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Development of a Fungal Spore Aerosol Generator: Test with *Cladosporium cladosporioides* and *Penicillium citrinum*

Lee, Byung Uk[1]*, Young Joong Kim[1], Chang Ho Lee[1], Sun Hwa Yun[1], Gwi-Nam Bae[2], and Jun-Ho Ji[3]

[1]*Aerosol and Bioengineering Laboratory, Department of Mechanical Engineering, Konkuk University, Seoul 143-701, Korea*
[2]*Center for Environmental Technology Research, Korea Institute of Science and Technology, Seoul 136-791, Korea*
[3]*Digital Appliance R&D Center, Samsung Electronics, Suwon 443-742, Korea*

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As the first step to develop efficient means to control fungal spore bioaerosols, we designed, manufactured, and evaluated a fungal spore aerosol generator. We studied the physical and biological properties of the fungal spore bioaerosols on two common fungal species. The results demonstrated that the fungal spore bioaerosol generator effectively produces fungal spore bioaerosols.

Keywords: Bioaerosols, fungal spores, *Cladosporium cladosporioides*, *Penicillium citrinum*, aerosol generator

Bioaerosols, which are airborne microorganisms, include airborne bacteria, viruses, pollens, fungal spores, and biological fragments [8]. Of all the airborne microorganisms, fungal spore bioaerosols have received particular attention because they are associated with public health problems and also because they constitute a major portion of airborne microorganisms in indoor and outdoor air [8, 16, 25, 26]. Fungal spores are associated with asthmatic symptoms, inflammation, the sick building syndrome, and respiratory diseases [2, 4, 7, 22]. Ambient fungal spore levels are associated with hospital visits for asthmatic symptoms [3] and an increase in respiratory symptoms [1]. Fungal spores also affect respiratory symptoms of children [24]. These public health problems have raised concerns about airborne fungal spores, and have highlighted the need for an effective method of controlling fungal spore bioaerosols.

Researchers have attempted to control bioaerosols, especially bacterial bioaerosols, by such means as ultraviolet (UV) irradiation [20, 21, 23], air electric ion emission [13–15], and thermal energy exposure [12, 17, 19]. However, few studies have focused on the control of fungal spore bioaerosols. The first step to develop methods to control bioaerosols is to develop equipment that can artificially generate a specific amount of bioaerosols in an experimental environment. However, in the case of fungal spores, few studies have been devoted to finding methods of artificially generating a specific amount of airborne fungal spores. As for the generation of bacterial bioaerosols, investigators have generally used a method of spraying solutions containing bacteria [10–12, 17]. On the other hand, the method of spraying fungal spores containing solutions has been criticized as being inadequate for simulating the dispersal of real environmental fungal spores, which usually involves blowing of a strong airflow over fungal cultures. Thus, the lack of an efficient method of artificially generating airborne fungal spores has been a stumbling block for the development of efficient methods of controlling airborne fungal spores.

We designed and manufactured a new artificial fungal aerosol generator and tested its capability of producing viable fungal spore particles under various conditions. There are a few studies on the measurement of fungal particles from indoor contaminated surfaces from a hygiene perspective [5, 6]. It should be noted that the idea of the measurement apparatus of the above studies, which involves an artificial airflow over contaminated surfaces [5, 6], was applied in the design of our new fungal spore aerosol generator.

Fig. 1 shows the shape and dimensions of our fungal spore aerosol generator, which is made of plastic. Fig. 2 shows the experimental setup. In Figs. 1 and 2, the airflow entering the generator accelerates as it passes through 80 nozzles, each of which has a diameter of 2 mm. The accelerated airflow passes over an agar plate of fungal cultures inside the generator. When the air flows, the fungal spores are detached from the fungal cultures because of frictional force between the airflow and the fungal cultures. The airflow then carries the detached fungal spores out of the agar plate. Hence, the exiting airflow from the generator contains the airborne fungal spore particles. The agar plate of fungal cultures is inserted between the upper and lower

*Corresponding author
Phone: 82-2-450-4091; Fax: 82-2-447-5886;
E-mail: leebu@konkuk.ac.kr

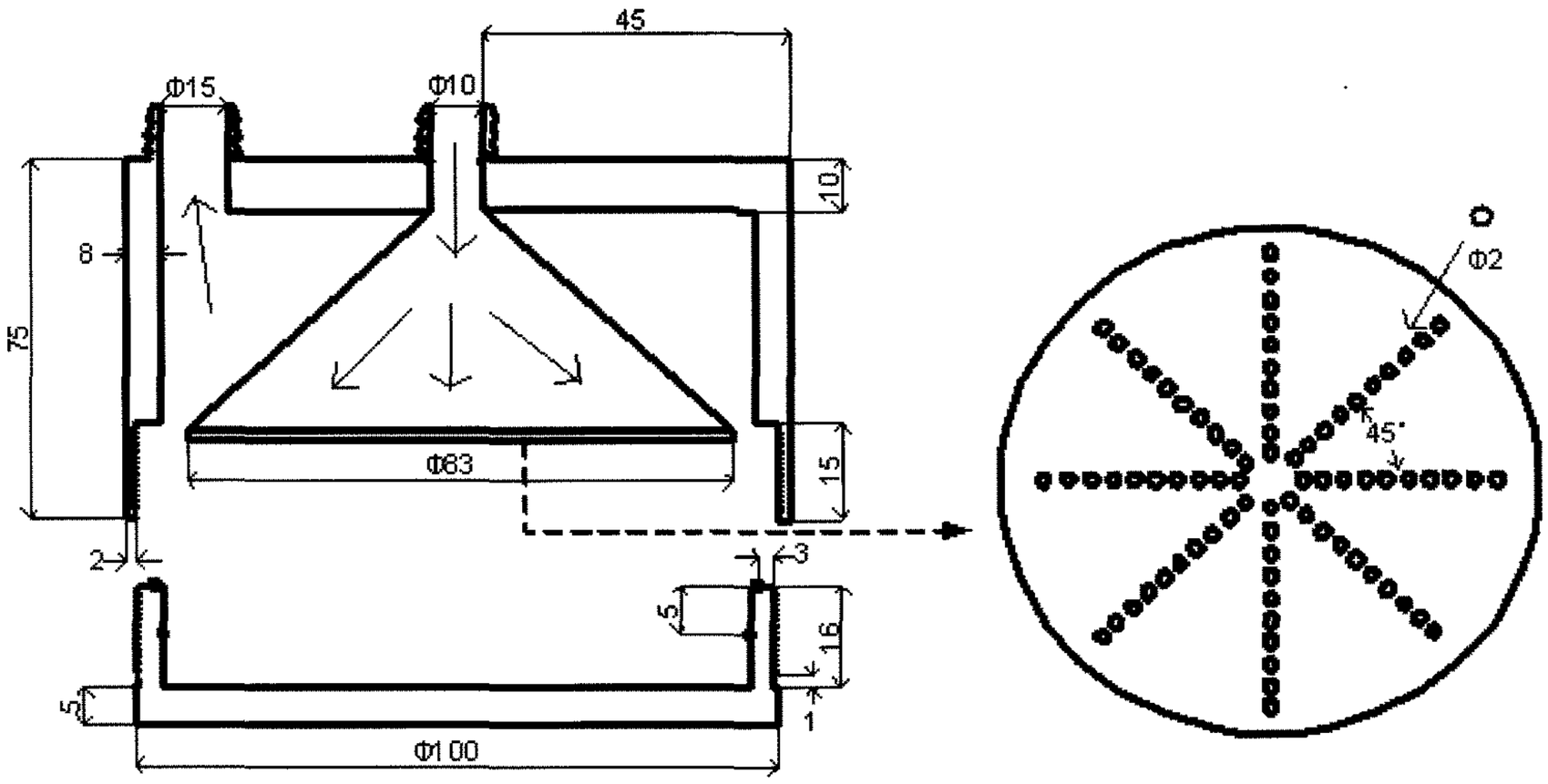

Fig. 1. Drawing of the manufactured fungal spore aerosol generator.
The gas flows from the upside through the center inlet of the upper part and passes through the 80 nozzles (right-side figure). This gas flow hits the fungal culture, which is assumed to be attached at the bottom part of the generator. The gas carries the detached fungal spores and flows to the outlet of the upper part of the generator.

parts of the generator. Rubber O-rings prevent any leakage at the connection between the upper and lower parts of the generator.

To evaluate the fungal spore aerosol generator, we used two common fungi genera: *Cladosporium cladosporioides* (KCTC 16680) and *Penicillium citrinum* (KCTC 6990). These fungi genera are common in the environment [16, 25, 26], and the species have been used as test microorganisms in microbiological studies including hygiene studies [6, 9, 18, 27].

The fungal strains were inoculated on malt extract agar plates and incubated at 25°C for 7 days before the aerosol generator was tested. To make the fungal plates for the tests, we mixed a part of the fungal cultures with 50 ml of sterilized water. The mixed solution was then shaken thoroughly by a vortex mixer, and injected into 25 malt extract agar plates with 2 ml of the mixture in each plate. The plates were incubated at 25°C for 2 to 4 days and inserted into the bioaerosol generator for the fungal spore bioaerosol generation. Therefore, the initial amount of fungal spores in 25 tested plates was maintained at a similar level.

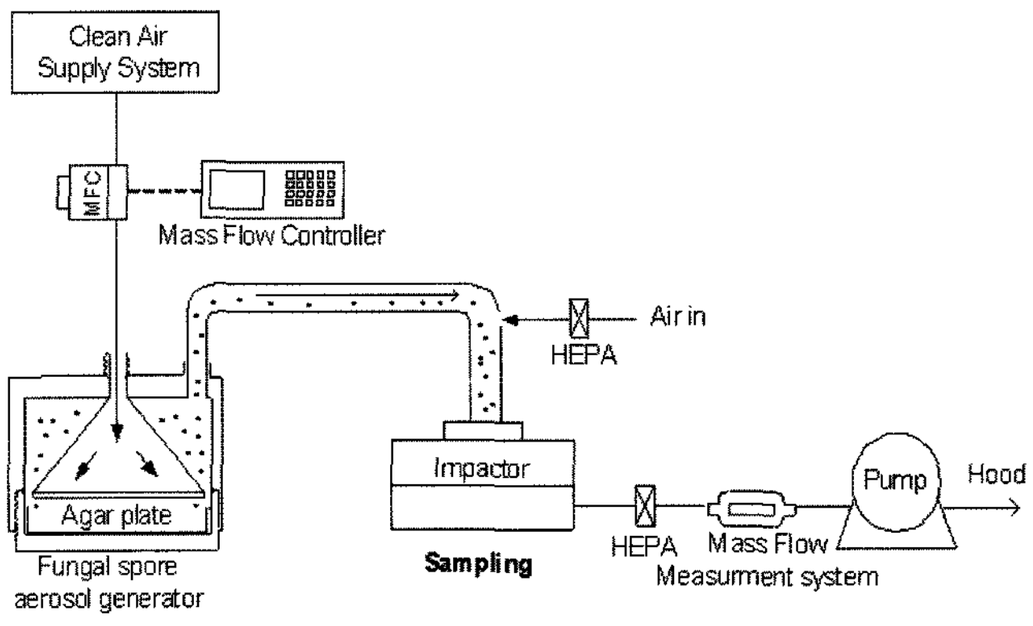

Fig. 2. Schematic diagram of the experimental setup.

The aerosolized fungal spores from the generator were sampled and measured by a Z-A6 Anderson impactor (Zefon, Arizona, U.S.A.), which was designed to sample and check the viability of aerosols from the air. The usage of the Anderson impactor for measuring bioaerosols has been described previously [17]. The airborne fungal spores were sampled on an agar plate inside the impactor and incubated for 1 to 2 days before the number of colonies was enumerated.

In the experimental system, the airflow had a temperature of 19.1°C±0.5°C and a relative humidity of 19%±1%. We tested two variables when evaluating the aerosol generator: the flow velocity over the agar plates, which was controlled by the flow rate through the aerosol generator; and the time sequence, namely the first 5 min, the second 5 min, and the final 5 min each of a 15 min sampling period.

Fig. 3 shows the number of viable fungal spores generated from the fungal spore aerosol generator at different air velocities above the fungal culture plate. We chose four typical air velocities for indoor and outdoor environments: indoor air (0.3 m/s), outdoor air (1.33 m/s, 2 m/s), and an intermediate value (0.6 m/s) [6]. The fungal spore aerosols were generated and sampled by the impactor for 3 min; the sampled fungal spores were incubated at 25°C for 24 to 48 h, and the number of colonies was enumerated. Three experimental replicates with different fungal cultures were conducted for each species. As shown in Fig. 3, the condition of the fungal cultures inserted into the fungal aerosol

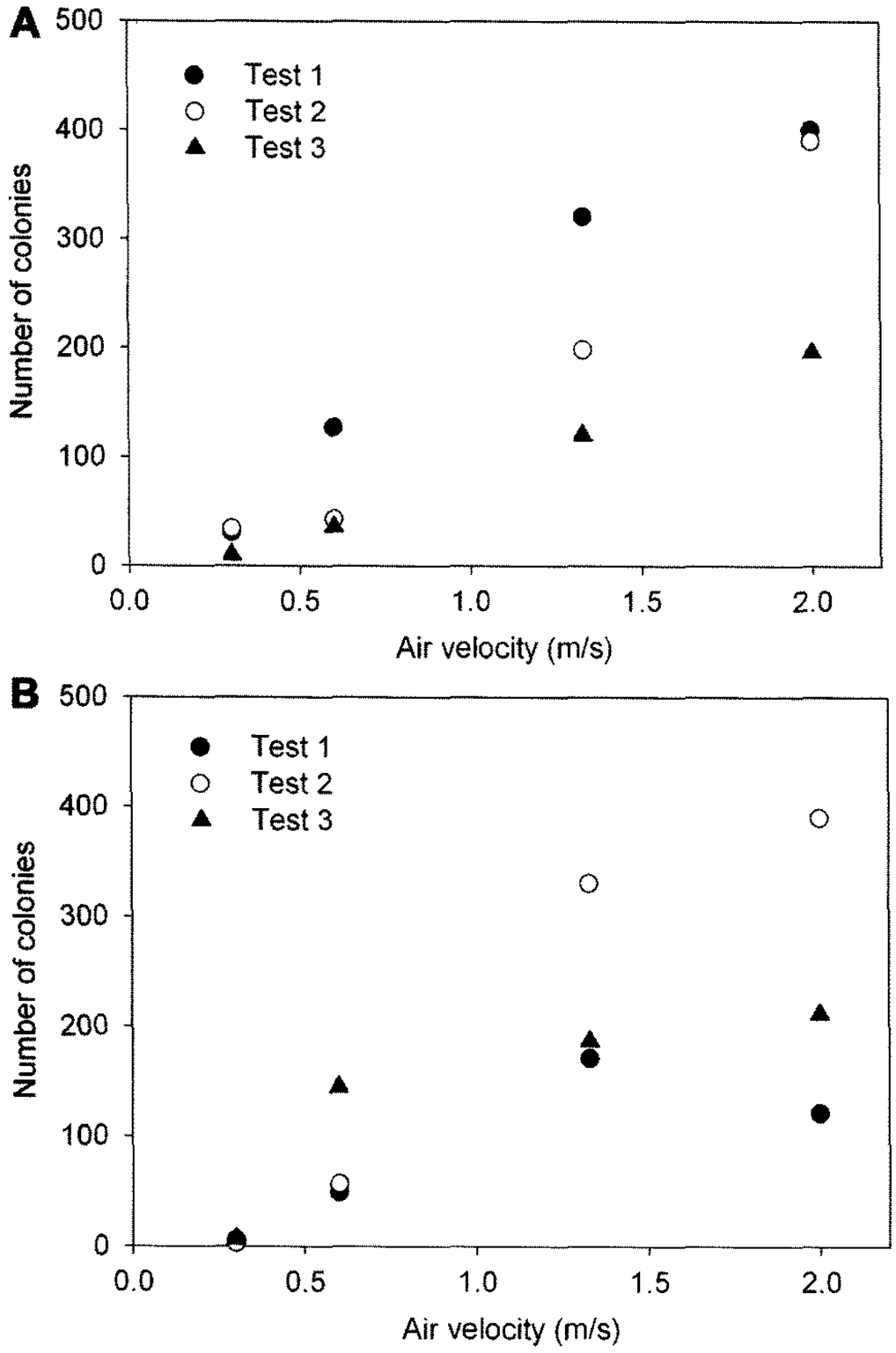

Fig. 3. Number of viable fungal spore aerosols generated by different air velocities over the fungal cultures.
A. *Cladosporium cladosporioides*, **B**. *Penicillium citrinum*. Three experimental replicates with different fungal cultures (Tests 1, 2, 3) were conducted for the same species.

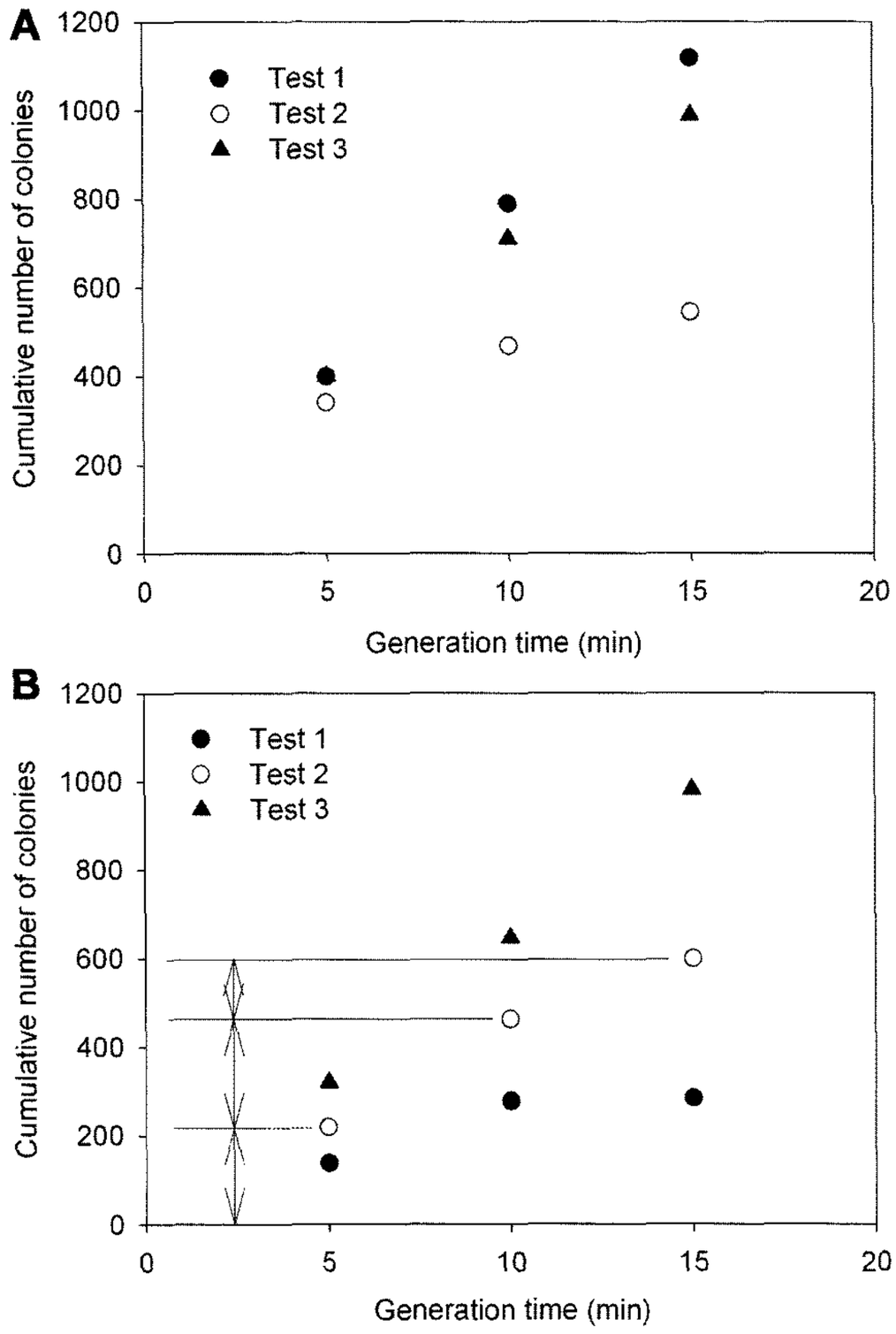

Fig. 4. Cumulative number of viable fungal spore aerosols generated during different generation periods.
A. *Cladosporium cladosporioides*, **B**. *Penicillium citrinum*. Three experimental replicates with different fungal cultures (Tests 1, 2, 3) were conducted for the same species.

generator was controlled to generate a similar number of fungal spores as expected by the experimental method. However, owing to a limitation of fungal experimentation, some deviations occurred in the number of fungal spore aerosols from each plate tested. In Fig. 3, the number of viable fungal spores generated increased as the air velocity on the fungal culture increased. This phenomenon seen in Fig. 3 can be explained by the fact that frictional force between the airflow and the fungal culture increases as the air velocity increases. For the *Cladosporium cladosporioides*, the number of fungal colonies at the sampled plates increased from the range of 10 to 30 for the indoor air velocity (0.3 m/s) to the range of 200 to 400 for the outdoor air velocity (2 m/s). For the *Penicillium citrinum*, the number of fungal colonies increased from less than 10 colonies for the indoor air velocity (0.3 m/s) to 400 colonies for the outdoor air velocity (2 m/s). Overall, the number of generated fungal spores for the outdoor air velocity (2 m/s) was more than 10 times greater than the corresponding number for the indoor air velocity (0.3 m/s). In real environments, the levels of fungal spore concentration keep changing. These experimental results, therefore, confirm that the fungal aerosol generator can be used to simulate and generate various fungal spore concentrations in fungal spore control experiments through adjustments of the air velocity on the fungal cultures.

To check the timeline for maintaining the rate at which the generator generates fungal spores, we sampled fungal spores at 5 min intervals of a 15 min operational period and enumerated the incubated colonies. In this experimentation, to find out the minimum timeline for maintaining the fungal spore generation, we experimented on the outdoor air velocity (2 m/s); as shown in Fig. 3, this condition generates the largest amount of fungal spores. Fig. 4 shows the cumulative number of sampled fungal spore colonies for a different sequence of timelines. In Fig. 4B, the lowest, the middle, and the highest intervals for the condition of test 2 refer to the number of viable fungal spores generated

during the first 5 min, the second 5 min, and the last 5 min, respectively. In most cases, the amounts of fungal spores during the first 5 min and the second 5 min reached similar levels. These results indicate that the fungal spore generator maintains the rate of spore generation for 10 min. However, the number of fungal spores generated in most cases decreased for the last 5 min, suggesting that most spores are detached during the initial 10 min, and that a smaller number of spores are detached after the initial 10 min under the present experimental condition. Hence, fungal spores can be generated homogeneously for 10 min under the present experimental condition; namely, the outdoor air velocity condition (2 m/s). The period of homogeneous generation is expected to exceed 10 min for a slower air velocity.

In summary, we have designed and manufactured an artificial fungal spore bioaerosol generator that can produce various amounts of fungal spores in an experimental system by adjusting the flow rate and operational period. However, because these experimental results are based on a fungal culture condition, the generator needs to be calibrated before being used in other culture conditions. Our fungal spore aerosol generator can be used in studies on the development of effective control methodology for airborne fungal spores.

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References

1. Bornehag, C. G., G. Blomquist, F. Gyntelberg, B. Jarvholm, P. Malmberg, L. Nordvall, A. Nielsen, G. Pershagen, and J. Sundell. 2001. Dampness in buildings and health. *Indoor Air* **11:** 72–86.
2. Cooley, J. D., W. C. Wong, C. A. Jumper, and D. C. Straus. 1998. Correlation between the prevalence of certain fungi and sick building syndrome. *Occup. Environ. Med.* **55:** 579–584.
3. Dales, R. E., S. Cakmak, R. T. Burnett, S. Judek, F. Coates, and R. J. Brook. 2000. Influence of ambient fungal spores on emergency visits for asthma to a regional children's hospital. *Am. J. Respir. Crit. Care Med.* **162:** 2087–2090.
4. Dutkiewicz, J. 1997. Bacteria and fungi in organic dust as potential health hazard. *Ann. Agric. Environ. Med.* **4:** 11–16.
5. Gorny, R. L., G. Mainelis, S. A. Grinshpun, K. Willeke, J. Dutkiewicz, and T. Reponen. 2003. Release of *Streptomyces albus* propagules from contaminated surfaces. *Environ. Res.* **91:** 45–53.
6. Gorny, R. L., T. Reponen, S. A. Grinshpun, and K. Willeke. 2001. Source strength of fungal spore aerosolization from moldy building material. *Atmos. Environ.* **35:** 4853–4862.
7. Gravesen, S. 1979. Fungi as a cause of allergic disease. *Allergy* **34:** 135–154.
8. Hinds, W. C. 1999. *Aerosol Technology*, Chapter 19. John Wiley & Sons, New York.
9. Kim, H. J., J. H. Kim, C. H. Lee, and H. J. Kwon. 2006. Gentisyl alcohol, an antioxidant from microbial metabolite, induces angiogenesis *in vitro*. *J. Microbiol. Biotechnol.* **16:** 475–479.
10. Lee, B. U., S. H. Kim, and S. S. Kim. 2002. Hygroscopic growth of *E. coli* and *B. subtilis* bioaerosols. *J. Aerosol Sci.* **33:** 1721–1723.
11. Lee, B. U. and S. S. Kim. 2003. Sampling *E. coli* and *B. subtilis* bacteria bioaerosols by new type of impactor with cooled impaction plate. *J. Aerosol Sci.* **34:** 1097–1100.
12. Lee, B. U. and Y. H. Lee. 2005. Usage of the heating tube to control *E. coli* bacteria bioaerosols. *Particle Aerosol Res.* **1:** 39–45.
13. Lee, B. U., M. Yermakov, and S. A. Grinshpun. 2005. Filtering efficiency of N95- and R95-type facepiece respirators, dust-mist facepiece respirators, and surgical masks operating in unipolarly ionized indoor air environments. *Aerosol Air Qual. Res.* **5:** 25–38.
14. Lee, B. U., M. Yermakov, and S. A. Grinshpun. 2004. Unipolar ion emission enhances respiratory protection against fine and ultrafine particles. *J. Aerosol Sci.* **35:** 1359–1368.
15. Lee, B. U., M. Yermakov, and S. A. Grinshpun. 2004. Removal of fine and ultrafine particles from indoor air environments by the unipolar ion emission. *Atmos. Environ.* **38:** 4815–4823.
16. Lee, T., S. A. Grinshpun, D. Martuzevicius, A. Adhikari, C. M. Crawford, J. Luo, and T. Reponen. 2006. Relationship between indoor and outdoor bioaerosols collected with a button inhalable aerosol sampler in urban homes. *Indoor Air.* **16:** 37–47.
17. Lee, Y. H. and B. U. Lee. 2006. Inactivation of airborne *E. coli* and *B. subtilis* bioaerosols utilizing thermal energy. *J. Microbiol. Biotechnol.* **16:** 1684–1689.
18. Lim, J. S., S. W. Park, J. W. Lee, K. K. Oh, and S. W. Kim. 2005. Immobilization of *Penicillium citrinum* by entrapping cells in calcium alginate for the production of neo-fructooligosaccharides. *J. Microbiol. Biotechnol.* **15:** 1317–1322.
19. Mullican, C. L., L. M. Buchanan, and R. K. Hoffman. 1971. Thermal inactivation of aerosolized *Bacillus subtilis* var. *niger* spores. *Appl. Microbiol.* **22:** 557–559.
20. Nicas, M. and S. L. Miller. 1999. A multi-zone model evaluation of the efficacy of upper-room air ultraviolet germicidal irradiation. *Appl. Occup. Environ. Hyg.* **14:** 317–328.
21. Noakes, C. J., L. A. Fletcher, C. B. Beggs, P. A. Sleigh, and K. G. Kerr. 2004. Development of a numerical model to simulate the biological inactivation of airborne microorganisms in the presence of ultraviolet light. *J. Aerosol Sci.* **35:** 489–507.
22. Pieckova, E. and Z. Jesenska. 1999. Microscopic fungi in dwellings and their health implications in humans. *Ann. Agric. Environ. Med.* **6:** 1–11.
23. Riley, R. L., M. Knight, and G. Middlebrook. 1976. Ultraviolet susceptibility of BCG and virulent tubercle bacilli. *Am. Rev. Respir. Dis.* **113:** 413–418.
24. Stark, P. C., H. A. Burge, L. M. Ryan, D. K. Milton, and D. R. Gold. 2003. Fungal levels in the home and lower respiratory tract illnesses in the first year of life. *Am. J. Respir. Crit. Care Med.* **168:** 232–237.
25. Wu, P. C., J. C. Tsai, F. C. Li, S. C. Lung, and H. J. Su. 2004. Increased levels of ambient fungal spores in Taiwan are associated with dust events from China. *Atmos. Environ.* **38:** 4879–4886.
26. Yeo, H. and J. Kim. 2002. SPM and fungal spores in the ambient air of west Korea during the Asian dust (Yellow sand) period. *Atmos. Environ.* **36:** 5437–5442.
27. Zhang, D., X. Li, J. S. Kang, H. D. Choi, J. H. Jung, and B. W. Son. 2007. Redoxcitrinin, a biogenetic precursor of citrinin from marine isolate of fungus *Penicillium* sp. *J. Microbiol. Biotechnol.* **17:** 865–867.