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Size-fractionated (1→3)-β-D-glucan concentrations aerosolized from different moldy building materials

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ABSTRACT

Release of submicrometer-sized fungal fragments (<1.0 μm) was discovered in earlier studies, which investigated the aerosolization of spores from moldy surfaces. However, the contribution of fungal fragments to total mold exposure is poorly characterized. The purpose of this study was to investigate the size-fractionated concentrations of particulate (1→3)-β-D-glucan and numbers of particles aerosolized from the surface of artificially mold-contaminated materials using a novel sampling methodology. *Aspergillus versicolor* and *Stachybotrys chartarum* were grown on malt extract agar and building materials (ceiling tiles and gypsum board) for one to six months. Fungal particles released from these materials were collected size-selectively by a newly developed Fragment Sampling System, and (1→3)-β-D-glucan in air samples was analyzed by *Limulus* Amebocyte lysate (LAL) assay. The concentrations of (1→3)-β-D-glucan varied from 0.4×10^0 to 9.8×10^2 ng m^{-3} in the fragment size and from 1.0×10^1 to 4.7×10^4 ng m^{-3} in the spore size range. Numbers of submicrometer-sized particles aerosolized from 6-month old cultures were always significantly higher that those from 1-month old ($P<0.001$). This can be attributed to increased dryness on the surface of material samples and an increase in fungal biomass over time. The average fragment to spore ratios both in particle numbers and (1→3)-β-D-glucan mass were higher for *S. chartarum* than for *A. versicolor*. The results indicate that long-term mold damage in buildings may lead to increased contribution of fragments to the total mold exposure. Therefore, the health impact of these particles may be even greater than that of spores, considering the strong association between numbers of fine particles and adverse health effects reported in other studies. Furthermore, the contribution of fragments may vary between species and appears to be higher for *S. chartarum* than for *A. versicolor*.

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1. Introduction

Indoor exposures to molds, especially in water-damaged buildings, contribute to occupant respiratory disease and symptoms such as allergic rhinitis, asthma, and hypersensitivity pneumonitis (Institute of Medicine of the National Academies, 2004). However, spore concentrations in buildings with mold problems have not shown strong associations with health outcomes (Rao et al., 1996; Cooley et al., 1998; Meklin et al., 2002). Recently, smaller-sized fungal fragments (<1.0 μm) have been suggested as potential contributors to adverse health effects since they contain biologically active agents such as fungal antigens, mycotoxins, and (1→3)-β-D-glucan (Górny et al., 2002; Brasel et al., 2005; Seo et al., 2007). Nonetheless, the contribution

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of fungal fragments to exposure and adverse health outcomes associated with these particles are poorly characterized.

Due to their small size of fungal fragments, they can stay in the air longer compared to intact spores or their aggregates, and can also penetrate and be deposited deeply into the alveolar region when inhaled. Cho et al. (2005) reported that the respiratory deposition of *Stachybotrys chartarum* fragments for adults was 230 times higher than that of spores, and the deposition ratios (numbers of deposited fragments divided by those of deposited spores) for infants were 4–5 fold higher than those for adults.

Simultaneous release of submicrometer-sized fungal fragments in large quantities together with intact spores from mold-contaminated surfaces has been demonstrated in earlier studies (Górny et al., 2002, 2003; Cho et al., 2005). Several investigations have reported that adverse health outcomes (e.g., respiratory and cardiac responses) are strongly associated with number concentrations of ultrafine particles (<0.1 μm), rather than with mass or number concentrations of larger particles (Peters et al., 1997; Penttinen et al., 2001; von Klot et al., 2002). Thus, measurement of submicrometer-sized fungal particles should be included when assessing mold exposure. Recently developed methodology for separating and analyzing submicrometer-sized fungal fragments (Seo et al., 2007) has a potential to characterize submicrometer-sized particles. Among the methods that could be used for analyzing fungal fragments, quantification of (1→3)-β-*D*-glucan by *Limulus* Amebocyte lysate assay seems to be suitable as it is characterized by a low limit of detection (2.54 pg ml^{-1}) and sufficient sensitivity for the analysis of airborne fungal fragments (Seo et al., 2007). Furthermore, (1→3)-β-*D*-glucan has been used as an indicator of total mold exposure (Chew, 2001; Rao et al., 2004), and associated with immuno-modulating health effects (Iossifova et al., 2007).

In an attempt to better understand the release of (1→3)-β-*D*-glucan, we have recently assessed its total mass concentrations on the surfaces of contaminated materials as well as in particles aerosolized from moldy building materials (Seo et al., 2008). It was found that the total mass of aerosolized (1→3)-β-*D*-glucan did not consistently follow the same trends as the overall numbers of aerosolized particles, measured with an optical particle counter in the size range of 0.3–20 μm. It was speculated that this was caused by the contribution of (1→3)-β-*D*-glucan mass in fragment-sized particles. The present study has generated size-selective data on the release of aerosol particles and particulate (1→3)-β-*D*-glucan in the submicrometer size range from moldy building materials.

2. Materials and Methods

2.1. Preparation of material samples

Test materials for experiments were prepared as described by Seo et al. (2008). Briefly, an isolate of *Aspergillus versicolor* (RTI 367; Research Triangle Institute International, Research Triangle Park, NC) and an isolate of *Stachybotrys chartarum* (JS51-05, National Institute for Occupational Safety and Health, Morgantown, WV) that was characterized as non-toxic by Jarvis et al. (1998) were grown on 2% malt extract agar for one week for preparation of fungal suspensions. Autoclaved glass microbeads (0.4–0.6 mm in diameter) were utilized to harvest spores from cultures, and fungal spores were extracted from microbeads after transferring into a sterile tube containing sterile deionized water with 0.05% Tween 80. Spore concentrations were adjusted to 10^6 spores ml^{-1} using a bright-line hemacytometer with less than 20% of the coefficient of variation (C.V.), and 0.1 ml of aliquots from fungal suspension were used to inoculate culture plates and building materials for aerosolization experiments.

Three material types were prepared to serve as fungal growth media: 2% malt extract agar (MEA; 5 ml/Petri plate), white ceiling tiles (Armstrong World Industries, Lancaster, PA), and wall-papered gypsum board (National Gypsum Company, Buffalo, NY) (Seo et al., 2008). The ceiling tile and gypsum board pieces were first cut to the same round shape and dimensions as a Petri plate (diameter—8.7 cm; height—0.7 cm). Pre-cut pieces of building materials were autoclaved and placed into sterile Petri plates for inoculation and fungal cultivation.

2.2. Fungal inoculation and incubation

Fungal cultivation was conducted as described by Seo et al. (2008). Briefly, an aliquot (0.1 ml) of each fungal suspension was inoculated onto 2% MEA, ceiling tiles and gypsum boards. Autoclaved pieces of each building material were first allowed to absorb 10 ml of sterile deionized water to establish high water activity (a_w), and then received 1 ml of malt extract broth (20 g l^{-1}) for simulating external nutrient source in settled dust on the real material surfaces. After inoculation, the Petri plates containing the material samples were placed in six different 5.3-liter chambers. The incubation chambers were kept at room temperature (21–24 °C) and aerated with filter-sterilized air (0.2 μm of pore size; GE Osmonics Inc., MN) once a day for 10 min at a flow rate of 0.53 l min^{-1} (Murtoniemi et al., 2003). Relative humidity of 97–99% inside the chambers was achieved by a K_2SO_4 solution. In addition, temperature and humidity in each chamber were monitored once a day by a traceable humidity-temperature pen (Fisher Scientific Company, Pittsburgh, PA), and moisture content on the surface of materials samples was measured by a moisture meter (Protimeter®: Model BLD5800; General Electric, MA) immediately before using the materials for the experiment. The material samples were incubated for 1, 2, 3, 4, 5, or 6 months. However, numbers of aerosolized particles after 2–5 months of incubation did not reveal any deviation from the trends observed for particle numbers between materials incubated for one and six months (Seo et al., 2008). Therefore, the size-selective (1→3)-β-*D*-glucan samples were analyzed only for samples with the shortest (one month) and the longest (six months) incubation period.

2.3. Collection of size-selective fungal particles aerosolized from material samples

A Fungal Spore Source Strength Tester (FSSST) with a flow-rate of 20.5 l min^{-1} was utilized to aerosolize fungal particles from material samples (Sivasubramani et al., 2004; Seo et al., 2007). Aerosolized particles were collected using a newly developed

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