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Development of a size-selective sampler combined with an adenosine triphosphate bioluminescence assay for the rapid measurement of bioaerosols

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ABSTRACT

In this study, a size-selective bioaerosol sampler was built and combined with adenosine triphosphate (ATP) bioluminescence assay for measuring the bioaerosol concentration more rapidly and easily. The ATP bioaerosol sampler consisted of a respirable cyclone, an impactor to collect bioaerosols onto the head of a swab used for ATP bioluminescence assay, a swab holder, and a sampling pump. The collection efficiency of the impactor was tested using aerosolized sodium chloride particles and then the particle diameter corresponding to the collection efficiency of 50% (cut-off diameter) was evaluated. The experimental cut-off diameter was 0.44 μm. The correlations between ATP bioluminescence (relative light unit; RLU) from commercially available swabs (UltraSnap and SuperSnap, Hygiena, LLC, U.S.A.) and colony forming unit (CFU) were examined using *Escherichia coli* (*E. coli*) suspension and then the conversion equations from RLU to CFU were obtained. From the correlation results, the R^2 values of UltraSnap and SuperSnap were 0.53 and 0.81, respectively. The conversion equations were the linear function and the slopes of UltraSnap and SuperSnap were 633.6 and 277.78, respectively. In the lab and field tests, the ATP bioaerosol sampler and a conventional Andersen impactor were tested and the results were compared. In the lab tests, concentrations of aerosolized *E. coli* collected using the sampler were highly correlated to those from the Anderson impactor ($R^2 = 0.85$). In the field tests, the concentrations measured using the ATP bioaerosol sampler were higher than those from the Andersen impactor due to the limitations of the colony counting method. These findings confirm the feasibility of developing a sampler for rapid measurement of bioaerosol concentrations, offering a compact device for measuring exposure to bioaerosols, and an easy-to-use methodological concept for efficient air quality management.

1. Introduction

Bioaerosols are aerosol particles of biological origins (e.g., bacteria, fungi, fungal spores, pollen, fragments of biofilm, etc.) (Delort and Amato, 2017; Humbal et al., 2018). Since bioaerosols can be suspended in the air for a long period, they are ubiquitous in indoor and outdoor air (Jaenicke et al., 2007; Matthias-Maser et al., 2000). Typically, a human adult inhales about 10^4–10^5 colony forming unit (CFU) through 1 m^3 of air in the outdoor environment (Li et al., 2011; Wu et al., 2018). In some contaminated places, bioaerosols could easily exceed 10^8 CFU/m^3 (Codina et al., 2008; Toivola et al., 2002). Inhaled by a human, they would be deposited in the respiratory tract, and potentially cause irritation, allergies, contagious infectious disease, acute toxic effects, cancer, and even death if the concentrations of bioaerosols are high (Aimanianda et al., 2009; Douglas et al., 2018; Kim et al., 2018; Xu et al., 2011). For example, legionellosis, influenza, measles, and tuberculosis, are often spread by aerosols especially in poorly ventilated environments (Kundsin, 1980; Gao et al., 2016; Samake et al., 2017). Some occupational places such as composting sites, wastewater treatment plants, food industries, livestock farms, health care centers have a high concentration of bioaerosols (Delort and Amato, 2017; Gao et al., 2018; Hsiao et al., 2020; Poh et al., 2017; Walser et al., 2015). At agricultural workplaces, high concentrations (10^5–10^7 CFU/m^3) of bacteria and fungi have caused infections and allergies, even death to farmers (Delort

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and Amato, 2017; Liang et al., 2013; Samake et al., 2017). Moreover, people working in healthcare facilities are at higher risk of infections such as gastroenteritis and damaged skin and mucous membranes damages because of the spread of bacterias in the air (Bonifait et al., 2015; Iturriza-Gómara and Lopman, 2014; Pothier and Kaiser, 2014).

To protect humans and the environment from biological threats, bioaerosol concentration should be measured even though standards for acceptable levels of bioaerosols still have not been established (Walser et al., 2015). Currently, assessment of the concentrations and content of bioaerosols involves collection into culture media by samplers (e.g., impactors and impingers) and quantification via colony counting (Lindsley et al., 2017). The most common collection methods for bioaerosols are inertial impaction methods (Kuo, 2015; Yoon et al., 2010). Six-stage Andersen cascade impactors are commonly used to measure the concentration of bioaerosols by size. Each stage consists of a multi-jet nozzle plate and a Petri dish containing agar media as the impaction plate. Bacteria can be directly collected onto these agar plates, then transferred for incubation and enumeration (Lindsley et al., 2017). However, there are several difficulties in using the Andersen impactor. Its operational flow rate of 28.3 L/min is relatively high for high concentration environments, which will easily cause an overlap of bacteria on agar plates in several minutes (Lindsley et al., 2017). Moreover, the Andersen impactor requires a heavy external vacuum pump to achieve the operational flow rate. Other difficulties include limitations on maximum CFU value due to the number of impactor nozzles (e.g., 400 holes per stage for Andersen cascade impactor) and requirements for a knowledgeable selection of agar. Viable bacteria sampling is limited to short sampling times for reducing the loss of viability and may introduce significant measurement error. The inconveniences of traditional inertial impactors also involve quantifying methods. Cultivation of viable microorganisms on agar media is the most widely used technique stated formerly (Byeon et al., 2008; Yoon et al., 2010). However, it is time-consuming, and difficult to be applied for in-situ bioaerosol monitoring. After collecting bioaerosols in the sampling location, samples have to be taken back to labs and incubated for at least 24 h (Chen and Godwin, 2006; Yoon et al., 2010). Moreover, this method is only able to measure the culturable species. Taking into account the defects of colony counting after sampling, the development of a bioaerosol sampler, which could be rapid, reliable, and portable would be extremely required.

The adenosine triphosphate (ATP) bioluminescence monitor which quantifies bioaerosol by measuring the light produced through ATP's reaction with enzyme luciferase, appears more desirable (Møretrø et al., 2019; Stewart et al., 1997). The ATP bioluminescence assay is originally designed to measure the bacterial concentration on the surface without incubation. It has been increasingly studied and utilized for monitoring microorganisms in different fields (Nante et al., 2017; Lappalainen et al., 2000; Vilar et al., 2008). The efficiency of the ATP bioluminescence assay has been evaluated in many research and some of these studies are done by comparison experiments with the colony counting method (Nante et al., 2017; Chen and Godwin, 2006; Huang et al., 2015). However, most of these tests are conducted for surface hygiene other than bioaerosol. Further research on the evaluation of ATP bioluminescence for measuring bioaerosols and comparison to the culture method should be needed. In our preliminary work, a sampler collecting bioaerosols directly onto a swab head used for bioluminescence monitoring was designed and built (Kim et al., 2019). This device consists of a single-stage impactor for collecting particles between 2.5 μm and 10 μm on to swab head and a cell-lysis spray system for reducing the sampling time. However, this system is still large and a little complex to operate because of the spray system. Moreover, single bacteria or bacteria combined with particles smaller than 2.5 μm cannot be collected onto a swab head using the system.

To address these deficiencies, a size-selective bioaerosol sampler was developed and evaluated in this study. The bioaerosol sampler consisted of a respirable cyclone as a size-selective inlet, an impactor for collecting bioaerosols, a swab holder to place the test swab used for ATP bioluminometer, and a sampling pump. The protocol to calibrate the test swabs and measure the bioaerosol concentration were also developed. The bioaerosol sampler and a conventional sampler were tested in the lab and field and the results from both methods were compared.

2. Materials and methods

2.1. Design and fabrication of ATP bioaerosol sampler

The schematic of the new sampler is shown in Fig. 1. The sampler consisted of a respirable cyclone, an impactor, and a swab holder. The respirable cyclone (225-01-01, SKC, U.S.A.) was used to remove airborne particles bigger than 4 μm. The impactor was designed to collect particles onto the head of a swab inserted in the holder. Particle-laden air was drawn into the respirable cyclone and the impactor using a vacuum pump at the flow rate of 2.5 L/min.

An impactor nozzle was designed using equations from Marple and Willeke (1976) and Hinds (1999). The diameter at which half of the particles can be collected is called the cut-off diameter (d_{50}). The target d_{50} of the impactor was set to 0.5 μm to collect even a single small cell (~1 μm). The theoretical d_{50} was calculated as follows:

$$d_{50}C_c^{0.5} = \left(\frac{9\pi\mu n_j D_j (Stk_{50})}{4\rho_p Q}\right)^{0.5} \quad (1)$$

where C_c is the Cunningham correction factor, μ is the air viscosity, n_j is the nozzle number, D_j is the nozzle diameter, Stk_{50} is the Stokes number at a 50% collection efficiency, ρ_p is the particle density, Q is the flow rate. The Reynolds number (*Re*) was also calculated as follow:

$$Re = \frac{4\rho_p Q C_c d_{50}^2}{\pi\mu n_j D_j} \quad (2)$$

To control *Re* between 2000 and 3000, the values for n_j, D_j, and Q were set as 3, 550 μm, and 2.5 L/min respectively. The theoretical pressure drop (ΔP) was calculated as the following equation:

$$\Delta P = \frac{1}{2}\rho_a V_j^2 \quad (3)$$

where V_j is the jet velocity and ρ_a is the air density. The MatLab® R2017 (ver. 9.3.0, MathWorks, Inc., U.S.A.) with Simulink (ver. 9.3., MathWorks, Inc., U.S.A.) was used to calculate the equations. The theoretical *Re*, d_{50}, and ΔP were 2104, 0.527 μm, and 2 kPa, respectively. A distance between the centers of nozzles was 1.3 mm to concentrate the three-jets onto the swab head (cross-sectional diameter of 3.6 mm).

Based on these parameters, three impactor nozzles were machined on the polyether ether ketone (PEEK) plate (diameter of 25 mm) as shown in Fig. 1. Each nozzle was examined using an optical microscope.

2.2. Collection efficiency of impactor

The experimental setup used to measure the collection efficiency of the impactor is shown in Fig. 2. To determine particle collection efficiency as a function of particle size, two aerosol generation systems were used to produce particles with different size distributions. A stream of particle-free compressed air from a dry particle-free air supply system consisting of an oil filter, an air dryer, and a high-efficiency particulate air (HEPA) filter was controlled by a mass flow controller (MFC; MC-10SLPM-TFT, Alicat, U.S.A.) and delivered to a vibrating mesh nebulizer (Aeroneb Solo, Aerosgen, Ireland) or a bubbler (225-36-2, SKC, U.S.A.). Sodium chloride (NaCl) 10% solution (weight/volume) was filled in both nebulizer and bubbler. The NaCl aerosols from the nebulizer or the bubbler were passed through a diffusion dryer for removing water and a neutralizer (3088, TSI Inc., U.S.A.) for neutralizing the particles. Subsequently, the NaCl aerosols were conveyed to the sampling chamber

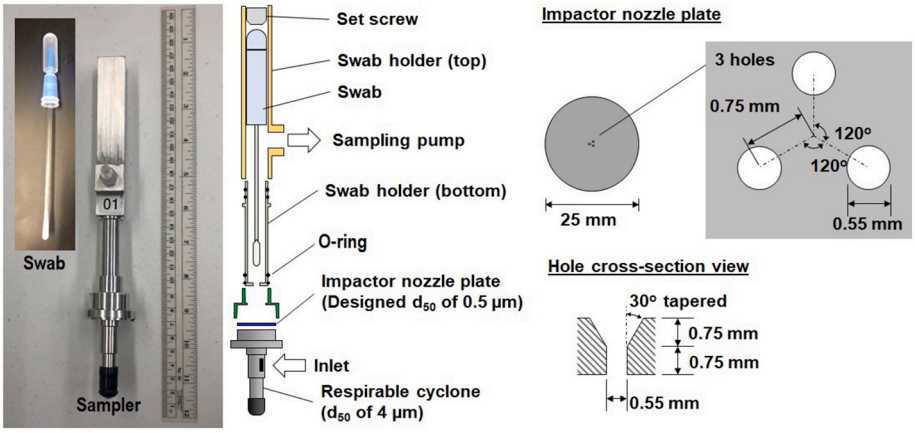

Fig. 1. Schematic of ATP bioaerosol sampler.

and were pulled through a three-way valve to passing through samplers. The sampler linked in the top line did not have the impactor nozzle plate and the swab while the other sampler in the bottom line had both of them. The respirable cyclone was not installed in the sampler during the collection efficiency test. Particle number concentrations at downstream of samplers were measured using a scanning mobility particle sizer (SMPS; 3938NL76, TSI Inc., U.S.A.) or an optical particle sizer (OPS; 3330, TSI Inc., U.S.A.). Test particles produced from the nebulizer and the bubbler were measured using the SMPS (0.05–0.8 μm) and OPS (0.5–2 μm), respectively. The SMPS consisted of a classifier controller (3082, TSI Inc., U.S.A.), a differential mobility analyzer (DMA; 3081A, TSI Inc., U.S.A.), a condensation particle counter (CPC; 3776, TSI Inc., U.S.A.), and an aerosol neutralizer (3088; TSI Inc., U.S.A.). The mobility diameter (d_m) measured using the SMPS was converted to volume equivalent diameter (d_{ve}) as following equations (Peters et al., 1993):

$$d_{ve} = d_m \times \frac{C_c(d_{ve})}{\chi \times C_c(d_m)} \tag{4}$$

where χ is the dynamic shape factor and assumed to be 1.08 for NaCl particles. The d_{ve} was then converted to aerodynamic diameter (d_{ae}) as follows:

$$d_{ae} = d_{ve} \times \sqrt{\frac{\rho_p \times C_c(d_{ve})}{\chi \times \rho_0 \times C_c(d_{ae})}} \tag{5}$$

where ρ_0 is the unit density (= 1000 kg/m^3) and ρ_p is the particle density. The ρ_p was assumed to be 2160 kg/m^3 for NaCl particles. The optical diameter measured using the OPS has been assumed to be equal to d_{ve} (Peters et al., 2006) and converted to d_{ae} using the equation (5). The collection efficiency by size was calculated by the following equation:

$$\eta_c(d_{ae}) = 1 - \frac{C_s(d_{ae})}{C_b(d_{ae})} \tag{6}$$

where C_s and C_b are the number concentration of particles passing through the samplers with and without impactor nozzle plate and the swab. The measurement occurred in the following sequence: C_{b1}-C_{s1}-C_{b2}-C_{s2}-C_{b3}-C_{s3}-C_{b4}. For the equation (6), values of $(C_{b1} + C_{b2})/2$, $(C_{b2} + C_{b3})/2$ and $(C_{b3} + C_{b4})/2$ were used and then average of three η_cs was calculated. All the tests for the collection efficiencies were performed in the same method.

A pressure drop of the impactor was measured using a differential pressure gauge (Magnehelic 2000-1000PA, Dwyer Instruments, U.S.A.).

2.3. Calibration of swabs and biolumometer with bacterial suspension

The swab calibration procedure is described in Fig. 3. Two different swabs having different sensitivity were selected and examined. A sensitive swab (SuperSnap, Hygiena, LLC, U.S.A.) and a less sensitive swab (UltraSnap, Hygiena, LLC, U.S.A.) were tested and compared.

Escherichia coli (*E. coli*, width: 0.5 μm, length: 2 μm), as a common Gram-negative bacterium, was selected to calibrate the swab used in ATP bioluminescence method. *E. coli* suspension was made from freeze-dried *E. coli* pellet (ATCC® 11775TM). *E. coli* pellet was added to 30 mL broth (Difco™ nutrient broth 23400, BD, U.S.A.) and then grown in a shaking incubator (J-NSIL-R-110, JISICO, Korea) at 37°C for 24 h. The prepared *E. coli* suspension was stored at 4°C. During the experiment, temperatures of *E. coli* suspension and nutrient broth were measured by an infrared thermometer and maintained between 20 and 25°C.

In order to examine the correlation between ATP bioluminescence and CFU, the *E. coli* numbers in test suspensions were analyzed by the two different methods: 1) ATP bioluminometer (EnSURE, Hygiena, LLC, U.S.A.) and 2) visible spectrometer (VIS 721, Yoke Instrument Co., Ltd., China). Original *E. coli* suspension was diluted by nutrient broth to separately 0–100% and then 0.01 mL of each diluted suspension was pipetted onto the swab head. Test swab was inserted into the swab tube and the luciferase enzyme was released and reacted with diluted suspension. After several seconds, the swab was placed in the ATP bioluminometer. The amount of light, as measured by the ATP bioluminometer, is expressed in relative light units (RLUs). Simultaneously, 1 mL of each diluted suspension was transferred to a cuvette to measure the optical density at a wavelength of 600 nm (OD_{600}) by the visible spectrophotometer. The *E. coli* concentration ($C_{e.coli}$) was calculated from OD_{600} by the following equation:

$$C_{e.coli} = OD_{600} \times 8 \times 10^8 \ \ \text{cells/mL} \tag{7}$$

CFU of *E. coli* was calculated from $C_{e.coli}$ and volume of suspension pipetted on the swab head. The conversion curve between RLU and CFU was obtained after calculation.

2.4. Evaluation of ATP bioaerosol sampler by comparison with andersen impactor

In the lab test, bioaerosols were collected using ATP bioaerosol samplers and a conventional sampler. The experimental setup is shown in Fig. 4. *E. coli* suspension was made as described in the previous step and then washed three times with sterile deionized water using a centrifuge (CL4M, Waverly, U.S.A.) at 6000 RPM for 15 min to remove the components of the nutrient broth. A vibrating mesh nebulizer (Aeroneb Solo System, Aerogen, Ireland) was used to aerosolize the *E. coli* in deionized water. For the collection of the aerosolized *E. coli*, two identical ATP bioaerosol samplers were placed in the sampling chamber (9 L) and connected to the personal sampling pumps (APB-926000, Libra, U.S.A.) pulling the air at the flow rate of 2.5 L/min. Two

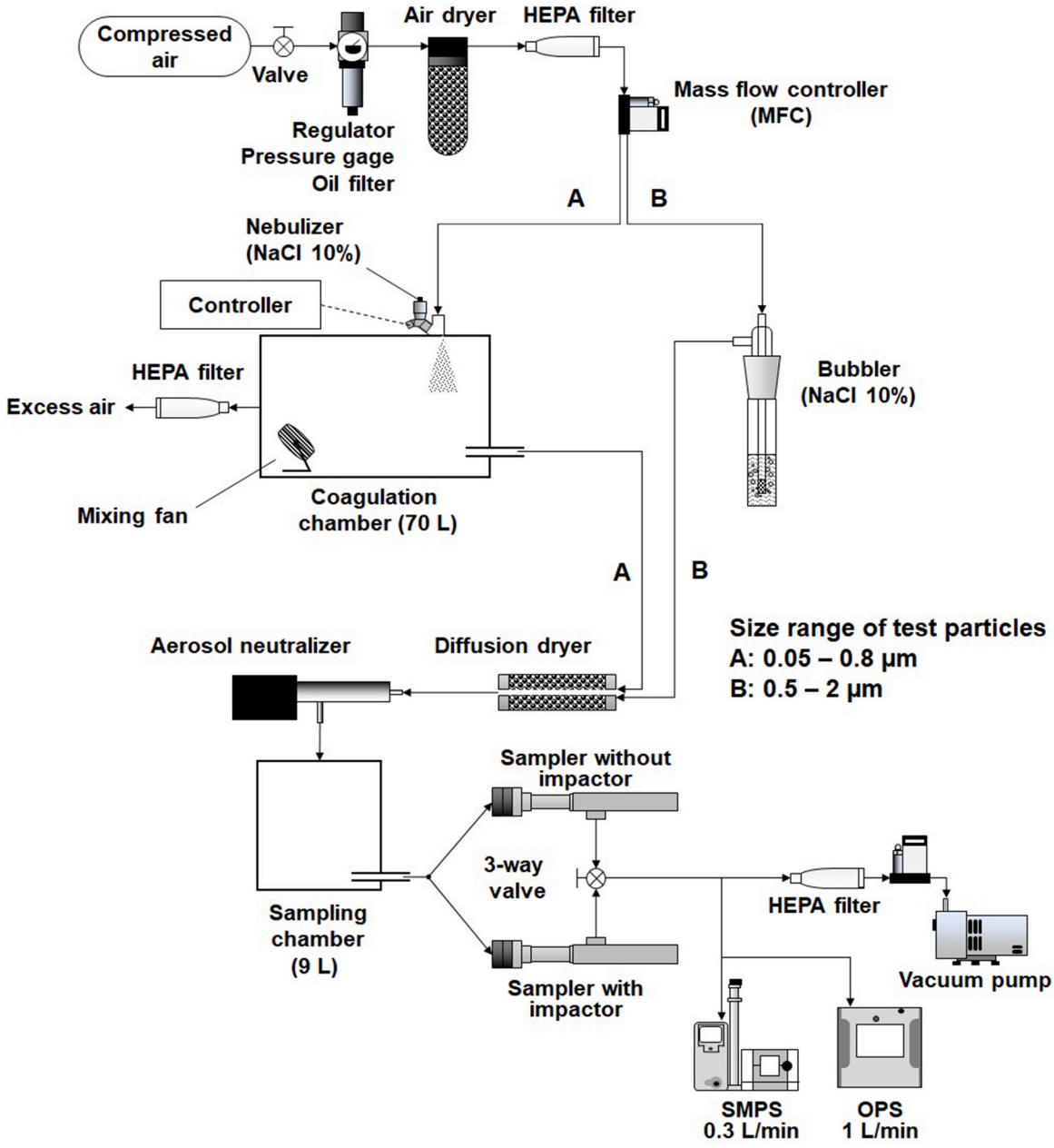

Fig. 2. Experimental setup for collection efficiency.

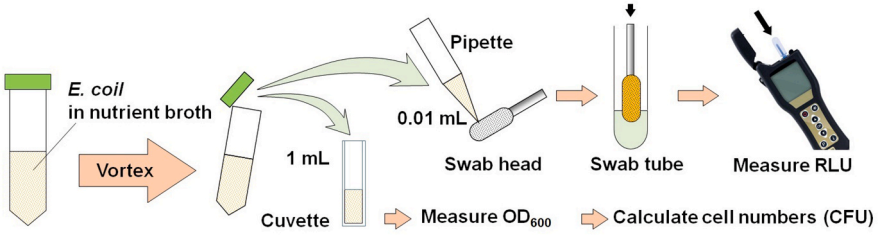

Fig. 3. Procedure for swab calibration.

different swabs, UltraSnap (Hygiena, LLC, U.S.A.) and SuperSnap (Hygiena, LLC, U.S.A.) were set in the samplers. After sampling the *E. coli* onto the swab head for 2 h, the swab was placed into the swab tube. The swab tube was shaken for several seconds after washing the swab head and mixing the collected bacteria with an ATP releasing reagent and a luminescence reagent for the light-generating reaction. The swab tube was placed in a bioluminometer (EnSURE, Hygiena, LLC, U.S.A.) to measure the RLUs from the sampled *E. coli*. RLUs were converted to CFUs using the conversion equation obtained from the swab calibration test. Since respirable cyclone was used in the ATP bioaerosol

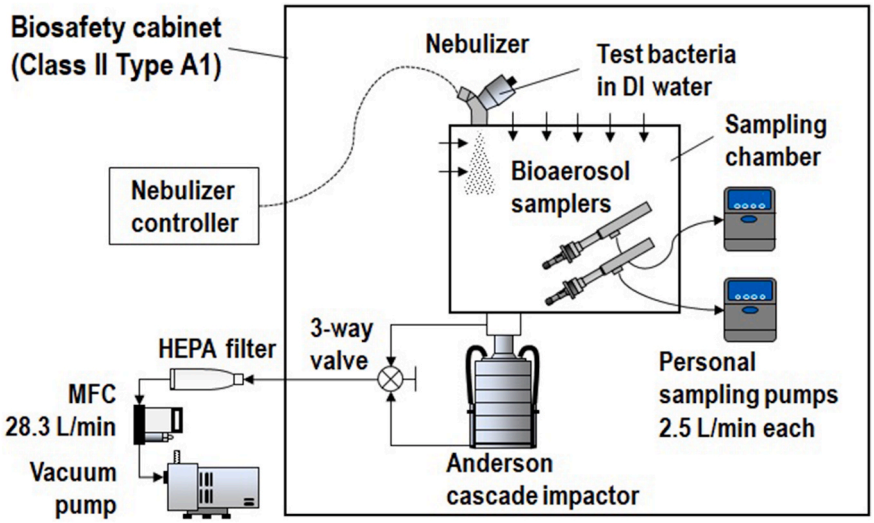

Fig. 4. Experimental setup for bioaerosol sampling in the lab.

sampler, results from the sampler were expressed in respirable CFU (CFU_{resp}) concentrations. For calculating the concentration, CFU_{resp}s were divided by sampling volume (= sampling flow rate × sampling time). The sampling flow rates of ATP bioaerosol samplers were calibrated using a volumetric airflow calibrator (BGI tetraCal®, Mesa Labs, Inc., U.S.A.) before and after sampling.

For the comparison, a six-stage Andersen cascade impactor (TE-10-800, Tisch Environmental, U.S.A.) was employed as a conventional sampler. This instrument divides the bioaerosols into six fractions, in accordance with their aerodynamic diameters, as follows: ≥ 7.0 μm (1st stage), 7.0–4.7 μm (2nd), 4.7–3.3 μm (3rd), 3.3–2.1 μm (4th), 2.1–1.1 μm (5th), and 1.1–0.65 μm (6th). A Petri dish (diameter of 100 mm) containing agar (Difco™ nutrient agar 213000, BD, U.S.A.) was utilized as the impaction substrate for each stage of the Andersen impactor. The sampling flow rate of the Andersen impactor was set to 28.3 L/min using an MFC (MCP-50SLPM-TFT-30PSIA, Alicat, U.S.A.) and calibrated using a volumetric airflow calibrator (BGI tetraCal®, Mesa Labs, Inc., U.S.A.) before and after sampling. The sampling time, incubation temperature, and incubation time were 1 min, 37°C, and 24 h, respectively. After incubation, the CFU of each stage was counted and adjusted using the following equation (Hinds, 1999):

$$CFU_{adj} = CFU_{ct} \times \left(\frac{1.075}{1.052 - \frac{CFU_{ct}}{400}} \right)^{0.483} \quad \text{for} \quad CFU_{ct} < 380 \tag{8}$$

where CFU_{adj} and CFU_{ct} are adjusted and counted CFUs, respectively. For the comparison, CFU of respirable bioaerosol was calculated as follows:

$$CFU_{resp} = \sum_{i=1}^{6} \left(CFU_{adj,i} \times F_r(d_i) \right) \tag{9}$$

where i is the stage number and F_r is the respirable fraction at midpoint size of stage i (d_i). Then, CFU_{resp} was divided by sampling volume to calculate the CFU_{resp} concentration. CFU_{resp} concentrations from ATP bioaerosol samplers and Andersen impactor were then compared.

2.5. Field application of ATP bioaerosol sampler

The sampling station consisting of two samplers and an Andersen impactor was used in field tests. Field tests were carried out at two different sites. The first sampling location was at Purdue West Lafayette campus (Indiana, U.S.A.; site A). The grassland approximately 30 m from the Engineer Fountain was chosen as the sampling spot. Fountains are a source of miscellaneous microorganisms because the water in a fountain can be easily contaminated with fecal bacteria such as *streptococci*, *E. coli*, and coliform from the activities of people and animals (birds, dogs, etc.) (Burkowska-But et al., 2013). Ambient air in site A was sampled by ATP bioaerosol samplers for 60 min and Andersen impactor for 30 min. The other sampling location was stable (in Shelbyville, Indiana, U.S.A., site B) consisting of multiple stalls (3.66 m × 3.66 m each). The stall had a half-size opening gate and a quarter-size window that allowed the horse to extend head and neck into the barn aisle. An electrical fan was installed on the window and pushing the air from aisle to stall. The sampling station was placed on the aisle floor underneath the window. A high concentration of bioaerosols could be expected because of the proximity of the sources such as the animals, their feces, feed, and litter (Wolny-Koładka et al., 2018). Ambient air in site B was sampled by ATP bioaerosol samplers for 60 min. The sampling time of the Andersen impactor was set to 3 min to prevent over deposition of bioaerosols. Similar to the lab experiments, the sampling flow rates of ATP bioaerosols samplers (2.5 L/min) and Andersen impactor (28.3 L/min) were calibrated using a volumetric airflow calibrator (BGI tetraCal®, Mesa Labs, Inc., U.S.A.) before and after sampling.

After sampling, the swab in ATP bioaerosol sampler was immediately inserted into the swab tube and examined using the bioluminometer by the same method used in lab tests. Each Petri dish in the Andersen impactor was sealed with parafilm tape (PARAFILM® M, Bemis, U.S.A.) and taken back to the lab to incubate at 37°C for 24 h. Three field blanks were prepared during sampling and the average blank value was subtracted before calculating equation (8) and RLU-CFU conversion.

2.6. Statistical analysis

The average and standard deviation of three collection efficiencies calculated from equation (6) were computed and then plotted (section 2.2.). The correlations between RLU and CFU (section 2.3.) and between CFU_{resp}s from ATP bioaerosol samplers and Andersen impactor (section 2.4.) were investigated using linear regression models. The regression coefficients, standard errors, and 95% confidence interval (CI) were calculated using a Stata (ver. 13.1, StataCorp, U.S.A.). Values of $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Collection efficiency of impactor

The collection efficiency results were presented in Fig. 5. The particle sizes corresponding to the 50% collection efficiency were interpolated from the collection efficiency data as the experimental d_{50}, which was 0.44 μm. The experimental d_{50} was in good agreement with the theoretical d_{50} (0.527 μm). The experimental d_{50} was 16.5% smaller than the theoretical d_{50}. The pressure drop of the fabricated impactor was 2.876 kPa and higher than the designed one. A smaller nozzle diameter than the designed size could create faster jet speed and result in a decrease of d_{50}. Since bioaerosols are generally larger than 1 μm in diameter and the primary concern is the respirable-size (<10 μm) (Löndahl, 2014), the fabricated impactor is capable of collecting the majority of bioaerosols.

3.2. Calibration and conversion plots of test swabs

The swab calibration results were shown in Fig. 6. RLU values from SuperSnap were proportional to CFU values when the *E. coli* numbers are smaller than 2.2×10^6 CFU. When the *E. coli* number increased from 2.2×10^6 to 5.2×10^6 CFU, RLU values from SuperSnap were not increased and kept about 8000 RLU. This means that 2.2×10^6 CFU of *E.coli* is the maximum limit measured using the SuperSnap. UltrsSnap shows similar linearity when the *E. coli* numbers are smaller than 3.3×10^6 CFU. UltraSnap shows a larger maximum measurable limit than SuperSnap. Results in linear sections of both test swabs were reorganized as a conversion plot in Fig. 6(b). The results of linear regression analysis are summarized in Table 1. SuperSnap ($R^2 = 0.81$) shows better linear regression than UltraSnap ($R^2 = 0.53$). The slope value of SuperSnap (633.60) and larger than one of UltraSnap (277.78) which means SuperSnap is 2.3 times more sensitive than UltraSnap for *E. coli*. The slope also means a minimum resolution of the swab. For example, one RLU measured using the SuperSnap is equivalent to 277.78 CFU of *E. coli*. Since the occupational bioaerosol concentrations are often higher than environmental bioaerosol concentrations, the UltraSnap could be used for measuring bioaerosols in occupation settings and the SuperSnap could be used in both environmental and occupational settings.

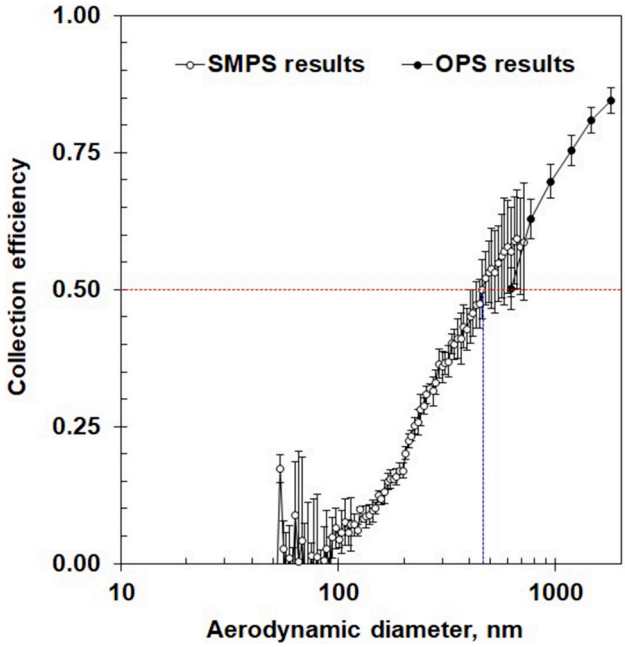

Fig. 5. Collection efficiency of three-jet impactor.

3.3. Comparison of ATP bioaerosol sampler and andersen impactor using the aerosolized E. coli in the lab

The CFU_{resp} concentrations measured using the ATP bioaerosol samplers and the Andersen impactor are shown in Fig. 7. Most colonies were found in the 1st and 6th stages of the Andersen impactor in every case. Aerosolized test *E. coli* were directly settled onto the 1st stage plate through the open inlet of the Andersen impactor and other aerosolized *E. coli* were collected onto the 6th stage plate because the aerodynamic diameter of single *E. coli* is about 1 μm and larger than d_{50} of 6th stage. The CFU value from the 1st stage did not significantly affect the results since the respirable fraction at the d_i of the 1st stage is about 0.002 (Equation (9)). The CFU_{resp} concentrations measured using SuperSnap and UltraSnap were proportional to those measured using the Andersen impactor. The results of linear regression analysis are documented in Table 1. The R^2 of SuperSnap (0.85) was higher than that of UltraSnap (0.74). However, the slopes of both linear regressions were slightly larger than 1 (Fig. 7). These deviations were presumably attributed to the sampling limitations of Andersen impactor such as desiccation stress and overloading problems (Stewar et al., 1995; Willeke et al., 1995; Xu et al., 2013). Specifically, the impaction process might decrease the viability of bacteria due to impact damage and desiccation (Haig et al., 2016). The conditions of the agar plate could also affect the culturability of collected bacteria (Therkorn and Mainelis, 2013).

3.4. Results of field applications

The results for field tests are documented in Table 2. SuperSnap shows higher respirable RLU (RLU_{resp}) concentrations than UltraSnap in both sampling locations. The RLU_{resp} is proportional to ATP concentration from the sampled bacteria. Therefore, the RLU_{resp} can be expressed in ATP concentration from the CFU_{resp} of *E. coli*. The RLU_{resp} concentrations were converted to CFU_{resp} concentrations using the conversion plot (Fig. 6(b)). In site A, CFU_{resp} concentrations measured using UltraSnap and SuperSnap were 106,290 ± 53,075 CFU_{resp}/m^3 and 95,749 ± 31,766 CFU_{resp}/m^3, respectively, which were higher than the result measured using Andersen impactor (33 ± 27 CFU_{resp}/m^3). In site B, Andersen impactor also underestimated the concentration of bacteria compared to both UntraSnap and SuperSnap results. The main reason could be that ambient bioaerosols including fungi and plant cells contain more ATP than *E. coli* (Bajerski et al., 2018). Another reason could be the limitations of Andersen impactor already explained in the previous section. The Andersen impactor could underestimate the concentrations because of the low viability and cultivability of sampled bacteria. The CFU_{resp} concentrations of bioaerosols sampled in the site B were similar to those in site A while the CFU_{resp} concentration in the site B measured by Andersen impactor was 13.4 times higher than one in site A. The sampling time (30 min) for site A was longer than the sampling time (3 min) for site B. Longer sampling time can decrease the viability of sampled bacteria. The type of agar plate also can make a difference in results. Site B has more various bioaerosols including the vast majority of different bacteria, endotoxin, viruses, parasites, fungi, mycotoxin, insect parts (Millner, 2009). Stables also pose a high level of culturable microorganisms (Samadi et al., 2009). Moreover, the stable environment promotes the proliferation and growth of these microorganisms by a humid and warm microclimate.

The CFU_{resp} concentrations of site B measured using the UltraSnap and SuperSnap were similar to those of site A which means that the ATP concentrations extracted from respirable bioaerosols sampled in both sites A and B are similar. The site B result of SuperSnap was 50% higher than the one of UltraSnap. One possible reason could be that SuperSnap has better and more chemicals to extract ATP from the bacteria in site B.

3.5. Implications and limitations

In our previous study, a sampler consisting of a single-stage impactor

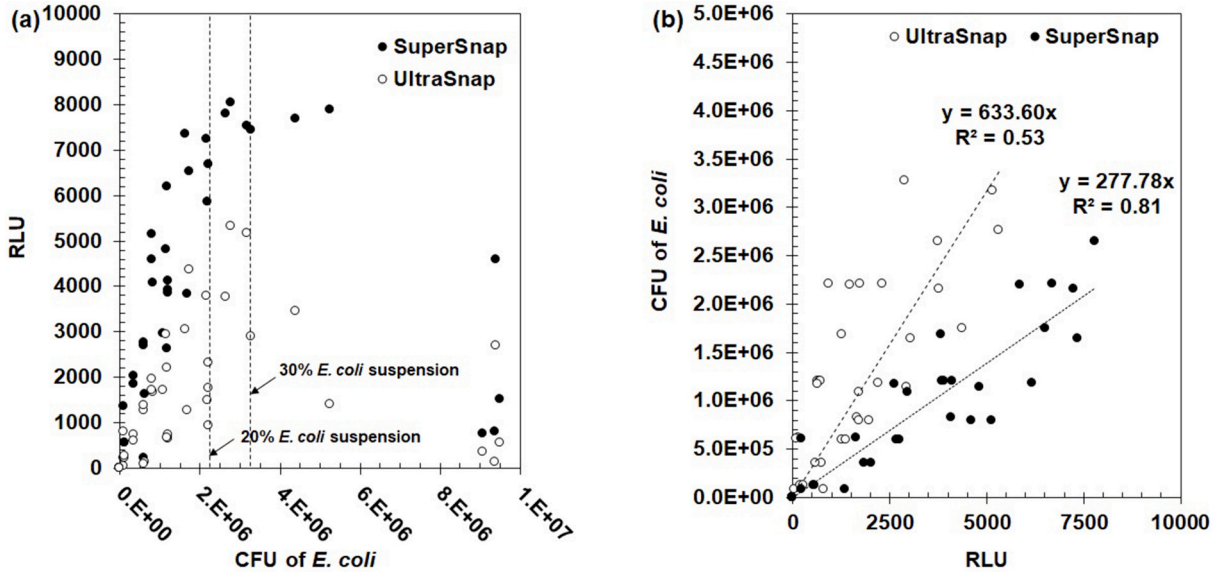

Fig. 6. (a) Swab calibration curve, (b) RLU-CFU conversion plot (The x and y axes are swapped; RLU: relative light unit; CFU: colony forming unit).

Table 1
Results of linear regression analysis for conversion plots and comparison tests in the lab.

Conversion plot (Fig. 6(b))			
Variable	Coefficient	Standard errors	95% Confidence interval
UltraSnap	633.60	50.77	530.42, 736.78
SuperSnap	277.78	14.40	248.32, 307.24
Comparison of ATP bioaerosol sampler and Andersen impactor in the lab (Fig. 7)			
Variable	Coefficient	Standard errors	95% Confidence interval
UltraSnap	1.14	0.14	0.83, 1.46
SuperSnap	1.08	0.10	0.86, 1.30

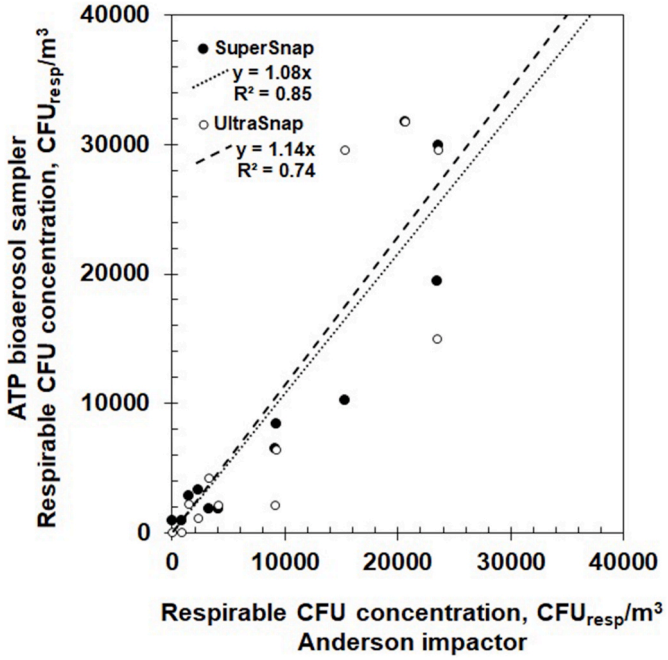

Fig. 7. Comparison of ATP bioaerosol sampler and Andersen impactor u sing the aerosolized *E. coli*.

for collecting particles between 2.5 μm and 10 μm on to swab head and a cell-lysis spray system for reducing the sampling time was introduced (Kim et al., 2019). In this study, an improved sampler was introduced. Specifically, the new ATP bioaerosol sampler utilized the impactor having smaller d_{50} and collecting even a single cell. A commercially available respirable cyclone was also utilized as a size-selective inlet. The respirable cyclone can be replaced with PM2.5 or PM10 impactor for environmental studies. The ATP bioaerosol sampler was designed for operating with a personal sampling pump that is also commercially available. This allows the sampler to be used for both personal samplings to assess individual exposures and area samplings to assess the background level of bioaerosols. The prototype sampler was made of aluminum and PEEK. The size and weight of the sampler can be reduced by the use of disposable plastics.

Currently, many researchers are developing and evaluating the ATP bioluminescence method for measuring bioaerosol concentrations. However, RLU values can not be directly compared among different swabs and bioluminometers since companies have defined their own RLU value from ATP concentration. There is no standard method to calibrate the swabs, interpret the RLU values among different luminometers and swabs, and convert RLUs to CFUs. To address this problem, a detailed method to calibrate the swabs using *E. coli* was introduced in this research. The calibration curve was also obtained. This calibration curve would be a reliable reference for researchers who want to know the contamination level from the RLUs. Using the calibration curve, RLUs can be defined as CFU of *E. coli* having the same amount of ATP. The sensitivities of different bioluminometers and swabs can be also compared easily using the calibration method.

Two commercially available swabs, the UltraSnap and SuperSnap were verified and compared. Since the SuperSnap showed better resolution than the UltraSnap, the SuperSnap can be used for both environmental and occupational samplings. The UltraSnap showed relatively low resolution but it can be still used for occupational sampling since occupational bioaerosol concentrations are often higher than environmental bioaerosol concentrations. The maximum detection limits of UltraSnap and SuperSnap were also evaluated. The 3.3×10^6 CFU and 2.2×10^6 CFU of *E. coli* are the maximum detection limit for UltrsSnap and SuperSnap, respectively (Fig. 6(a)). When the sampling time is assumed to be 1 h, measurable maximum concentrations for UltraSnap and SuperSnap are 2.2×10^7 CFU/m^3 and 1.67×10^7 CFU/m^3, respectively. When the bioaerosol concentration is higher than the maximum limits, sampling time should be shorter than 1 h. These

Table 2
Respirable concentrations of airborne bacteria sampled in field sites.

	ATP BIOAEROSOL SAMPLER WITH ULTRASNAP		ATP BIOAEROSOL SAMPLER WITH SUPERSNAP		ANDERSEN IMPACTOR
SAMPLING LOCATION	RLU_{resp}/m^3	CFU_{resp}/m^3[a]	RLU_{resp}/m^3	CFU_{resp}/m^3[a]	CFU_{resp}/m^3
SITE A (CAMPUS)	168 ± 84	106,290 ± 53,075	345 ± 114	95,749 ± 31,766	33 ± 27
SITE B (STABLE)	173 ± 105	109,824 ± 66,385	660 ± 240	168,284 ± 66,660	443 ± 168

[a] *E. coli* equivalent CFU_{resp}: RLUs were converted to CFUs using the conversion equations in Fig. 6(b).

measurable maximum concentrations are still much larger than the limits of the Andersen impactor. This deficiency of Andersen impactor was already discussed by other studies (Lindsley et al., 2017). The Andersen impactor can collect a maximum of 400 particles per each stage in ideal conditions. After applying the adjustment equation, the maximum CFU of each stage was calculated as 380 CFU (equation (8)). If the bioaerosol is monodisperse and the concentration is 1×10^5 CFU/m^3, sampling time has to be shorter than 8.06 s to avoid overload. If the sampling time is 1 min, the Andersen impactor can measure a maximum of 13,428 CFU/m^3. Consequently, the ATP bioluminescence method can measure the wider range of bioaerosol concentration than the Andersen impactor.

A limitation of this study would be sampling loss due to particle bounce on the impaction substrate of both ATP bioaerosol sampler and Andersen impactor. As shown in Fig. 5, particle collection efficiency was approximately 0.75 at 1 μm and increased by increasing size. The collection efficiency curve is less stiff than those of conventional impactors. The impaction substrate of the ATP bioaerosol sampler was a swab head made of cotton fibers instead of a solid plate coated with grease. Moreover, the collection efficiency was evaluated using NaCl particles which may cause the underestimation of collection efficiency since dried NaCl particles could bounce more than *E. coli* droplets or ambient bioaerosols. Even though the sampling loss was not significantly observed in both lab and field tests, better impaction substrates (e.g., porous plastic or metal foam) should be developed in future research.

4. Conclusions

The ATP bioaerosol sampler was developed and evaluated. The three-jet impactor was fabricated and tested with NaCl particles. The experimental d_{50} was 0.44 μm which indicated that the developed ATP bioaerosol sampler can collect bioaerosols since the majority of bioaerosol size is bigger than d_{50}. RLU values of two different swabs were defined with CFU values of *E. coli*. Their maximum measurable limits were also evaluated. In comparison with Andersen impactor, the ATP bioaerosol sampler was able to overcome the limitations of Andersen impactor. Specifically, the ATP bioaerosol sampler has a wider range of measurable concentrations of bioaerosols and reduces measurement time. The detailed calibration and conversion methods were provided which allows us to define RLU values based on CFU of *E. coli* and compare RLU values among different swabs and bioluminometers. The developed ATP bioaerosol sampler was proved to use in various sampling conditions, for example, personal and area samplings in both environmental and occupational settings. Further researches will be focused on applications of the sampler in other various fields.

Author contributions

Li Liao, Data curation, Formal analysis, Instrumentation, Methodology, Software, Validation, Roles/Writing - original draft, Writing – review & editing. Jeong Hoon Byeon, Conceptualization, Funding acquisition, Fabrication, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. Jae Hong Park, Conceptualization, Funding acquisition, Fabrication, Investigation, Methodology, Software, Project administration, Resources, Supervision, Roles/Writing - original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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