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Release and characteristics of fungal fragments in various conditions

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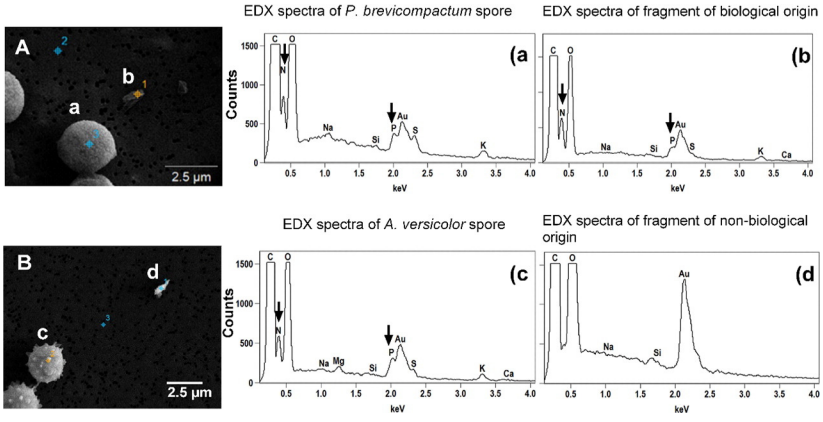
HIGHLIGHTS

- Increased air velocity increased fragment release, but decreased F/S-ratios.
- Fragments release may occur in conditions where spore release is not favorable.
- Mechanical break-up of fungal structures is the main process for fragment formation.
- Detection of N and P was used to differentiate the origin of fragments released.

GRAPHICAL ABSTRACT



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ABSTRACT

Intact spores and submicrometer size fragments are released from moldy building materials during growth and sporulation. It is unclear whether all fragments originate from fungal growth or if small pieces of building materials are also aerosolized as a result of microbial decomposition. In addition, particles may be formed through nucleation from secondary metabolites of fungi, such as microbial volatile organic compounds (MVOCs). In this study, we used the elemental composition of particles to characterize the origin of submicrometer fragments released from materials contaminated by fungi.

Particles from three fungal species (*Aspergillus versicolor*, *Cladosporium cladosporioides* and *Penicillium brevicompactum*), grown on agar, wood and gypsum board were aerosolized using the Fungal Spore Source Strength Tester (FSSST) at three air velocities (5, 16 and 27 m/s). Released spores (optical size, $d_p \geq 0.8$ μm) and fragments ($d_p \leq 0.8$ μm) were counted using direct-reading optical aerosol instruments. Particles were also collected on filters, and their morphology and elemental composition analyzed using scanning electron microscopes (SEMs) coupled with an Energy-Dispersive X-ray spectroscopy (EDX).

Abbreviations: MVOCs, microbial volatile organic compounds; SEM, scanning electron microscope; FSSST, Fungal Spore Source Strength Tester; EDX, Energy Dispersive X-ray spectroscopy; OPS, optical particle sizer; PBOA, primary biogenic organic aerosol; NADPH, nicotine adenine dinucleotide phosphate; NAHA, β-N-acetylhexosaminidase; UVAPS, Ultra Violet Aerodynamic Particle Sizer; WHO, World Health Organization; PM, particulate matter; ME, malt extract agar; DG18, dichloran glycerol 18% agar; LAS-X, laser aerosol spectrometer.

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Among the studied factors, air velocity resulted in the most consistent trends in the release of fungal particles. Total concentrations of both fragments and spores increased with an increase in air velocity for all species whereas fragment–spore (F/S) ratios decreased. EDX analysis showed common elements, such as C, O, Mg and Ca, for blank material samples and fungal growth. However, N and P were exclusive to the fungal growth, and therefore were used to differentiate biological fragments from non-biological ones. Our results indicated that majority of fragments contained N and P.

Because we observed increased release of fragments with increased air velocities, nucleation of MVOCs was likely not a relevant process in the formation of fungal fragments. Based on elemental composition, most fragments originated from fungi, but also fragments from growth material were detected.

1. Introduction

Moisture-damaged building materials and other indoor furnishes are the major sources of indoor mold (Meklin et al., 2004). Mold growth in indoor environments due to water damage can cause deterioration of indoor air quality and be harmful for human health (Ettenauer et al., 2012; IOM, 2004; WHO, 2009). Until recently, assessment of mold exposure was focused on spores only. However, no clear association has so far been documented between fungal spore concentrations and adverse health effects in indoor environments (Mendell et al., 2011).

Released fungal particles have been shown to be heterogeneous in nature and comprise of intact spores and submicrometer size fragments (Górny et al., 2002; Górny, 2004; Kildesø et al., 2003; Madsen et al., 2006). Fragments may arise from breakdown of spores and hyphal materials (Cho et al., 2005; Madelin and Madelin, 1995). When fungus grows on building materials it decomposes the material by fungal enzymes and acids in order to release nutrients (Adan, 1994; Davis, 2001). Therefore, it can be hypothesized that fragment particles released during fungal growth and sporulation may not all be from the growth but also from the growth material. Fragment particles may also originate by nucleation of by-products of fungal metabolism, such as microbial volatile organic compounds (MVOCs) (Fiedler et al., 2001; Górny et al., 2002; Korpi et al., 1997; Pasanen et al., 1997).

There is increased interest in the role of aerosolized fungal fragments in adverse effects considering the strong association between numbers of fine particles and adverse health effects (Gold et al., 2000; Green et al., 2006; Magari et al., 2001; Pekkanen et al., 2002). The submicrometer fragments are of utmost importance, because they tend to stay longer in the air, are easily inhaled and the smallest fragments (<0.1 μm) can deposit deep in the respiratory tract having the potential for causing adverse health effects (Cho et al., 2005; Frankel et al., 2014; Seo et al., 2008). Furthermore, the large surface area of the fragments relative to their mass may evoke high biological activity (Frankel et al., 2014). The high number of released fungal fragments in combination with their potential to deliver harmful antigens and mycotoxins to the alveolar region of the lung suggests the need for their characterization. The characterization of fungal fragments is important to help us understand the potential health effects associated with the exposure (Cho et al., 2005; McGinnis, 2007).

Several laboratory studies have been conducted to characterize particle size of aerosolized fungal fragments. These studies utilized direct-reading aerosol instruments, such as particle counters or aerodynamic particle sizers (Brandl et al., 2008; Cho et al., 2005; Górny et al., 2002; Seo et al., 2007). The disadvantage of these devices is that they do not distinguish biological particles from non-biological ones. The Ultra Violet Aerodynamic Particle Sizer (UVAPS) has been used to detect and count fungal particles from aerosol mixtures (Kanaani et al., 2008; Lee et al., 2010). This method employs the presence of fluorescence materials (nicotine adenine dinucleotide phosphate (NADPH) and riboflavin) in the biological propagules. Despite its ability to distinguish biological from non-biological materials, UVAPS may not be useful for characterizing fungal fragments because submicrometer size fragments give off very little or no fluorescence compared to larger spores (Kanaani et al., 2008; Saari et al., 2014).

Fungal fragments have also been characterized microscopically using morphology or chemically analyzing specific components such as (1 → 3)-β-D-glucan and β-N-acetylhexosaminidase (NAHA) (Adhikari et al., 2013; Afanou et al., 2014, 2015; Górny and Ławniczek-Wałczyk, 2012; Górny et al., 2002; Kanaani et al., 2008; Kildesø et al., 2003; Madsen et al., 2005; Seo et al., 2009). Other studies using immunochemical methods have also attempted to characterize fungal particles of various sizes to determine their biological activity (Górny et al., 2002; Schmechel et al., 2003). Also microscopic methods have been combined with immunostaining to increase the specificity (Green et al., 2005). Cascade impactors, e.g., Andersen impactor (Górny et al., 2002), and electrical low pressure impactor (ELPI) (Cho et al., 2005) have been used in other studies to characterize fungal fragments by aerodynamic sizes. These studies are informative but did not attempt to distinguish biological and non-biological fragments.

Bioparticles can be identified by microscopic methods. However, the different sources and similarities in particle appearance with particles from other sources may lead to difficulties in quantification (Wittmaack et al., 2005). Matthias-Maser and Jaenicke (1991, 1994) developed criteria for determining primary biogenic organic aerosols (PBOAs) in atmospheric samples. PBOAs were determined based on the particle elemental composition, the particle morphology and the particle behavior during EDX analysis. Morphology of the biological particles ranged from rod shaped, through elongated shaped to curved particles, while behavior of the samples was mainly changes in shape of particle either by shrinking or disappearing during EDX. They found that PBOAs contain minor amounts of K, P, S, Na and Ca (usually <10% of relative element of X-ray intensity of the particle) and thereby EDX analysis can be used to separate PBOA from other types of particulate matter (PM) in ambient air samples (Matthias-Maser and Jaenicke, 1994; Matthias-Maser et al., 2000). This criterion was recently adopted by Coz et al. (2010) to characterize PBOA in the atmosphere.

To our knowledge, there are no previous studies characterizing the origin of submicrometer fragments released from mold contaminated building materials based on their elemental composition. The aim of this study was to characterize the origin of fungal fragments released from contaminated building materials using their chemical composition. The amount of spores and fragments was measured with direct-reading aerosol instruments to determine the optimal experimental conditions for the collection of EDX samples.

2. Materials and methods

2.1. Test microorganisms

Three fungal species, *Aspergillus versicolor* (Culture collection of the Institute for Health and Welfare, Finland: HT31), *Cladosporium cladosporioides* (German collection of Microorganisms and Cell Cultures: DSMZ 62121), and *Penicillium brevicompactum*, (American Type Cell Collection: ATCC 58606) were used in the experiments. These species are common in indoor air worldwide (Hyvärinen et al., 2002; Méheust et al., 2013; Reponen et al., 2012). *A. versicolor* strain used was previously isolated from indoor air samples collected in Finnish homes.