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Key determinants of the fungal and bacterial microbiomes in homes

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ABSTRACT

Background: The microbiome of the home is of great interest because of its possible impact on health. Our goal was to identify some of the factors that determine the richness, evenness and diversity of the home's fungal and bacterial microbiomes.

Method: Vacuumed settled dust from homes ($n=35$) in Cincinnati, OH, were analyzed by pyrosequencing to determine the fungal and bacterial relative sequence occurrence. The correlation coefficients between home environmental characteristics, including age of home, Environmental Relative Moldiness Index (ERMI) values, occupant number, relative humidity and temperature, as well as pets (dog and cat) were evaluated for their influence on fungal and bacterial communities. In addition, linear discriminant analysis (LDA) was used for identifying fungal and bacterial genera and species associated with those housing determinants found to be significant.

Results: The fungal richness was found to be positively correlated with age of home ($p=0.002$), ERMI value ($p=0.003$), and relative humidity ($p=0.015$) in the home. However, fungal evenness and diversity were only correlated with the age of home ($p=0.001$). Diversity and evenness (not richness) of the bacterial microbiome in the homes were associated with dog ownership.

Linear discriminant analysis showed total of 39 putative fungal genera/species with significantly higher LDA scores in high ERMI homes and 47 genera/species with significantly higher LDA scores in homes with high relative humidity. When categorized according to the age of the home, a total of 67 fungal genera/species had LDA scores above the significance threshold. Dog ownership appeared to have the most influence on the bacterial microbiome, since a total of 130 bacterial genera/species had significantly higher LDA scores in homes with dogs.

Conclusions: Some key determinants of the fungal and bacterial microbiome appear to be excess moisture, age of the home and dog ownership.

1. Introduction

The factors that determine the make-up of the home's fungal and bacterial microbiomes are of growing interest. However, the home itself has its own microbiome and this microbiome may also be important for adverse human health effects such as immune disorders (Parker et al. 2013) influencing allergy and asthma incidence. Therefore, the factors that determine the make-up of the home's fungal and bacterial microbiomes are of growing interest.

Although there have been many studies that have measured specific fungal species or groups of fungi in homes (e.g., Pitkäranta et al., 2011), the home's fungal microbiome has been considered in only a few studies (Nonnenmann et al., 2012; Adams et al., 2013a,2013b; Adams et al., 2014). In addition, many studies have measured specific bacteria or groups of bacteria (e.g. Gram-positive or negative bacteria) in homes (e.g., Adhikari et al., 2014) but the home's (or other indoor environments') only a few studies have been conducted on bacterial microbiome (Kembel et al., 2012; Qian et al., 2012; Kembel. et al. (2014); Adams et al., 2014).

Recently, Adams et al. (2014) showed that outdoor air had a major input into the richness of the home's fungal microbiome but human inputs dominated the home's bacterial microbiome. This is consistent with a recent study of the bacterial microbiome that demonstrated that the bacterial content of the home was linked to

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the human and animal occupants, and components of the occupants' bacterial microbiome were readily transferred to newly occupied homes (Lax et al., 2014). However, these studies did not consider the microbiome in water-damaged versus non-water-damaged homes as well as other possible environmental determinants of bacteria considered in the present study.

In this study, we examined the bacterial and fungal microbiome of homes associated with increased-risk of asthma, i.e., homes with higher Environmental Relative Moldiness Index (ERMI) values, compared to homes with low ERMI values (Reponen et al., 2011, 2012). Our goal was to determine the factors associated with the home that influence the composition of both the bacterial and fungal microbiome. By examining household factors, such as age of home, ERMI, occupant number, relative humidity, temperature, and the presence of pets (dog or cat), we hope to expand our understanding of factors that create and influence the microbiome of homes, especially homes that are associated with a higher risk of asthma.

2. Methods

2.1. Study Homes

The protocols for collection and analysis of household dust samples previously approved by the Institutional Review Board at the University of Cincinnati were followed during the home sampling. The families recruited for this study ($n=35$) were a subset of the cohort participating in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) undertaken in Cincinnati, Ohio and Northern Kentucky in 2001 (LeMasters et al., 2006). The ERMI values for these homes were determined previously (Adhikari et al., 2014). Based on the ERMI values, homes were selected for inclusion in a low ERMI (<2) group ($n=17$) or a high ERMI (≥ 2) group ($n=18$).

2.2. On-site home Visit and sampling

On-site home visits were performed by two-person teams at child's age eight. Floor-dust samples were obtained for the assessment of bacteria and fungi by vacuuming the floor in the child's primary activity room (Cho et al., 2006). Dust samples for carpeted floor were collected from an area of 2 m^2 at a vacuuming rate of 2 min/m^2. For non-carpeted floor (hard wood, linoleum, tile, or sheet floor), the sample was collected from the entire room at a rate of 1 min/m^2. Large dust particles were removed by sieving (355 μm mesh sieve), and the resulting fine dust was stored at −20 °C before analyses. Temperature (°C) and relative humidity (%) were recorded in each home by a portable thermo-hygrometer (Fisher Scientific, Pittsburgh, PA) and data on the number of occupants, age of the homes, and pet ownership were collected through a questionnaire survey.

2.3. DNA extraction from environmental samples

DNA was extracted from 50.0±0.1 mg of the indoor dust samples into 100 μl solutions, using a protocol developed at the U. S. EPA for quantitative analysis of DNA from dust. However, we did not add the external standard (Haugland et al., 2002) because the addition of a large number of external cells could influence the results. Extracted DNA samples were shipped to Research and Testing Laboratories, LLC (Lubbock, TX) for pyrosequencing.

2.4. Pyrosequencing primers

Extracted DNA samples were amplified for pyrosequencing using forward and reverse fusion primers. For bacteria, the forward primer was constructed with (5′-3′) the Roche A linker (CCATCTCATCCCTGCGTGTCTCCGACTCAG), an 8–10 bp barcode (>2 nucleotide differences between primers), and the 28F primer (5′-GAGTTTGATCNTGGCTCAG-3′). The reverse fusion primer was constructed with (5′–3′) a biotin molecule, the Roche B linker (CCTATCCCCTGTGTGCCTTGGCAG TCTCAG), and the universal eubacterial 519R primer (5′-GTNTTACNGCGGCKGCTG-3′). For fungal DNA, the same approach was used, however, the fungal specific ITS1 primer (CTTGGTCATTTAGAGGAAGTAA) replaced 28F in the forward primer and the fungal specific ITS4 (TCCTCCGCTTATTGATATGC) replaced the 519R in the reverse primer.

2.5. Amplification

Amplifications were performed in 25 μl reactions with Qiagen HotStart Taq Master Mix (Qiagen Inc., Valencia, CA), 1 μl of each 5 μM primer, and 1 μl of template on ABI Veriti thermocyclers (Applied Biosytems, Foster City, CA) under the following thermal profile: 95 °C for 5 min, then 35 cycles of 94 °C for 30 s, 54 °C for 40 s, 72 °C for 1 min, followed by one cycle of 72 °C for 10 min and 4 °C hold. Amplification products were visualized with eGels (Life Technologies, Inc., Grand Island, NY) and pooled equimolar. Each pool was cleaned with Diffinity RapidTip (Diffinity Genomics, West Henrietta, NY), and size selected using Agencourt AMPure XP (BeckmanCoulter, Brea, CA) following Roche 454 protocols (454 Life Sciences). Size selected pools were quantified and 150 ng of DNA hybridized to Dynabeads M-270 (Life Technologies, Inc., Grand Island, NY) to create single stranded DNA following Roche 454 protocols (454 Life Sciences, Branford, CT). Single stranded DNA was diluted and used in emPCR reactions. Sequencing was accomplished following established manufacturer protocols (454 Life Sciences, Branford, CT) on FLX Sequencers using titanium chemistry.

2.6. Putative identifications of bacteria and fungi

Operational taxonomic unit (OTU) clusters for the fungi and bacteria were determined using Kraken as the classifier to assign taxonomy (Wood and Salzberg, 2014). The sequences were sorted using FASTA formatted files and then clustered into OTU clusters with 96.5% identity (3.5% divergence) using USEARCH (Nucleotide, 2011). The file was then queried against a database of high quality sequences derived from NCBI using a distributed.NET algorithm that utilizes BLASTN+ version 0.7.4 (KrakenBLAST; www.krakenblast.com). The BLASTn+ outputs were compiled using a.NET 4.5 standards and C# 5.0 analysis pipeline.

2.7. Data reduction

The data reduction analysis was performed, as previously described (Dowd et al., 2008; Callaway et al., 2009; Callaway et al., 2010). Based upon the above BLASTn+ derived sequence identity (percent of total length query sequence which aligns with a given database sequence) and validation using taxonomic distance methods, the bacteria and fungi were putatively classified at the genus and species taxonomic levels. Sequences representing approximately 82,584 bacterial and 14,438 fungal species were present in the database (Nucleotide, 2011). Sequences with identity scores were compared to known or well-characterized ribosomal DNA sequences. Sequence identities greater than 97% ($<3\%$ divergence) were resolved at the species level and between 95 and 97% at the genus level (Nonnenmann et al., 2012). Any match below this percent identity was discarded. In addition, the High Score Pair was at least 75% of the query sequence or it was discarded, regardless of identity (Browning and Browning, 2013).