a poultry layer house. *J Appl Poult Res* 13:433–442
- Wu Y, Yao M (2010) Inactivation of bacteria and fungus aerosols using microwave irradiation. *Journal of Aerosol Science* 41:682–693

- Xu Z, Yao M (2011) Analysis of culturable bacterial aerosol diversity obtained using different sampling and cultivation methods. *Aerosol Sci Tech* 45:1143–1153
- Yao M, Mainelis G (2006) Utilization of natural electrical charges on microorganisms for their collection by electrostatic means. *Journal of Aerosol Science* 37:513–527
- Yao M, Mainelis G (2007) Analysis of portable impactor performance for enumeration of viable bioaerosols. *J Occup Environ Hyg* 4:514–524
- Yao M, Mainelis G, An HR (2005) Inactivation of microorganisms using electrostatic fields. *Environ Sci Technol* 39:3338–3344
- Zhen S, Li K, Yin L, Yao M, Zhang H, Chen L, Zhou M, Chen X (2009) A comparison of the efficiencies of a portable BioStage impactor and a Reuter centrifugal sampler (RCS) high flow for measuring airborne bacteria and fungi concentrations. *Journal of Aerosol Science* 40:503–513