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Inflammatory potential of low doses of airborne fungi from fungal infested damp and dry gypsum boards

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ABSTRACT

This study has investigated the total inflammatory potential (TIP) of low concentration fungal samples from moisture-damaged and fungal infested gypsum boards. The fungal aerosols were generated from damp and dried surfaces, and sampled using filter sampling and liquid impingement. The TIP of the samples was analysed using a granulocyte assay based on differentiated HL60 cells. The study found a tendency to a J-shaped dose-response curve for fungal samples. Low concentrations of fungi were aerosolised from the gypsum boards, and the aerosols were dominated by *Aspergillus versicolor* and *Penicillium chrysogenum*. *Bacillus infantis* and *Paenibacillus* sp. were found on the gypsum boards, but not recovered in the aerosols. A significant correlation was found between the TIP of diluted and undiluted samples of fungal aerosols. However, diluted samples had a higher TIP than undiluted samples, and no significant association was found between concentration of fungi and the TIP of the samples. This is likely due to the J-shaped dose response curve. The aerosol samples from the dried gypsum boards had a significantly higher TIP compared to aerosols from the damp surfaces. However, the J-shaped dose-response curve weakens the conclusion on the influence of surfaces dampness, sampling time, fungal species or sampling methods. It could, however, be concluded that samples from both damp and dry surfaces induce inflammation in the HL60 cells, despite the low concentration of fungi. Thus, a dried fungal infestation in a building seem still to present a concern for the occupant.

1. Introduction

Inflammation is an underlying cause of some health symptoms associated with exposure to fungi in damp buildings. Thus, *in vivo* as well as *in vitro* studies have found indications that exposure to fungi causes respiratory inflammation [1–5]. Review studies conclude that there is an association between dampness or fungal growth in buildings and symptoms amongst occupants such as, colds, fatigue, skin irritation, concentration difficulties, and even asthma and allergies [6–8]. Thus, studies have investigated factors that might influence the concentration of airborne fungi in indoor air [9–13], as well as the potential health effect of airborne fungi in indoor air, by investigating the inflammatory potential of home dust samples or aerosol samples with fungi in *in vitro* assays [10,14,15].

Environmental samples for investigation of the inflammatory potential of microbes in the indoor air seem to contain lower fungal concentrations (fungal spores or Colony Forming Units (CFU)/ml) than aerosol samples produced in the laboratory for investigation of the inflammatory potential. Thus, *in vitro* studies of fungal samples generated in laboratory settings have exposed cells to 10^5–10^7 CFU fungi/ml [10,14,15], while samples from the indoor air often contain $<10^2$ CFU (- or spores)/ml [11,16]. It is, however, difficult to relate a concentration in CFU/ml to a human indoor exposure. Typical concentrations found in buildings with visual fungal growth or moisture damage seem only rarely to exceed 10^4 CFU/m^3 [17]. Low fungal concentrations of the indoor air ($<10^3$ CFU/m^3 [17]) may occur if fungi are growing on hidden surfaces within the building structure, e.g. in between multilayer wall constructions or underneath a parquet floor. In these cases, the structure behind which the fungi are growing works as a barrier for the fungal particles [18,19]. In spite of low fungal concentrations in the indoor air, caused by a fungal infestation, occupants still seem to experience symptoms associated with fungal exposure [20]. However, knowledge on the inflammatory potential of low doses of fungi from infested building materials is limited.

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A way to assess an impact of fungal components on the human immune system is through sensitive *in vitro* studies using human cell lines like e.g. the HL 60 cell line [21]. The HL 60 cells are sensitive to microorganisms [14,22]. Thus, a study of aerosol samples from biofuel plants shows that four microbial factors (endotoxin, fungal spores, β-D-glucan, and actinomycetes) contributed to the total inflammatory potential (TIP) of the samples [14]. Likewise, a study of mixed fungal species aerosolised from infested gypsum boards used HL 60 cells to investigate the TIP of the fungal samples and found that the relative humidity (RH) during the incubation process influenced the TIP of the samples [10]. Studies on the effects of aerosols of mixed fungal species on the TIP have recently been suggested [13], partly based on the facts that several fungal genera are present on indoor surfaces [23] as well as in indoor air [13,24], and partly based on the lack of knowledge on the influence of different species and species combinations on the TIP. With the development of methods which quickly can identify fungal species correctly [25], it seems now to be possible to gain more knowledge on health effects of fungi on species level.

Different methods can be used to sample airborne microorganisms. One method, liquid impingement, has been suggested to capture mycotoxins [26] in addition to fungal particle, which likely could be of interest in regards to the TIP and toxicity of an aerosol sample. E.g. a study comparing filter sampling and liquid sampling found a higher TIP of the liquid samples, despite a lower concentration [27]. Sampling time may affect the species richness of a fungal aerosol sample [24], which again may affect the TIP of an aerosol sample. Likewise, studies on fungal spore release from gypsum boards have shown a difference in the number of fungal spores released from damp and dry surfaces, as well as in the TIP of the aerosol samples [10].

The aim of this study was to get knowledge on the TIP of low concentrations of fungi released from water-damaged fungal infested gypsum boards. The study is designed to get knowledge on how the TIP of a fungal sample is affected by the sampling time, sampling method, species composition, and by drying out the infested gypsum boards.

2. Method

2.1. Design and generation of fungal samples for the TIP investigation

The fungal aerosol samples were generated from water-damaged gypsum boards, inoculated with fungi sampled in a moisture-damaged house. Gypsum boards are sensitive to damp conditions and a commonly used material in buildings. Further, they contain cellulose which supports fungal growth [28] and have previously been used in investigations of fungal growth on building materials and spore release [10,12,29–31]. The gypsum boards were incubated at approximately 95% RH during the growth process. After 13 weeks, one half of the boards were dried and further incubated at 35–40% RH. A detailed description of the incubation and growth process and an illustration of the setup can be found in Ref. [24]. The P-FLEC (Particle-Field and Laboratory Emission Cell, Chematec, Denmark) was used for generation of the fungal aerosols. A bar with ten 0.8 mm nuzzles rotated 1 cm over the surfaces of the infested gypsum boards and scanned an area of 130 cm^2 during one rotation of 60 s. The air jets from the nozzle bar are directed towards the surface at an angle of 45°. In the chamber of the P-FLEC an airflow was created, and the released aerosols were thereby transported to the outlet at the top of the chamber. Here either a GSP (GesamtStaubProbenahme) (CIS by BGI, INC, USA) sampler or an impinger (BioSampler, SKC, UK) was connected to sample the aerosols (Fig. 1). Further, data on the particle size distribution measured by an aerodynamic particle sizer (APS) can be found in a previous study [24].

2.2. Sampling

Four aerosol sampling scenarios using both impinger and GSP sampling were performed with three repetitions of each of the aerosol sampling scenarios (Table 1, scenario 1–4). In addition, controls with gypsum boards without fungal growth were conducted for each aerosol sampling scenario. The gypsum boards for the control samplings were the same as the ones inoculated with fungi, but without moisture and fungal growth. During sampling with both impinger and GSP, the P-FLEC was moved slightly over the surfaces in regular intervals, every 2 min for the 15 min sampling (in the following referred to as short sampling time) and every 30 min for the 8 h sampling (in the following referred to as long sampling time). This was to ensure sampling over the entire surfaces of the gypsum boards (196 cm^2). The GSP and impinger samplings were conducted at individual gypsum boards. Both sampling time and air velocity near the surface were adjusted simultaneously to ensure that the boards were exposed to the same air volume across all four aerosol sampling scenarios. In addition to the aerosol samples, surface scrapings were conducted on a total of six damp and six dry surfaces. The surface scrapings were each conducted on individual boards after the aerosol samplings, but on the same boards as the aerosol samplings.

The impinger samples aerosols at a flow of 12.5 l/min into 20 ml impinger liquid (0.0005% Tween20 solution). The total sampling air volume was thus 187.5 l and 6000 l for the short- and long term sampling, respectively. The GSP samples at a flow of 3.5 l/min, and sampled the aerosols onto polycarbonate filters with pore size 1 μm (Frisenette, USA). The total sampling air volume was thus 52.5 l and 1680 l for the short- and long term sampling respectively. Surface samplings were done by gently scraping a sterilized scalpel over the entire surfaces of the gypsum boards (196 cm^2), making sure not to scrape the same area more than once.

2.3. Extraction and dilution of samples

The polycarbonate filters were extracted in 5.6 ml 0.0005% Tween20 solution to account for the differences in sampling volume between the GSP and impinger. The filter was set to shake at 300 rpm for 1 h. The scrapings were likewise suspended in 5.6 ml 0.0005% Tween20 solution.

A total of 24 aerosol samples (undiluted and 10× diluted) and 12 surface scrapings (undiluted and 10× diluted) were produced for investigation of the TIP (Table 1). The samples were diluted using HBSS (Hanks Balanced Salt Solution, Biological Industries, USA).

2.4. Plate dilution

The suspensions from the surface scrapings, the filter extractions, and the impinger sampling were plate diluted on DG18 ager plates (MERCK, Germany) for quantification and identification. The DG18 agar was chosen since it has been proven to be more effective than other agar media both in regards to quantity and species richness [32]. One sample from each of the four aerosol sampling scenarios (Table 1, scenario 1–4) and one sample from both scraping scenarios (Table 1, scenario 5–6) were in addition plate diluted onto nutrient agar (OXOID, UK) for quantification and identification of bacteria in the samples.

2.5. TIP investigation

The TIP of the fungal samples was measured using the