

Effect of Vibration on Dispersal of *Cladosporium cladosporioides* Bioaerosols

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The vibration of fungal cultures was evaluated to determine its potential effect on the dispersal of airborne fungal microorganisms suspected of being pathogens. An artificial vibration system, which simulates the actual environmental vibration of fungal structures, was designed and constructed for this purpose. Experiments featured the use of low-frequency vibrations similar to those induced by earthquakes. Within the range of conditions tested, the vibration of fungal cultures was found to affect the airflow-driven generation of bioaerosols.

Keywords: Bioaerosols, fungal spores, vibration, earthquake, *Cladosporium cladosporioides*

Airborne fungal particles, termed fungal bioaerosols, have received particular attention because they are known to constitute a major portion of the ambient airborne microbial population [14, 15] and are associated with adverse health effects. In particular, fungal bioaerosols have been found to be associated with respiratory diseases, allergic symptoms, and sick building syndrome [2, 5, 6, 12]. Several reports describe their association with hospital visits for asthmatic symptoms [3] and an increase in respiratory symptoms [1]. Moreover, it has been reported that fungal spores affect the respiratory symptoms of children [13]. As concern over the adverse health effects of fungal bioaerosols grows, so does the demand for the elucidation of their dispersal mechanisms as well as related control methods [9, 10]. In an attempt to satisfy this demand, an investigation was experimentally conducted in the present study on the dispersal mechanisms of fungal bioaerosols, with a particular focus on the effect of the vibration of fungal structures on the generation of airborne particles.

Thus far, several mechanisms of fungal bioaerosol dispersal in natural environments are known. Some fungi are dispersed by rain splash, in which water droplets striking fungal

surfaces carry detached spores into the air [4]. Wind aerosolization is a common mechanism for detaching spores from hyphae and delivering them into the air environment [8]. In the present investigation, the vibration of fungal structures was postulated as an additional mechanism of dispersal; it is possible that vibration may weaken the hyphal structure, thereby facilitating the dispersal of fungal spores and particles. Interestingly, 185 of 1,200 survivors of an earthquake in Turkey were found to have skin disorders shortly after the disaster, most of which were parasitic, allergic, or microbial in origin [11]. If fungal dispersal is in fact enhanced by vibrations such as those generated by earthquakes, and thus increases the concentration of fungal bioaerosols, it may provide one possible explanation for this finding.

In the present study, a new system for artificially vibrating fungal cultures was designed and constructed. This system can vibrate fungal cultures on a plate at frequencies ranging from 0.1 to 100 Hz, and at various amplitudes, with a maximum of 0.6 mm. We measured the physical aerosol particle size distribution and the number of colonies of fungal bioaerosols that were generated by an airflow stimulus in the presence and absence of vibration.

Cladosporium cladosporioides (KCTC 16680) was used as the test organism in this study. *Cladosporium* is one of the most common genera found in the air environment [14]. *Cladosporium* spp. are nonpathogenic in humans, with the exception of immunocompromised patients. However, *Cladosporium* spp. are known to have the ability to trigger allergic reactions in sensitive individuals [7]. The fungal test strain was inoculated on malt extract agar plates and incubated at 25°C for 7 days prior to vibration experiments [8]. To make plates for the tests, we inoculated a portion of a fungal culture into 50 ml of sterilized water, and then mixed the solution thoroughly using a vortex mixer. We then inoculated each of 25 malt extract agar plates with 2 ml of the solution. The plates were incubated at 25°C for 2 to 4 days, and then subjected to experimentation using the vibration system [8].

Fig. 1 shows a schematic diagram of the experimental setup. We designed and manufactured a new vibration system

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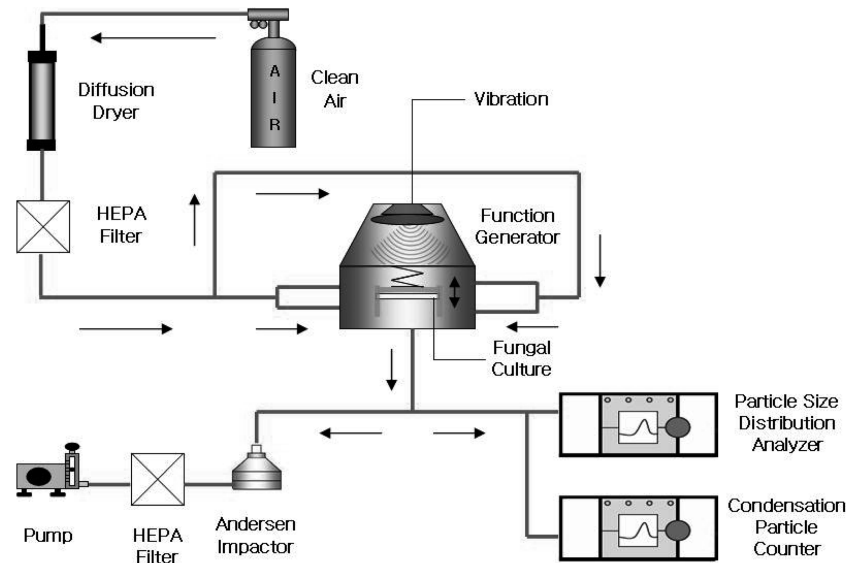


Fig. 1. Schematic diagram of the experimental setup.

for fungal cultures. The vibration system consists of a function generator (GWINSTEK GFG-3015, Seoul, Korea), a vibrator, a platform with a diameter of 87 mm and a height of 14 mm for accommodating a culture plate, a connecting structure between the vibrator and the platform, and the external housing. The function generator provides sine and square-type wave functions at frequencies from 0.1 to 100 Hz and supplies a maximum voltage of 10 V to the vibrator. The vibrator was constructed by combining an electromagnet and a fabric membrane. When the function generator sends a signal to the vibrator, the fabric membrane vibrates according to that signal, and fungal cultures on the petri plate tightly attached to the platform and the vibrator *via* the connecting structure will also vibrate according to that signal.

The vibrator and the attached petri plate are contained within the metal housing, which has inlets through which air enters and one outlet through which generated fungal bioaerosols exit.

In the experimental procedure, an airflow of 30 l per minute was directed at an attached culture plate, thereby generating fungal bioaerosols. Vibration of the fungal cultures was induced, and the resulting variation in the characteristics of airflow-driven fungal bioaerosols was observed. A particle size distribution analyzer (PSD3603; TSI, MN, U.S.A.) and a condensation particle counter (HCT 4312; HCT Co. Ltd., I-cheon, Korea) were employed to measure the particle size distribution and concentration of generated fungal bioaerosols. An Andersen impactor Z-A6 (Zefon International, FL, U.S.A.) [8, 10] and an incubation system were used to measure the concentration of viable fungal bioaerosols generated by the vibration system. Airborne fungal particles were sampled on a malt extract

agar plate inside the impactor and incubated for 1–2 days before the number of colonies was enumerated [8].

We first measured the particle size distributions of fungal bioaerosols generated with and without vibration. Since it was difficult to obtain culture plates having the same initial number of aerosolized fungal particles, we classified the fungal plates into several groups by considering their growth condition and shape, and then used each group for one type of experiment. We used one such group for experiments with vibration at 0, 0.5, 1, and 3 Hz, and another group at 0, 10, 20, and 30 Hz. Fig. 2A shows the aerosol particle size distributions of airflow-driven fungal bioaerosols generated at 0 Hz (no vibration) and 1 Hz (amplitude 0.6 mm). The concentration of fungal fragment particles, which were small pieces of debris or broken fungal particles with a size of less than 1 μm , was not affected by a vibration of 1 Hz (amplitude, 0.6 mm); however, the concentration of fungal spore particles, the size of which is around 2 μm , was increased as a result of vibration. Fig. 2B shows that vibration at 20 Hz (amplitude, 0.2 mm) affected the concentration of generated fungal fragments and spore bioaerosols simultaneously.

We also sampled fungal bioaerosols generated by square-type vibrations and calculated the concentration of culturable fungal bioaerosols based on enumeration of fungal colonies after incubation. As shown in Table 1, the concentration of culturable fungal bioaerosols was increased owing to vibration. In particular, vibration at 20 Hz (amplitude, 0.2 mm) had a relatively large effect.

In the above experiments, variation in the initial number of airflow-driven particles between culture plates was a major source of error when determining the effect of vibration on dispersal. To address this problem, we designed

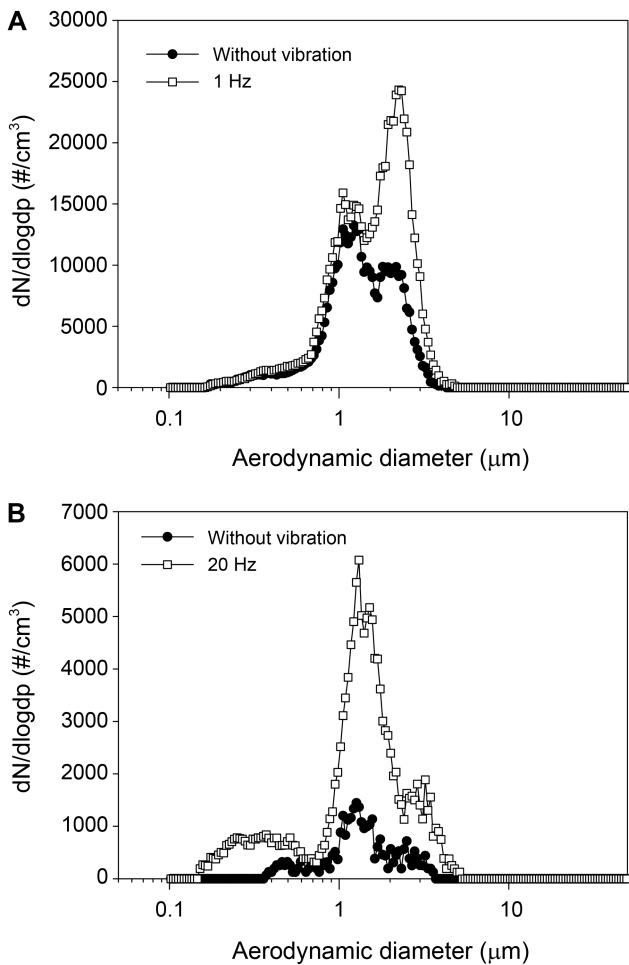


Fig. 2. Particle size distributions of airflow-driven fungal bioaerosols generated (A) with or without vibration at 1 Hz (amplitude, 0.6 mm), and (B) with or without vibration at 20 Hz (amplitude, 0.2 mm).

another experiment, the results of which are shown in Fig. 3. The rate of generation of aerosol particles from fungal culture plates decreased as the airflow passed over the plates, so that, in the absence of vibration, the total concentration of aerosolized particles was rapidly depleted; this result, as shown in Fig. 3, is in keeping with results from the literature [7]. If vibration affects the generation of fungal

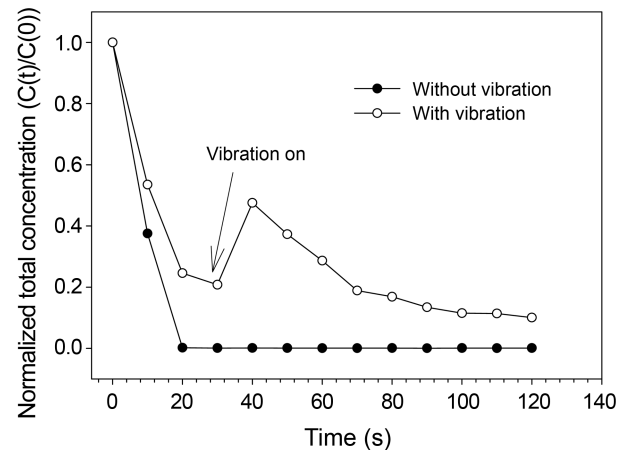


Fig. 3. Variation of the total concentration over time of airflow-driven fungal bioaerosols of *C. cladosporioides* following the sudden onset of vibration.

bioaerosols, the total concentration of bioaerosols should increase abruptly at the onset of vibration using our experimental system. Thus, as also shown in Fig. 3, when a square-type vibration at a frequency of 8 Hz (amplitude, 0.5 mm) was induced in fungal cultures 30 s after the airflow-driven generation of bioaerosols was initiated, we readily observed an abrupt increase in the total particle concentration from that point. Specifically, the total concentration of *C. cladosporioides* bioaerosols increased from 385 to 880 particles/cm³ upon exposure to vibration. Since we used only one plate to observe the variation in total particle concentration, the initial condition of the culture did not present a problem. Furthermore, we obtained the same result in terms of the abrupt increase in the number of generated particles using more than three different culture plates.

Overall, the results shown in Fig. 2 and 3 and Table 1 demonstrate that vibration affects the dispersal of bioaerosols from fungal culture plates under our experimental conditions. Thus, it is possible that other sources of low-frequency vibrations, such as earthquakes and other natural phenomena, may also provide a mechanism for the dispersal of fungal bioaerosols under actual environmental conditions.

Table 1. Variation of viable airflow-driven fungal bioaerosol concentration according to vibration stimulus.

| Vibration characteristics (frequency, amplitude) | Bioaerosol concentration (CFU/m ³) |
|--|--|
| 0 Hz, 0 mm | 141 |
| 18 Hz, 0.6 mm | 188 |
| 19 Hz, 0.5 mm | 223 |
| 20 Hz, 0.2 mm | 412 |
| 50 Hz, 0.2 mm | 223 |
| 100 Hz, 0.1 mm | 294 |

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