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Microbial content of house dust samples determined with qPCR

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ABSTRACT

This study was designed to produce information about microbial concentrations using qPCR and their variation in different seasons and home environments with analyses of two types of house dust samples. Also the correlations between the two types of samples and the reproducibility of the parallel subsamples were studied. Two types of vacuumed house dust samples, rug dust and vacuum cleaner bag dust, were collected in 5 normal urban homes in four different seasons ($N=20+20$). From all dust samples, five parallel subsamples were subjected to qPCR analyses of 17 microbial species or assay groups of microbes. The highest fungal concentrations were found for the *Penicillium*/*Aspergillus*/*Paecilomyces variotii* group, and for the species *Aspergillus penicillioides*, *Aureobasidium pullulans*, *Cladosporium cladosporioides* and *Cladosporium herbarum*. These species/groups were present in almost all samples. The two types of dust samples gave similar results for most microbial species or groups analyzed, but in general, concentrations were slightly higher in rug dust than in dust from vacuum cleaner bag. Microbial concentrations varied significantly between different seasons and hence the similarity of samples within home was mainly low. The concentrations varied significantly also between different home environments. The reproducibility of the parallel subsamples was good or moderate for most of the analyzed species or assay groups. However, further studies are needed to fully understand the factors causing variation in these methods. Nevertheless, in order to show actual differences in fungal concentrations between urban homes with no known microbial sources, all dust samples to be compared should be taken during the same season.

1. Introduction

The interest towards understanding the importance of indoor microbial exposures as either protective agents against allergy (Braun-Fahrländer and Lauener, 2003; Böttcher et al., 2003) or possible causal agents of adverse health effects (Pekkanen et al., 2007) has been increasing. This has emphasized the need to develop methods of microbial exposure assessment, a complex issue in many aspects. For detailed exposure analysis, microbial species and their concentrations can be determined either by culture or by DNA based analyses. Using culture-based methods, only viable microbes can be detected, which represent just a small part of the total microbial concentrations in environmental samples; the estimates vary from <1 to 79% of the total microbial biomass (Niemeier et al., 2006; Toivola et al., 2004). DNA based methods, especially quantitative PCR (qPCR) can be used for the detection of different microbial species or groups including both viable and non-viable microbial material. Several qPCR methods have recently been developed to analyze fungal or bacterial species and groups from house dust, one of the main sample matrix types from indoor environments (Haugland et al., 2004; Rintala and Nevalainen, 2006; Roe et al., 2001).

Dust samples have been widely used in epidemiological studies and in investigations of microbial indoor air problems. Analyses of house dust samples provide an indication of cumulative microbial populations in indoor environments (Chew et al., 2003). Chao et al. (2002) showed gradual increasing of fungal concentrations in floor dust suggesting that house dust serves as a reservoir of microbial contamination. Dust samples can be easily collected in large sample volumes and thus allow a number of different analyses (Chao et al., 2002; Chew et al., 2003; Hyvärinen et al., 2006; Tovey et al., 2003). To determine microbial concentrations and composition of the mycobiota and bacterial flora, house dust samples may be collected by vacuuming from floors, carpets, and rugs (Chao et al., 2002; Chew et al., 2003; Ellringer et al., 2000; Horner et al., 2004).

Seasonal variation in viable fungal types and concentration in indoor environments can be considerable in a climate with distinctly different seasons (Ren et al., 1999) which has been especially observed in subarctic climate (Reponen et al., 1992). However, seasonal variation in microbial content of house dust analyzed with qPCR has not been earlier studied. Verhoeff et al. (1994) observed that viable fungal concentrations were also affected by the type of flooring, the presence of pets, and the length of the time occupants spend daily at

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home. This suggests that each home may have varying sources for environmental microbes. On the other hand, the fungal profile of indoor environments determined with qPCR in a similar climate in USA and Britain was largely similar (Vesper et al., 2005).

The microbial concentrations of similar environments vary between sample types. Fungal concentrations have been higher in dust from textile covered floor than in samples from bare floor as shown both with culture-based analyses (Verhoeff et al., 1994) and by using ECP or β-glucan analyses (Chew et al., 2001). This indicates that the total amount of fungi (including non-viable fungal material) is more dominant in the dust of textile covered floor than in that of bare floor.

The representativeness and reproducibility of samples of microbial analyses from house dust has seldom been explored. Most of these studies have focused on endotoxin, the bioactive component of the cell wall of gram-negative bacteria. Hyvärinen et al. (2006) observed a good correlation of endotoxin between three types of dust samples while the correlation to air samples was poor, as also observed by Park et al. (2000). The reproducibility of bed dust samples for endotoxin has also been good (Hyvärinen et al., 2006; Park et al., 2000), while that of floor dust samples is more contradictory (Heinrich et al., 2003; Hyvärinen et al., 2006; Park et al., 2000). Giovannangelo et al. (2007) presented that the variation of endotoxin, extracellular polysaccharides, and three types of allergens in two parallel samples from mattresses and floors is lower than the between-home variance indicating good reproducibility for parallel samples.

It is expected that there is significant variation between seasons and homes in microbial concentrations in house dust. Assumably this variation can be observed with qPCR method. To test these hypotheses we studied the baseline concentrations and seasonal variation of 16 species or assay groups of fungi and a bacterial genus, *Streptomyces*, from two types of house dust samples using qPCR method. By collecting samples from five normal urban homes we also observed the variation in microbial concentrations between different home environments. We also tested the mutual correlation of the two sample types. While PCR analyses target the microbial DNA instead of viable cells (culture methods) or bioactive components (endotoxin or β-glucan), the previous data on reproducibility cannot be directly applied to this methodology. In order to provide the necessary background information for use of microbial qPCR for exposure assessment, we tested the reproducibility of the method isolating DNA in five parallel subsamples.

2. Materials and methods

2.1. Study sites and sampling

Dust samples were collected in the city of Kuopio, Finland, from five urban homes that had no dampness or microbial indoor air problems, thus, no unusual sources of microbes. The homes in the study were selected to represent different types of housing and family characteristics. The home characteristics are shown in Table 1. In each home, two different types of dust samples were collected in four different seasons. In Finland, wall-to-wall carpets are rare in private homes, and therefore the samples were taken from rugs. Rug dust samples were collected into a nylon sock with 25 μm pore size (Allied Filter Fabrics, Hornsby, Australia), commonly used in epidemiological studies (Schram-Bijkerk et al., 2006), by vacuuming the living room rug from a total area of 5 m^2 for 2 min each, thus the total sampling time was 10 min. If the area of living room rug was less than 5 m^2, the rest of the sample was vacuumed from the bedroom rug. Occupants were asked not to vacuum their rugs 24 h prior to the sampling. In addition, dust bag samples were taken from the vacuum cleaners which had been in normal household use of the occupants. Dust had been collected for 1–3 months, each period corresponding to one season (autumn, winter, spring, summer). The occupants were advised not to vacuum a car, a garage, or a balcony into the same vacuum cleaner bag during the sampling period. After the sampling period the whole dust bag was taken to further analyses. Rough and fibrous material was separated by sieving all the dust through a sterilized mesh (pore size about 1×1 [rug dust] and 1×2 mm [dust bag]). The samples were stored at −20 °C before analyses.

Table 1
Characteristics for the homes where the dust samples were collected.

	Housing type	Persons	Pets	Floor area/m²	Ventilation	Constr. year	Type of constr.
Home1	House	2	Yes	150	Mech[a]	1981	Wood
Home2	Apartment	2	No	57.5	Mech[a]	1965	Concrete
Home3	House	5	No	135	Mech[a]	1983	Wood
Home4	Apartment	2	No	84.5	Mech[a]	1994	Concrete
Home5	Apartment	3	Yes	74	Mech[a]	1987	Concrete

[a] Mechanical exhaust ventilation.

2.2. DNA extraction and PCR analyses

Total DNA from dust samples was extracted using the glass bead milling and glass milk adsorption method reported previously (Haugland et al., 2002). From all homogenized dust samples five parallel subsamples of 5 mg were analyzed. Dust samples were weighed in a sterile 2 ml extraction tube (Simport Plactics, Canada) containing 0.3 g glass beads (G-1277, Sigma-Aldrich, Germany). Ten microliter aliquots of 2×10^8 conidia/ml reference suspension of *Geotrichum candidum* in 0.5% Tween 80 and 100 and 300 μl of lysis and binding buffer, respectively from an Elu-Quick DNA Purification Kit (Schleicher and Schuell, Keene, NH) were added to the tubes. The tubes were shaken 1 min in Mini Bead-Beater (Biospec Products, USA) at a maximum speed. After that the tubes were held at 70 °C heat block (Thermomixer comfort, Eppendorf, Germany) for 10 min. Extraction tubes were centrifuged (Centrifuge 5415R, Eppendorf, Germany) for 1 min at 12000 rpm. Liquid above the beads was removed and placed in tubes containing 175 μl 99% ethanol. The tubes were transferred to a DNeasy™ mini spin column (Qiagen, Germany) on a 24-manifold system (Qiagen). The column was washed twice with 0.5 ml Qiagen AW1 buffer and once with 0.5 ml Qiagen AW2 buffer, then centrifuged for 3 min at 13000 rpm. The columns were transferred to a clean catch tube and the DNA was recovered by adding 100 μl Qiagen AE extraction buffer, centrifuging the column for 2 min at 8000 rpm and then repeating this step with another 100 μl of AE buffer. The DNA samples were stored at −80 °C.

PCR reactions were done using minor modifications of a previously reported method (Haugland et al., 2004). Reactions were prepared in a 96 well optical reaction plate (Applied Biosystems, USA). PCR reactions contained 6.25 μl 2×concentrated Universal Master Mix (Applied Biosystems), a mixture containing reverse and forward primers (final concentration 1 μM each, with an exception for *Aspergillus versicolor* forward primer 5 μM) and TaqMan probe (final concentration 80 nM) (Oligomer, Finland and Applied Biosystems), bovine serum albumin, BSA (final concentration 0.2 mg/ml) (New England BioLabs, USA) and nuclease free water. Sample DNA was added (2.5 μl) to complete the 12 μl total volume of reaction mixture.

Reactions were completed and monitored using Applied Biosystems ABIPrism model 7000 sequence detection system. Thermal cycles consisted of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C for template denaturation and 1 min at 60 °C for probe and primers annealing and template extension.

All dust samples were analyzed using the primers and probes, listed on the website www.epa.gov/nerlcwww/moldtech.htm, for 16 different species, genera or groups of fungi that were assessed to be relevant for indoor environments (U.S. Environmental Protection Agency, 2009). Analyzed species, genera or groups of fungi were *Aspergillus fumigatus*/*Neosartorya fischeri* (Afumi), *Aspergillus niger*/