The Behavior of Mold Spores Suspended in Indoor Air and an Alternative Method for Analyzing Filters Obtained from Sampling by Filtration

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Abstract
In indoor environments, air sampling normally involves drawing a sample from an air stream or room using equipment based on either the impaction or filtration methods. An international standard regarding sampling methods for the detection and enumeration of molds is about to be proposed. This ISO (ISO/TC 146/Sc 6/WG 10 N 27), entitled "Detection and enumeration of molds - sampling by filtration" suggests that the process of suspension is necessary in order to spread mold spores filtered on the surface of a filter onto a growth media. However, airborne microbes suspended in air are rarely in the state of naked particles. Therefore, by the suspension process specified by the ISO method, the number of colony forming units (CFUs) on a culture medium will not accurately express the number of bio-aerosols actually suspended in air. The present study was conducted to clarify the behavior of bio-aerosols suspended in air and to demonstrate the inadequacies of a filtration method that uses the suspension process to quantify fungal spores on a filter surface. The authors found that fungal spores suspended in air are coagulation particles and not naked particles, as demonstrated by both the Andersen sampler as well as electron microscopy of W.sebi spores.

Furthermore, by the process of suspension, the CFU number was several times the value of fungal spores actually suspended in air.

Keywords: bio-aerosol; fungal spore; behavior; size distribution; filtration method

1. Introduction
Until the 1980s there was no standard method for the sampling of microbiological agents suspended in air (WHO, 1988). With respect to molds, an international standard for the detection and enumeration of molds is about to be proposed (ISO 2006a, 2006b, 2006c). In indoor environments, air sampling normally involves drawing a sample from an air stream or room using the impaction or filtration methods. Air samplers based on the impaction method are used to provide a size distribution of microbes, to permit the collection of specific microbes directly onto growth media. In the filtration method, the air is filtered and the filter then immersed in saline solution in order to spread mold spores onto a growth medium (the suspension process). However, as airborne microbes suspended in air are rarely in the state of naked particles, by the suspension process specified by the ISO method the number of colony forming units on a culture medium will not accurately express the number of bio-aerosols actually suspended in air. This is because a CFU is defined as a unit by which the culturable number of microorganisms is expressed, and importantly, one colony forming unit may originate from a single microorganism, from aggregates of microorganisms as well as from one or many microorganisms attached to a particle.

This study was conducted to clarify the behavior of bio-aerosols suspended in air and to demonstrate the inadequacies of a filtration method that uses the suspension process to quantify fungal spores on a filter surface.

2. Methodology
2.1 Measurement system
Experiments were performed in a full-scale clean room of 3 m (L) × 3 m (W) × 2.2 m (H), with a floor area of 9 m² and a total volumetric area of 20 m³.

Fig.1. shows the measurement system. Fungal spores of Wallemia sebi on a plate, known as a "sensor piece" (Abe, 1993) were used in this study as the trace microbes. W.sebi spores were generated using an ultrasonic device, and then introduced into the clean
room by pressurized air. Before and during the test, the suspended particle concentration, airborne fungal concentration and size distribution of *W. sebi* spores were measured. In addition, a fan was used in the room to uniformly distribute any particulate or organisms matter during the experiment.

2. Measurement Equipment

**Fungal aerosols generator**

As shown in Fig. 1., a vibrating plate was set in a glass chamber, 80 mm wide and 170 mm deep, with a half wavelength extension rod. High intensity airborne ultrasound waves were generated in this chamber, as this has been reported to be a very effective way of diffusing microorganisms (Otsuka, 2007).

**Airborne fungal spore concentration**

In order to clarify the behavior of a bio-aerosol suspended in air, two sets of MD8 (Sartorius Co., Germany) equipped with a gelatin disposable filter were used to measure the airborne fungal spore concentration. The sampling rate of MD8 was 10 L (flow rate, 3 m³/h; sampling period, 12 seconds; flow velocity, 21.7 cm/s). The sampling efficiency of a gelatin filter is in excess of 95% for fungal spores with an aerodynamic diameter larger than 1 µm using a flow velocity at the filter of v=21.7 cm/s (ISO 2006a). In addition, the size distribution of *W. sebi* spores was measured using an Andersen sampler (Tokyo Dylic Co., Japan) with 6 stages. The size ranges in each stage of the Andersen sampler are 0.65-1.1, 1.1-2.1, 2.1-3.3, 3.3-4.7, 4.7-7, and 7 µm. Furthermore, a Sioutas cascade impactor (SKC Co., USA) was used to directly obtain an electron microscopy sample via carbon tapes at each stage. The size ranges at each stage of the Sioutas cascade impactor are 0.25-0.5, 0.5-1, 1-2.5, and 2.5 µm.

**Suspended particle concentration**

Suspended particles were measured using the optical particle counter, KR-12A (Rion Co., Japan) simultaneously with measurement of fungal spores. The KR-12A unit is used to measure the concentrations of suspended particles with different size distribution based on the light scattering technique. The size ranges in each channel of the KR-12A unit are 0.3-0.5, 0.5-0.7, 0.7-1, 1-2, 2-5, and 5 µm.

3. Test Procedures

Table 1. shows the test procedure. Before the test the clean room was cleaned for 30 minutes in order to reduce the indoor background concentration of particles, and during the test period the air-conditioning was turned off. After the generation of *W. sebi* spores, the indoor airborne fungal spore concentration was measured simultaneously using two sets of gelatin disposable filters after 1, 5, 10, 15, 20, 25, 30, 45 and 60 minutes of exposure to the clean room air. For each filter set, one filter was directly applied onto a DG18 agar plate while the other was immersed in saline solution. Decimal dilutions of the suspension were then prepared and aliquots spread on DG18 agar plates. All
plates were incubated at 25°C for 7 days.

The size distribution of indoor airborne fungal spores was measured using the Andersen sampler 10 minutes after the generation of *W.sebi* spores. Sampling by the Sioutas cascade impactor was performed 25 minutes after generation of *W.sebi*.

3. Results

3.1 Distribution of fungal spores

Fig.2. shows colonies of *W.sebi* on the surface of a DG18 agar plate following exposure using the direct method (non-suspension). Clearly the *W.sebi* spores were uniformly suspended in the clean room air.

Fig.3. shows the suspended particle concentration vs. elapsed time before, during, and after the generation of *W.sebi* spores. According to the concentrations of suspended particles larger than 2µm, which show a sharp rise during the generation period, the size of *W.sebi* spores suspended in air can be considered to relate to large particles. The fall in suspended particulate concentration after spore generation is due to gravity sedimentation.

Fig.4. shows the relationship between suspended particles and airborne *W.sebi* spore concentration. A significant correlation between concentrations of suspended particles larger than 2 µm and *W.sebi* spore was found.

3.2 Size distribution of *W. sebi* spores

Fig.5. shows the size distribution of *W.sebi* spores measured using the Andersen sampler. The size of fungal spores was distributed between 1.1µm and 7µm, and shows a log normal distribution with a median diameter of approximate 4µm. The size of fungal spores suspended in indoor environments has been reported to be distributed in a log normal distribution with a median diameter of approximately 3.5µm (AIJ, 2005). Thus, the generation of *W.sebi* spores by the ultrasonic device used in this study reproduced the size distribution of fungal spores suspended in normal environments.

Fig.6. suggests that spores suspended in air are coagulation particles and not naked particles, and this is supported by the electron microscopy findings obtained using a Sioutas cascade impactor (Fig.6.).

3.3 Concentrations of airborne *W.sebi* spores determined using the direct and indirect methods

Fig.7. shows the relationship between the direct method (suspension method) and indirect method (non-suspension method) obtained by paired sampling using gelatin disposable filters. Significant correlation was found and the CFUs obtained by the indirect method were 4.6 times higher than by the direct method.
4. Discussion

The authors examined the behavior of fungal spores suspended in air and evaluated the different methods of detecting spores in air. *W. sebi* spores were generated in a clean test room using an ultrasonic generator, which produced size distributions of spores that were largely in agreement with reported measurements of airborne mold spores in indoor environments. Therefore, the behavior of the fungal spores suspended in air in the present study can be considered to reflect the behavior of fungal spores in a real environment.

The authors findings indicate that fungal spores suspended in air are in the state of coagulation particles and not naked particles, as confirmed by both Andersen sampler results and electron microscopy. Moreover, this finding agrees with the significant correlations found between suspended particle concentrations larger than 2µm and *W. sebi* spore concentrations.

For accurate results to be obtained with the filter method, the ISO (ISO, 2005a) indicates that the process of suspension is necessary in order to spread mold spores filtered on the surface of a filter onto a growth medium. However, fungal spores suspended in air are coagulation particles, not naked particles, and thus by the suspension process specified by the ISO method, the number of CFUs on a culture medium will not accurately express the number of bio-aerosols actually suspended in air.

The number of CFUs obtained using the indirect method (suspension process) was several times greater than that obtained by the direct method (direct application of the filter onto a DG18 agar). This result is due to the size distribution of fungal spores; i.e., by the suspension method the coagulated spore collapses in many small spores.

Based on these findings, the authors recommend the direct method as a suitable alternative to the suspension method when analyzing the removal performance of airborne mold spores by an air filter.

5. Conclusions

The behavior of mold spores suspended in indoor air was investigated in a full-scale test room. *W. sebi* spores were used and were generated by an ultrasonic device. Using the Andersen sampler and electron microscopy of *W. sebi* spores, the authors found that the airborne spores suspended in air are in the state of coagulation particles.

The results obtained by comparing the direct method (direct placement of the filter onto a DG18 agar plate) with the indirect method (suspension in liquid medium of the filter obtained from sampling by filtration followed by plating on agar) indicate that the CFU obtained by the suspension process does not accurately express the number of bio-aerosols actually suspended in air.

Based on these findings, the authors recommend the direct method as a suitable alternative to the suspension method when analyzing the removal performance of airborne mold spores by an air filter.

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