# MICROBIAL FRAGMENTS – A NEW CHALLENGE FOR EXPOSURE ASSESSMENT OF BIOAEROSOLS

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## INTRODUCTION

Bioaerosol exposures are still poorly understood and a clear cause-and-effect relationship between the exposure and adverse health outcome has not been well established. This may be partially due to inadequate methods utilized for exposure assessment. Traditional sampling and analysis of airborne fungi and bacteria rely on measuring intact spores and bacterial cells whereas other types of microbial propagules have not been sufficiently explored. Our previous study on fungi showed that smaller-sized fungal fragments are released together with spores from moldy surfaces (Górny et al., 2002). In this study, we investigated the aerosolization of microbial propagules from contaminated sources in a wide size range of 0.02 – 10 μm. Two different aerosolization mechanisms were studied: aerosolization of dry microbial particles from surfaces and microbial aerosolization from liquids.

## METHODS

Fungi and bacteria were aerosolized in the laboratory from pure microbial cultures using newly developed laboratory scale simulators: a Fungal Spore Source Strength Tester (FSSST) and a Metal Working Fluid (MWF) simulator. The use of the FSSST represented the aerosolization of fungal spores from moldy surfaces into indoor environments, and the use of the MWF simulator represented the aerosolization of bacteria from MWFs into industrial environments. Two microorganisms, *Aspergillus versicolor* and *Pseudomonas fluorescens* were selected to represent common microorganisms in moldy buildings and in MWFs, respectively.

*A. versicolor* was grown on Malt Extract agar (MEA) plates at 25 C° for one month. After incubation, fungal propagules on agar plates were released by HEPA-filtered air at a flow-rate of 27 L/min using the FSSST, which is designed to aerosolize fungal propagules from contaminated surfaces by high-speed air jets (Grinshpun et al., 2002).

*P. fluorescens* culture was grown in a Trypticase Soy broth at 28°C for 18 h. The cells were washed twice with sterile deionized water by centrifugation at 7000 rpm for 7 min and diluted with the test MWF or with sterilized deionized water to the final concentration $10^8$ cells/mL. A commercially available semi-synthetic MWF was selected for this study. The following three fluids were tested: 1) Pure MWF, 2) *P. fluorescens* suspension in water, and 3) *P. fluorescens* suspension in MWF. MWF was used as 5% solution (consisting of 95% water and 5% of MWF concentrate) similar to its use in factories. Aerosol generation in the MWF simulator took place when a liquid pump ejected MWF through a nozzle against a rotating aluminum rod (simulating a grinding wheel). The fluid application rate was 1.0 L/min. The rotation speed of the rod was 8000 rpm, which is typical for grinding operations.

The airborne fungi and bacteria were continuously monitored using an electrical low-pressure impactor (ELPI; 3935 series, Dekati Ltd., Tampere, Finland) as an aerodynamic particle sizer.

## RESULTS

Figure 1 shows that the size distribution of fungal propagules aerosolized from a moldy surface by the FSSST was bimodal. One mode was in the size range of 2.45-3.97 μm representing intact spores, and the other was in the range of 0.06-0.10 μm representing fungal fragments. Minimum fungal particle concentrations observed at 0.17-0.25 μm separated the spore and fragment peaks. Separate experiments including microscopic observations of collected propagules confirmed that the particles in the fragment size range did not include bounced-off spores from the upper stages.

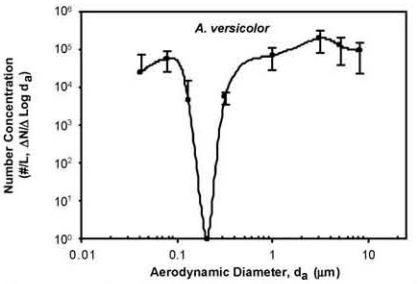


Figure 1. Size distribution of fungal propagules aerosolized from a moldy surface by the FSSST.

Figure 2 shows the size distribution of particles aerosolized from the three fluids by the MWF simulator. The particulate concentrations in all the size ranges were highest for the fluid containing *P. fluorescens* in MWF. The size distribution mode for particles aerosolized from the *P. fluorescens* suspension in water was in the size range of 0.64-0.99 μm representing the aerodynamic diameter of intact *P. fluorescens* cells. The mode of the two other size distributions (pure MWF and *P. fluorescens* suspension in MWF) was between 0.24 μm and 0.39 μm.

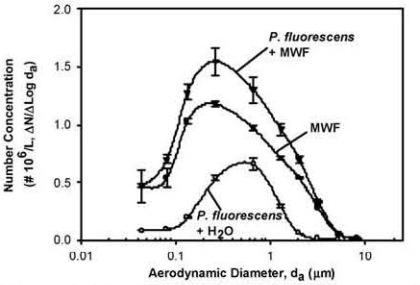


Figure 2. Size distribution of particles aerosolized from three fluids by the MWF simulator.

## CONCLUSIONS

The results suggest that aerosolization of high concentrations of microbial fragments in the fine and ultrafine particle size ranges may occur in microbiologically contaminated indoor and industrial environments. The role of microbial fragments is particularly interesting in light of recent epidemiological studies that have established a strong relationship between the concentration of outdoor fine particles (<2.5 μm) and adverse health outcomes. Microbial fragments may potentially contribute to the inexplicable adverse health effects associated with microbial exposures. This creates a need to develop field-compatible methods for sampling and analysis of microbial fragments.

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