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Impact of sampler selection on the characterization of the indoor microbiome via high-throughput sequencing

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ABSTRACT

Concerns regarding the potential health effects of microorganisms in the indoor environment paired with recent advances in DNA sequencing technologies have led to a rapid expansion in microbial studies of the built environment. The objective of this study is to compare the microbial communities recovered from six different samplers placed in the same building to assess how sample selection can impact the interpretation of the indoor microbiome. To this end, pyrosequencing was used to delineate the fungal and bacterial communities recovered from six samplers placed in an occupied retail building over two consecutive sampling events spaced one week apart. The microbial communities (335K+ sequences) were much more diverse in the settled dust and heating, ventilation, and air-conditioning (HVAC) filter dust samples than the communities recovered from the shorter term, composite samples collected in four different bioaerosol samplers. The bacterial communities recovered from a given sampler were in general more similar to communities from the same samplers than to communities recovered during the same sampling event. Only 14% of the bacterial OTUs and 44% of the fungal OTUs detected were shared in all four bioaerosol samplers, despite the fact that the samplers were collocated and sampled the indoor air simultaneously. These results indicate that sample type should be considered when interpreting results, particularly when comparing results across multiple studies that use different sampling techniques.

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1. Introduction

Rapid advancements in DNA-based molecular analyses coupled with improvements in computational software and data storage have led to a renewed focus on indoor sampling, and with it, the capability for direct cross-comparison studies [1,2]. High-throughput sequencing (e.g. pyrosequencing) efforts have yielded an increasing number of bacterial and fungal datasets for a wide range of indoor built environments including residences, hospitals, classrooms, and offices [3–6]. These sequence sets are readily downloadable from several depositories (e.g. NCBI, QIIME, MG-RAST, EBI) and yield useful insight into the indoor microbiome, particularly if datasets from one environment can be compared to those obtained from another environment [4,7,8]. However, the sampling techniques employed in indoor studies are not standardized and, in some cases the detailed sampling protocols are not readily available. Thus, while techniques such as pyrosequencing may provide considerable insight into the microbial community present, the effect of sampling methodology on the indoor microbiome inferred in a given building is not well understood.

Both short-term bioaerosol and time-integrated dust sampling approaches have been utilized in microbiological studies of the indoor environment [3,5,9]. Bioaerosol samplers actively draw an air sample through a capture device for a selected period of time. Many of these samplers are limited to relatively short sampling periods (e.g., 5–30 min) and generally sample only a small fraction of the total air volume present in an indoor environment. In an outdoor study utilizing one such bioaerosol sampler (BioSampler®), significant temporal differences in the airborne microbial communities detected via Sanger sequencing indicated that repeated

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sampling would be required to more accurately reflect the biological community present [10]. Such temporal variability in bioaerosol composition is also expected in many indoor environments where occupant activity [5] or changes in ventilation can alter the community microorganisms [4]. Some bioaerosol samplers address this concern by altering the design to yield higher sample airflow rates. For example, a wetted-wall cyclone can now be operated at 1250 L/min as compared to many bioaerosol samplers which operate at 4–10 L/min [11]. In contrast to most bioaerosol samplers, collection of microbial-laden dust provides a time-integrated sample of the microbial community collected over experimental periods ranging from days to months or even longer. Settled dust samples are the most common indoor sample collected in many exposure studies [12–14]. More recently, the dust recovered from heating ventilation and air conditioning (HVAC) filters has been utilized as another sampling approach for assessing the airborne microbial community [3,15].

Culture-based analytical methods have been utilized to compare the indoor microbial communities collected in different types of samplers [16–20]. However, culture-based studies potentially skew the analysis since as few as 0.3% of the bacterial species present in the samples may be recovered via culturing [12]. The advent of molecular methods and sequencing techniques such as pyrosequencing allow further investigation into the microbial community structures recovered in different samplers. For instance, Noris et al. (2011) and Hospodsky et al. (2012) [3,5] found that the distribution of phyla recovered from HVAC filter dust differed considerably from that found in settled dust samples collected from residences and a classroom, respectively. The variability may in part be due to differences in aerodynamic diameter which can selectively alter the microorganisms observed in the air versus settled dust [21,22]. Among bioaerosol samplers, collection efficiency is nonstandard and dependent upon particle size [18]. For example, in a qPCR-based study of four bioaerosol samplers deployed in the same indoor environment (i.e. a laboratory), Li (2011) [23] observed that the total bacterial counts varied by a factor of one to five between samplers [23]. Another potential difference between samplers is their effect on effect on viability and DNA integrity [24].

In the present study, the microbial communities in four simultaneously collected, bioaerosol samples and two integrated dust samples were investigated in an indoor environment over two consecutive sampling weeks. The objective of the study was to use pyrosequencing to provide a more thorough comparison of the bacterial and fungal communities recovered from six samplers in an indoor environment to assess how sample selection may impact the interpretation of the microbiome present. This study is one of the first to use a high throughput sequencing technique to compare the bacterial and fungal microbiome recovered from collocated bioaerosol samplers that included: a BioSampler® (BIO) (SKC Inc., Eighty Four, PA), a button sampler (BS) (SKC Inc., Eighty Four, PA), a personal environmental monitor with a nominal cut size of 2.5 μm (PEM) (SKC Inc., Eighty Four, PA), and a wetted-wall cyclone (WWC) (TSI Inc., Shoreview, MN). In addition, settled dust collected from a high surface (SD) and airborne dust recovered from an HVAC filter (HVAC) were collected to compare the communities recovered from these longer-term samples to those recovered from the shorter-term bioaerosol samplers.

2. Material and methods

2.1. Sample collection

Microbial sampling was conducted at an occupied 55,000 m^3 retail store over two consecutive weeks. The space was well mixed and measured air exchange rates were 0.49 hr^{-1} during the Week 1 sampling event and 0.75 hr^{-1} during the second week sampling event. Further details on the building factors are included in the Additional Information and published elsewhere [25].

For the HVAC filter dust sample, a new minimum efficiency reporting value (MERV) 7 HVAC filter (Tri-Dim Filter Corporation, Louisa VA) was placed in an air handling unit (AHU) at the beginning of each the two weeks of testing. Although a higher efficiency filter would have captured more microorganisms [26], the efficiency of the filter used in the testing was similar to that typically found in retail stores. Airflow across the filters was primarily from recirculated indoor air as the outdoor dampers were purposely closed during the two sampling events. During AHU operation, the flow rate through each HVAC filter was approximately 50,000 L/min across the 0.37 m^2 filter surface area. After collecting airborne particulate matter and microorganisms for seven days, the HVAC filter was removed from the AHU and the dust was recovered for DNA extraction and sequencing. For the settled dust sample, high surface dust was collected from the highest shelf on the retail floor (2.5 m off floor), adjacent to the bioaerosol samplers. The dust was vacuumed into a DNA-free 3-piece dust sampling cassette preloaded with a 0.47 μm nominal pore size filter (DustChek, Fenton UK) on the same day the HVAC filter was removed. No attempt was made to clean the shelf before sampling so, as with most settled dust samples collected in indoor studies, the time of dust collection on the shelf was unknown.

With respect to the bioaerosol samplers, the four indoor air samplers were collocated 1 m above the floor and operated for three consecutive 15-min intervals on day seven of the HVAC filter testing each week. The three 15-min samples for each sampler were composited into one sample for DNA analysis and thus represented 45 min sampling time in total. The four bioaerosol samplers included the following: button sampler (4 L/min; SKC Inc., Eighty Four PA), BioSampler® (12.5 L/min; SKC Inc., Eighty Four PA), personal environmental monitor 2.5 μm (10 L/min; SKC Inc., Eighty Four PA), and wetted-wall cyclone (100 L/min; prototype manufactured by TSI Inc. (Shoreview, MN) for Texas A&M). Each sampler was operated at the standard sample flow rate recommended by their respective manufacturers. It is recognized that standardizing the total sampling time to 45 min for each bioaerosol sampler resulted in the samplers collecting different volumes of air. However, it was desirable for all the bioaerosol samplers to collect samples simultaneously due to the temporal variability in airborne microbial communities. Also, sampling times of 15–45 min are fairly typical for bioaerosol samplers used in indoor studies [4,5,20].

The BS and PEM samplers were loaded with a DNA-free gelatin filter with a nominal pore size of 3.0 μm (SKC Inc., Eighty Four PA), and sampled for 15 min. Immediately following each of the three 15-min sampling periods, the filters were placed into 45 mL DNA-free phosphate buffer solution (PBS; 10 g/L NaCl, 0.25 g/L KCl, 1.43 g/L Na_2HPO_4, 0.25 g/L KH_2PO_4) in a DNA-free 50 mL centrifuge tube (Thermo Fisher Scientific Inc., Waltham MA). The BIO sampler was filled with 20 mL PBS during each 15 min sampling period and, after each sampling period, the solution was deposited into DNA-free 50 mL centrifuge tubes. The WWC uses tangential impaction into a DNA-free 0.01% Tween-20 liquid, condensing the sample to a few milliliters per 15 min sampling period. The liquid sample from the WWC for all three 15-min samples was combined into one DNA-free 50 mL centrifuge tube. All the samples were stored at 4 °C for less than three days prior to DNA extraction. Reagents, filters, and equipment were tested for contamination by DNA extraction followed by PCR amplification using universal bacterial primers (8F, 1491R) and fungal primers (ITS1F, ITS4R). No amplification was detected.