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

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ARTICLE DATA

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

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 $(1\rightarrow3)$-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, $(1\rightarrow3)$-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 $(1\rightarrow3)$-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 $(1\rightarrow3)$-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 $(1\rightarrow3)$-β-D-glucan mass in fragment-sized particles. The present study has generated size-selective data on the release of aerosol particles and particulate $(1\rightarrow3)$-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$_2$SO$_4$ 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 $(1\rightarrow3)$-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

Fragment Sampling System as described in detail by Seo et al. (2007). Briefly, the Fragment Sampling System consists of two Sharp-Cut cyclones (PM$_{2.5}$ and PM$_{1.0}$ with modified cut-off sizes; BGI, Inc., Waltham, MA) and a 25-mm after-filter (preloaded endotoxin-free polycarbonate filter with pore size of 0.4 μm; Fisher Scientific Company, Pittsburgh, PA). Aero-solized fungal particles were separated into three distinct size fractions: (i) >2.25 μm (spores); (ii) 1.05–2.25 μm (mixture of spores and fragments); and (iii) <1.05 μm (submicrometer-sized fragments).
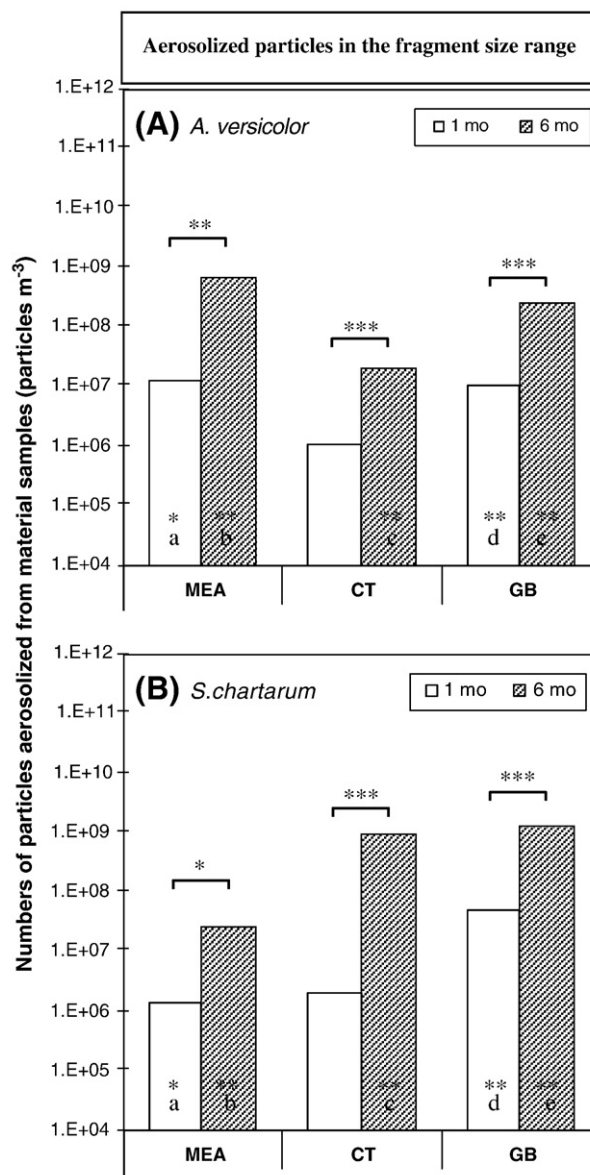
Fungal particles from each material sample were aero-solized for three minutes, and 3–4 material samples were used for the collection of one air sample. During the aerosolization experiments, numbers of aerosolized particles were measured in real time by two optical particle counters (OPC: Model 1.108; Grimm Technologies, Inc., Douglasville, GA) located up- and downstream of the PM$_{2.5}$ cyclone and with a condensation nucleus counter (CNC: P-Trak®, Model 8525; TSI inc., Shore-view, MN) located downstream of the PM$_{1.0}$ cyclone. Numbers of aerosolized particles in the spore size range were calculated as the difference between the particle numbers obtained with the two OPCs and corresponding values in the submicrometer size range were directly measured by the CNC. In addition, numbers of particles measured by the OPC located upstream of the PM$_{2.5}$ cyclone were used to determine case by case the maximum number of material samples, which can be used for the experiment without overloading the collection system (Seo et al., 2007). The aerosolization experiment was repeated three times for each combination of fungal species and material type (2 species×6 incubation times×3 replicate experiments×3–4 material samples/aerosolization experiment). The aerosolization and sampling, as well as fungal inoculation and sample preparation were performed inside a Class II Biosafety cabinet (Sterilchem Gard; Baker Company, Inc., Sanford, ME).

2.4.   (1→3)-β-D-glucan assay

The size-fractionated aerosol samples were analyzed for (1→3)-β-D-glucan using the *Limulus* Amebocyte lysate (LAL) assay as described by Seo et al. (2007). Collected particles were extracted from the two cyclones and from the after-filter into 0.05% Tween 80 (in pyrogen-free water) by 2-min vortexing and 10-min ultrasonic agitation. An aliquot of 0.5 ml of each sample extract was further extracted with 0.5 ml of 0.6 M NaOH by mechanical shaking (Wrist-action® Shaker: Model 75; Burrell Scientific, Pittsburgh, PA) for 1 h. Finally, 25-μl aliquots were transmitted to microwell plates and 50 μl of assay reagent (specific (1→3)-β-D-glucan lysate) were added. The microwell plate was incubated for 150 min in an absorbance Microplate Reader (ELx808TM; Bio-Tek Instruments, Inc., Winooski, VT) and the kinetics of the ensuring purple color reaction (optical density) was read at 405 nm every 30 s.

2.5.   Statistical analysis

The concentrations of particulate (1→3)-β-D-glucan as well as numbers of airborne particles were shown to have log-normal distributions as determined by the Shapiro–Wilk and Kolmo-

Fig. 1 – Numbers of particles (particles m^{-3}) in the fragment size range aerosolized from each material sample (MEA = malt extract agar; CT = ceiling tile; GB = gypsum board): (A) *A. versicolor*; (B) *S. chartarum*. Histograms present geometric means, and error bars present geometric standard deviations of three repeats. Significant difference between the two species is indicated by a letter symbol (the same letters present a significant difference). Solid lines indicate significantly different geometric means between the two incubation periods. Asterisks present the significance level of statistical difference (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$). Note: The following significant differences were found between material types; (A) For *A. versicolor*, ceiling tile incubated for one and six months was lower than MEA and gypsum board; (B) for *S. chartarum*, gypsum board incubated for one month was higher than MEA and ceiling tile and MEA for six month was lower than ceiling tile and gypsum board.

gorov–Smirnov tests. Therefore, geometric means (GM) and geometric standard deviations (GSD) were calculated to describe the center and spread of the data. Different species, incubation times, and material samples were examined in order to investigate their effects on log-transformed concentrations of size-fractionated $(1{\rightarrow}3)$-β-D-glucan and airborne particles using a general linear model (GLM). The variability of three experimental repeats was estimated by modeling replicate measurements as a random effect. A Pearson correlation coefficient was obtained to estimate the correlation between concentrations of particulate $(1{\rightarrow}3)$-β-D-glucan and numbers of airborne particles.

The analysis of variance (ANOVA) tested the differences of airborne particle numbers (or the concentrations of particulate $(1{\rightarrow}3)$-β-D-glucan) released from three material types. These tests were followed by investigation of differences between species and incubation times for each material type. Tukey's adjustment for multiple comparisons was applied in order to maintain overall 5% significance levels for hypothesis testing. Statistical Analysis System (SAS) software (SAS for Window version 9.1; SAS Institute Inc., Cary, NC) was used and a significance level (α) of 0.05 was applied unless indicated otherwise.

3. Results

3.1. Particle numbers aerosolized from material samples

Fig. 1 shows numbers of particles in the fragment size range aerosolized from the three material types incubated for one and six months. The single measurement values ranged from 1.0×10^6 to 1.6×10^9 particles m^{-3}. The highest and lowest values were measured for *S. chartarum* grown on gypsum boards for six months and on MEA for one month, respectively. The generalized liner models (GLM) demonstrated that numbers of airborne fragment-sized particles were statistically significantly influenced by incubation period, species, and material type ($P<0.001$).

Geometric mean numbers of aerosolized particles in the fragment size range from all 6-month old cultures were significantly higher than the respective values from 1-month old cultures. This difference was more pronounced for fungi grown on building materials (ceiling tiles and gypsum board) than on MEA. Differences between the two species were observed for all data sets, except ceiling tile incubated for one month. For MEA, numbers of aerosolized particles from *A. versicolor* grown for one and six months were significantly higher than those from *S. chartarum*. In contrast, corresponding values aerosolized from building materials were significantly higher for *S. chartarum* than for *A. versicolor*.

Comparisons among three material types (ANOVA) showed that there were differences between the material types for all data sets. For *A. versicolor*, numbers of particles released from MEA and gypsum boards incubated for one and six months were significantly higher than those released from ceiling tile ($P<0.01$). For *S. chartarum*, corresponding values for gypsum board incubated for one month were higher than those for 1-month old MEA and ceiling tile, but respective data for fungi grown on MEA for six months were lower than those of ceiling tile and gypsum board with the same incubation period ($P<0.01$).
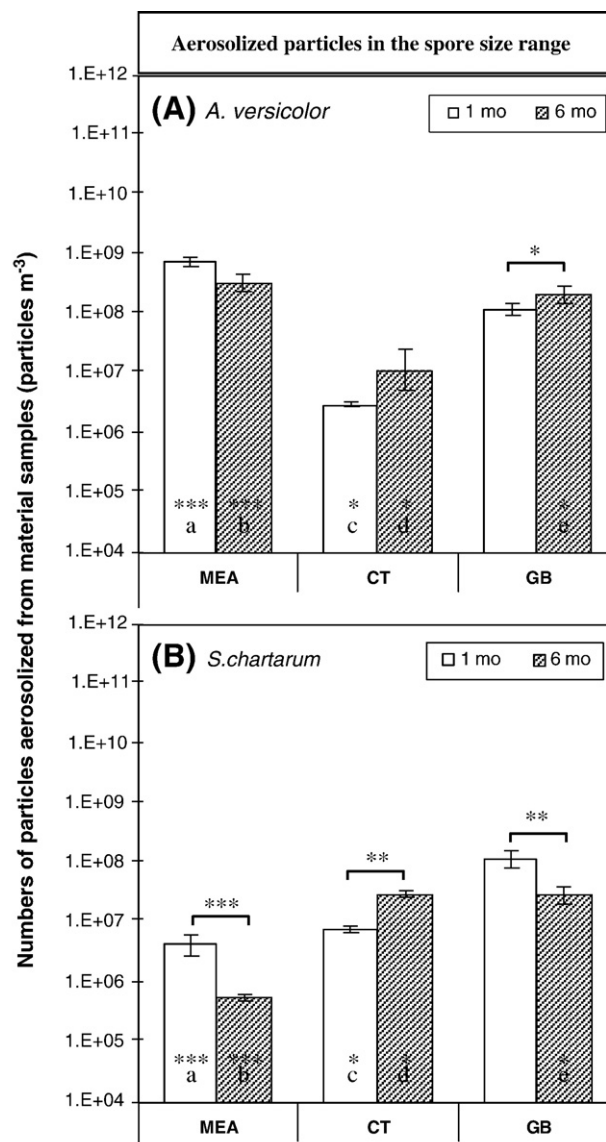

Fig. 2 – Numbers of particles (particles m^{-3}) in the spore size range aerosolized from each material sample (MEA = malt extract agar; CT = ceiling tile; GB = gypsum board): (A) *A. versicolor*; (B) *S. chartarum*. Histograms present geometric means, and error bars present geometric standard deviations of three repeats. Significant difference between the two species is indicated by a letter symbol (the same letters present a significant difference). Solid lines indicate significantly different geometric means between the two incubation periods. Asterisks present the significance level of statistical difference (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$). Note: The following significant differences were found between material types: (A) For *A. versicolor*, ceiling tile incubated for one and six months was lower than MEA and gypsum board; (B) for *S. chartarum*, gypsum board incubated for one month was higher than MEA and ceiling tile and MEA for six months was lower than ceiling tile and gypsum board.

Numbers of aerosolized particles in the spore size range from the three material types are shown in Fig. 2. The single measurement values ranged from 0.5×10^6 to 8.6×10^8 particles

Table 1 – Ratios of the particle numbers in the fragment to those in the spore size fractions (particle F/S-ratio) for the two fungal species

		MEA	CT	GB	average
A. versicolor	1 month	0.019	0.365	0.099	0.161±0.04
	6 months	2.274[a]	2.038[b]	1.287[c]	1.867±0.97[d]
	Average	1.146± 1.426	1.202± 1.260	0.693± 0.705	1.014±0.82[e]
S. chartarum	1 month	0.352] ***	0.289] ***	0.446] ***	0.363± 0.19] ***
	6 months	60.65[a]	32.00[b]	46.02[c]	46.21± 22.17[d]
	Average	30.50± 43.34	16.13± 17.49	23.23± 27.61	23.29±22.33[e]

Average±standard deviation of three repeats.
Note: significant difference between the two species is indicated by a letter symbol (the same letters present a significant difference). Asterisks present the significance level of statistical difference between two incubation times (***: $P<0.001$).

m^{-3}. The highest value was observed for *A. versicolor* grown on MEA for one month and the lowest value was found for *S. chartarum* grown on MEA for six months. The GLM demonstrated that similar to fragment-sized particles, numbers of spore-sized particles were statistically significantly influenced by incubation periods, species, and material type ($P<0.001$).

For *S. chartarum*, the differences in particle numbers between the two incubation times were found for all material types. Numbers of aerosolized particles from ceiling tile increased with time, but corresponding values for MEA and gypsum board decreased with time. For *A. versicolor*, the only significant increase with time was observed for gypsum board. Differences between the two fungal species occurred for almost all material types. For MEA, numbers of aerosolized particles from *A. versicolor* grown for one and six months were significantly higher than those from *S. chartarum*. For building materials, corresponding values from *S. chartarum* grown on ceiling tiles for one and six months were higher than those from *A. versicolor*, while opposite results were obtained for gypsum board incubated for six months.
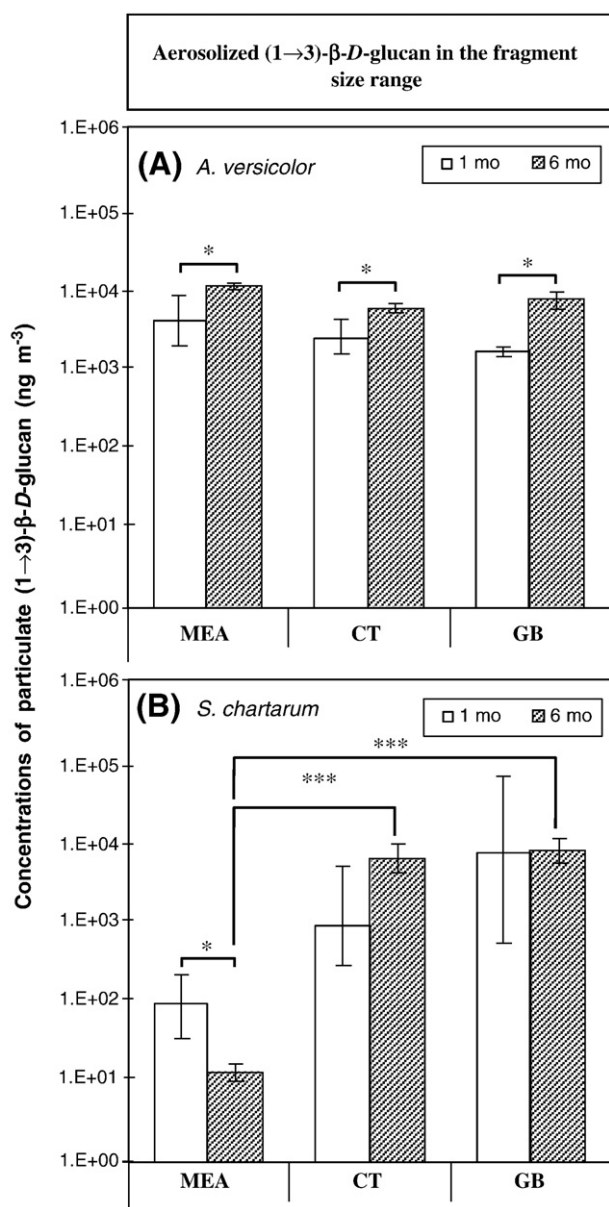
Significant differences among the three material types (ANOVA) were observed. Released particle numbers from *A. versicolor* grown on MEA and gypsum board were statistically significantly higher than those from ceiling tile ($P<0.001$). For *S. chartarum*, the particle numbers in the spore size range showed similar trends as numbers of particles in the fragment size range. Corresponding values for gypsum board incubated for one month were higher than those for MEA and ceiling tile ($P<0.01$), and after six months of incubation, numbers of aerosolized particles from ceiling tile and gypsum board were higher than those aerosolized from MEA ($P<0.001$).

The ratio of particle numbers in the fragment size range to those in the spore size range (particle F/S-ratio) is shown in Table 1 and varied from 0.011 to 104.2. The highest value was measured for *S. chartarum* grown on MEA for six months and the lowest for *A. versicolor* grown on MEA for one month. Average particle F/S-ratios were calculated as 1.01±0.82 (average±standard deviation of three repeats) for *A. versicolor*

and 23.29±22.33 for *S. chartarum*. A significant difference between the two incubation times was observed for *S. chartarum* grown on all three material types ($P<0.001$). Furthermore, for all material types incubated for six months, particle F/S-ratios for *S. chartarum* were significantly higher than those for *A. versicolor* ($P<0.001$).
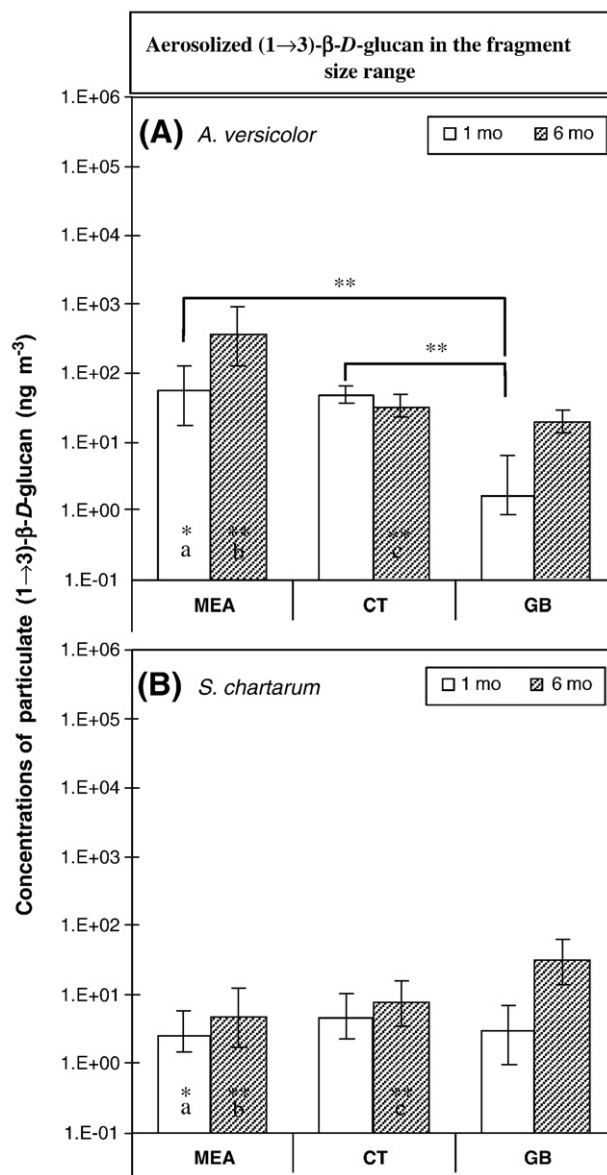
Fig. 3 – Airborne concentrations of particulate $(1{\rightarrow}3)$-β-D-glucan (ng m^{-3}) in the fragment size range aerosolized from each material sample (MEA = malt extract agar; CT = ceiling tile; GB = gypsum board): (A) *A. versicolor*; (B) *S. chartarum*. Histograms present geometric means, and error bars present geometric standard deviation of three repeats. Significant difference between the two species is indicated by a letter symbol (the same letters present a significant difference). Solid lines indicate significantly different geometric means between material types. Asterisks present the significance level of statistical difference (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$).

and on gypsum board for one month, respectively. The GLM showed that the $(1\rightarrow3)$-β-D-glucan concentrations of fragment-sized particles were influenced by the species and the material type ($P < 0.001$).

Species differences were found for MEA incubated for one and six months and ceiling tile for one month. The concentrations of $(1\rightarrow3)$-β-D-glucan aerosolized from A. versicolor were significantly higher than those from S. chartarum. Differences among the three material types were found only for A. versicolor grown for one month. The concentrations of $(1\rightarrow3)$-β-D-glucan released from MEA and ceiling tile were higher than the corresponding concentrations from gypsum board.

The concentrations of particulate $(1\rightarrow3)$-β-D-glucan in the spore size range are shown in Fig. 4. The single measurement values ranged from 1.0×10^1 to 4.7×10^4 ng m^{-3}. The highest and the lowest values were measured for S. chartarum grown on gypsum board for one month and on MEA for six months, respectively. The GLM demonstrated that the $(1\rightarrow3)$-β-D-glucan concentrations of spore-sized particles were influenced by the incubation period and material type ($P < 0.001$).

Differences in the $(1\rightarrow3)$-β-D-glucan concentration between the two incubation times were mainly observed for A. versicolor: the concentrations of $(1\rightarrow3)$-β-D-glucan increased with time for all three material types. In contrast, the corresponding values for S. chartarum grown on MEA decreased with time. Differences among the three material types (ANOVA) followed by Tukey's range test were observed for S. chartarum grown for six months: the concentrations of $(1\rightarrow3)$-β-D-glucan released from ceiling tile and gypsum board were significantly higher than the respective concentrations from MEA.

The ratio of $(1\rightarrow3)$-β-D-glucan concentrations in the fragment size range to those in the spore size range ($β_G$ F/S-ratio) is shown in Table 2 and varied from 0.2×10^{-3} to 6.7×10^{-1}. The highest value was measured for S. chartarum grown on MEA for six months and the lowest for S. chartarum grown on gypsum board for one month. The average $β_G$ F/S-ratios were 0.02 ± 0.02 for A. versicolor and 0.06 ± 0.16 for S. chartarum. A significant difference between the two incubation times was observed for A. versicolor grown on ceiling tiles only.

3.3. Correlation analyses

According to the results presented above, the concentrations of particulate $(1\rightarrow3)$-β-D-glucan did not always follow the

Fig. 4 – Airborne concentrations of particulate $(1\rightarrow3)$-β-D-glucan (ng m^{-3}) in the spore size range aerosolized from each material sample (MEA = malt extract agar; CT = ceiling tile; GB = gypsum board): (A) A. versicolor; (B) S. chartarum. Histograms present geometric means, and error bars present geometric standard deviation of three repeats. Solid lines indicate significantly different geometric means between the two incubation periods and between the material types. Asterisks present the significance level of statistical difference (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

3.2. $(1\rightarrow3)$-β-D-glucan concentrations aerosolized from material samples

Fig. 3 presents the concentrations of particulate $(1\rightarrow3)$-β-D-glucan in the fragment size range aerosolized from the three material types. The single measurement values ranged from 4.3×10^{-1} to 9.8×10^2 ng m^{-3}. The highest and lowest values were measured for A. versicolor grown on MEA for six months

Table 2 – Ratios of $(1\rightarrow3)$-β-D-glucan concentrations in the fragment to those in the spore size fractions ($β_G$ F/S-ratio) for the two fungal species

		MEA	CT	GB	average
A. versicolor	1 month	0.020	0.021]*	0.001	0.014±0.01
	6 months	0.045	0.006	0.003	0.018±0.03
	Average	0.032± 0.03	0.013± 0.01	0.002± 0.01	0.016±0.02
S. chartarum	1 month	0.066	0.010	0.001	0.026±0.05
	6 months	0.410	0.002	0.006	0.105±0.23
	Average	0.204± 0.27	0.006± 0.01	0.003± 0.004	0.063±0.16

Average±standard deviation of three repeats; asterisk presents the level of statistical difference (*: $P < 0.05$).
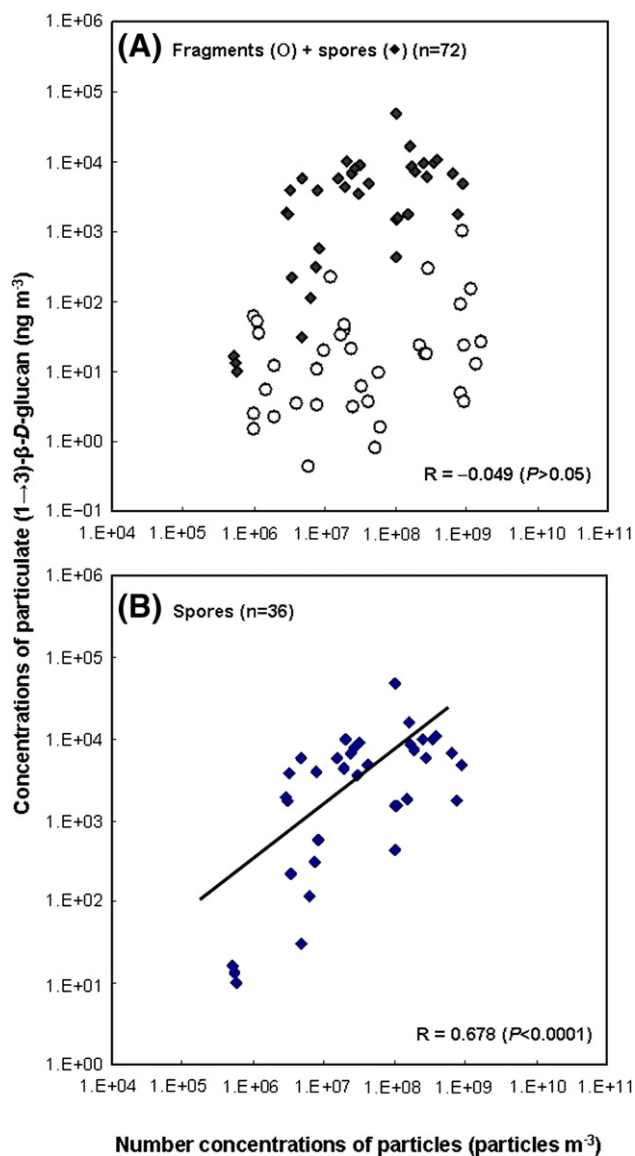
Fig. 5 – Scatter plot of numbers of aerosolized particles (particles m^{-3}) versus airborne concentrations of particulate $(1\rightarrow3)$-β-D-glucan (ng m^{-3}): (A) fragments+spores; (B) spores. Solid line indicates a linear regression line, and *n* is the number of samples.

same trends as numbers of particles aerosolized from material samples. Furthermore, the results in the fragment size range had different patterns from those in the spore size range. The scatter plots of the particle numbers versus the concentrations of particulate $(1\rightarrow3)$-β-D-glucan are shown in Fig. 5. The correlation analysis including data in the fragment and spore size range ($n=72$) failed to show correlation between measurements (Fig. 5(A): $r=-0.05$, $P>0.05$). Additionally, analysis including data in the fragment size range only ($n=36$) revealed low association between these variables ($r=0.31$, $P>0.05$). In contrast, the data in the spore size range ($n=36$) showed relatively good correlation between the particle numbers and the concentration of particulate $(1\rightarrow3)$-β-D-glucan (Fig. 5(B): $r=0.68$, $P<0.0001$). Poor correlations were found between the fragment and spore

results with respect to either particle numbers ($r=-0.04$, $P>0.05$) or particulate $(1\rightarrow3)$-β-D-glucan mass ($r=0.14$, $P>0.05$).

4. Discussion

Overall, the particle numbers released from mold-contaminated surfaces increased with incubation time. This was most clearly seen with numbers of fragment-sized particles, which were always significantly higher for 6-month old cultures than for 1-month old cultures. This trend was also observed in many cases in the spore size range. The increased release of particles can be explained by the changes in fungal biomass and moisture content. Total spore numbers on the surface increased about up to 320 times for *A. versicolor* and up to 26 times for *S. chartarum* when the incubation time increased from one to six months (Seo et al., 2008). At the same time, the average moisture content on the surface of material samples was 17.9% for 1-month old cultures and decreased to 5.3% for 6-month old cultures (Seo et al., 2008). Dryness on the surface can increase the release of fungal particles by reducing adhesion forces among fungal structures and enhancing these structures to become brittle. However, numbers of particles in the spore size range released from *S. chartarum* grown on MEA and gypsum board decreased with time. This finding was consistent with the trend in total particle numbers and total mass of particulate $(1\rightarrow3)$-β-D-glucan aerosolized from material samples reported by Seo et al. (2008) and may be associated with the exudates (i.e., slimy materials) produced by *S. chartarum* when it grew. Furthermore, the microscopically observed extent of aggregation of exudates surrounding the spores was highest on MEA and lowest on ceiling tile (Seo et al., 2008).

Generally, size-fractionated concentrations of $(1\rightarrow3)$-β-D-glucan aerosolized from material samples had increasing trend with increase in the incubation time, but not as clearly as with numbers of airborne particles. In contrast to particle numbers, the increase in the aerosolized $(1\rightarrow3)$-β-D-glucan was more notable in the spore size range than in the fragment size range. The concentrations of particulate $(1\rightarrow3)$-β-D-glucan in the spore size range aerosolized from *A. versicolor* grown on all material types for six months were significantly higher than the respective values for one month; however, the opposite trend was found for *S. chartarum* grown on MEA. This is consistent with the decreased numbers of particles in the spore size range aerosolized from *S. chartarum* grown on MEA. This is also supported by a good correlation between the airborne concentrations of particulate $(1\rightarrow3)$-β-D-glucan and numbers of aerosolized particles in the spore size range.

When all the data in the fragment and spore size ranges were combined, airborne $(1\rightarrow3)$-β-D-glucan mass and numbers of aerosolized particles did not correlate. This occurred due to poor correlation between these two parameters in the fragment size range. This helps explaining why in the concurrent study (Seo et al., 2008), the total mass of aerosolized $(1\rightarrow3)$-β-D-glucan did not consistently follow the same trends as the total numbers of aerosolized particles. The difference in the correlation may be due to the different characteristics of particles in the spore and fragment size ranges. Fungal spores aerosolized from material samples have

relatively monodisperse size distribution (Reponen et al., 1998; Kanaani et al., 2007), and thus, spore numbers are expected to be associated with the concentration of (1→3)-β-D-glucan originating from pure fungal cultures. On the other hand, fungal particles in the fragment size range are derived from intra- and extracellular structures of fungal colonies when aerosolized (Górny et al., 2002). Fungal fragments originating from intracellular components are expected to contain less (1→3)-β-D-glucan than those originated from the cell wall. Fungal fragments vary in size more than fungal spores (Cho et al., 2005) causing additional variation in the (1→3)-β-D-glucan mass per particle. Thus, (1→3)-β-D-glucan content per particle can vary more in the fragment than in the spore size range, and results in poor correlation between numbers of fragment-sized particles and the mass concentrations of (1→3)-β-D-glucan. In addition, biodegradation of building materials, which is caused by fungal growth, may contribute to this difference. When fungi utilize a cellulose-containing building material as a nutrient source, biodegradation of the material occurs (Gutarowska and Piotrowska, 2007) and may result in the generation of small particles, which may not contain (1→3)-β-D-glucan. Thus, these particles cannot contribute to (1→3)-β-D-glucan concentrations in air samples and further reduce the correlation between the two parameters.

Significant differences between the two species were observed for the particle numbers and (1→3)-β-D-glucan mass concentrations. The latter is supported by Iossifova et al. (2008), who showed that the (1→3)-β-D-glucan content varies widely between fungal species and is lower for *S. chartarum* than for *A. versicolor*. However, the extent of the differences varied depending on the incubation time and material type. The most pronounced difference was found for fungal particles in the spore size range released from MEA: both the particle numbers and concentrations of particulate (1→3)-β-D-glucan in the spore size range were lower for *S. chartarum* than for *A. versicolor*. For *S. chartarum*, the differences between building materials and MEA were more pronounced than for *A. versicolor*. This finding is consistent with the aerosolization ratios of non-size-fractionated particulate (1→3)-β-D-glucan previously reported by Seo et al. (2008). The results suggest that the use of laboratory media such as MEA is likely to underestimate the release of *S. chartarum* spores from real building materials.

The average particle F/S-ratios were slightly higher for *S. chartarum* (0.36) than for *A. versicolor* (0.16) for 1-month old cultures. This difference was more pronounced for 6-month old cultures: the particle F/S-ratios of *S. chartarum* grown for six months were up to 36-fold higher than those of *A. versicolor*. Generally, the β_G F/S-ratios were also higher for *S. chartarum* (0.06) than for *A. versicolor* (0.02). These results indicate that spores were the main contributor to the total (1→3)-β-D-glucan mass aerosolized from mold-contaminated materials under laboratory conditions. The contribution of fragments to the total (1→3)-β-D-glucan mass was higher for *S. chartarum* than for *A. versicolor*. The spores are also the main contributor to the total particle numbers released from young cultures (F/S-ratio <1). For older cultures, however, the contribution of fragments became more pronounced than that of spores (F/S-ratio ≫1).

In summary, the release of particles and particulate (1→3)-β-D-glucan generally increased with the age of the fungal culture. This increase was most consistent for particle numbers in the submicrometer size range. Particle numbers correlated with the mass concentrations of particulate (1→3)-β-D-glucan only in the spore size range. This is possibly due to variation in the (1→3)-β-D-glucan content per fragment particle attributed to a wide size range and test-specific size distribution of fungal fragments as well as the variation in the origin of the fragments within the fungal structure. Results of β_G F/S-ratios indicate that the contribution of fragments to the total mold exposure was higher for *S. chartarum* than for *A. versicolor*. The present study indicates that long-term mold damage in buildings may increase the contribution of submicrometer-sized fungal fragments to the overall mold exposure. 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 (Gold et al., 2000; Magari et al., 2001, 2002; Pekkanen et al., 2002). As the fragment results did not correlate with the corresponding spore results, fragment concentrations cannot be estimated based on spore data. Therefore, assessment of submicrometer-sized fungal particles should be conducted along with other mold measurements and analyses when determining exposure in moldy indoor environments.

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