Sensors and Actuators B 260 (2018) 274–281

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

The development of paper discs immobilized with luciferase/D-luciferin for the detection of ATP from airborne bacteria

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ARTICLE INFO

Article history:
Received 10 August 2017
Received in revised form
15 December 2017
Accepted 2 January 2018
Available online 3 January 2018

Keywords:
Immobilization
Luciferase
D-luciferin
Paper disc
Airborne bacteria
ATP

ABSTRACT

The presence of various microbes in the air is one of the main causes of respiratory diseases or ailments affecting the health of humans and livestock. Therefore, early identification and detection of microorganisms in the air are key to preventing the risk associated with microbial infection. In this study, we prepared paper discs (diameter = 0.5 cm) immobilized with luciferase/D-luciferin and found that these discs could be used to determine the adenosine triphosphate (ATP) directly from the heat-lysates of airborne bacteria. The repetition of sequential adsorption/drying of the mixture solution (luciferase and D-luciferin) was performed using paper disc. The storage stability of the paper discs at room temperature was maintained for one month following their preparation while the storage stability of the liquid-based ATP assay not maintained even for one day. The paper discs could detect ATP extracted from aerosolized *Escherichia coli* (*E. coli*) as low as 1.17×10^3 CFU/mL in pure bacteria samples or 2.32×10^3 CFU/mL in bacteria samples containing dust (1 mg/mL). ATP evaluation using the paper discs for detection of aerosolized bacteria may reduce the detection time to be less than 7 min after sampling. Novel paper discs immobilized with luciferase/D-luciferin will be valuable for the development of fast and sensitive sensors for early detection and enumeration of airborne microorganisms without preparation of enzyme solution for ATP assay.

1. Introduction

Airborne microorganisms including bacteria, fungi, and viruses are a major threat to public health and frequently cause public panic due to the transmission of pathogens through the air without a barrier [1,2]. Our society remains vulnerable and defenseless against unexpected wide-spreading of airborne pathogens. Bacterial infection or viral flus transmission via the air are serious threats worldwide. Due to their small sizes, they can easily be transported long distances (up to several kilometers) by the wind and can enter the lungs via inhalation, which can cause significant public health concerns non-infectious diseases such as hypersensitivity pneumonitis [3], allergies [4], and asthma [5], and infectious diseases such as legionellosis, influenza [6] and sputum-positive tuberculosis [7]. Therefore, rapid detection or identification methods of airborne pathogens are important. Currently, there are a number of conventional methods for the detection and identification of airborne microbes in order to regulate and control the air quality, including culture-based methods [8–10], microscopy [11–14], immunoassay [15–17], and polymerase chain reaction (PCR) [18–20]. However, the real-time detection of airborne bacteria remains challenging due to experimental complexity and costs [1]. Conventionally, the suggested regulation to identifying airborne microbes is the culture based method; however, it takes several days to grow the bacteria in culture medium until colony formation [21,22]. Furthermore, with this method, only a small proportion of microorganisms [2,23], about less than 10%, can be culturable and the remained proportion is not culturable [24]. Conversely, the considerable detection methods of airborne microorganisms to identify the exact species use highly specific

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https://doi.org/10.1016/j.snb.2018.01.009
0925-4005/

antibodies or DNA or RNA gene probes for PCR [25,26]; however, this method requires several hours of preparation, complicated procedures, and operation of experts [1]. Additionally, direct methods have been used that detect the microorganisms based on the adenosine triphosphate (ATP)-driven luciferase/D-luciferin photoemission with high sensitivity and specificity of ATP [21,27]. In luciferase/D-luciferin photoemission, D-luciferin reacted with ATP in the presence of luciferase as a catalyst, molecular oxygen, and Mg^{2+} ions to produce oxyluciferin, adenosine monophosphate, and light. The relaxation of the electronically excited oxyluciferin to the ground state resulted in the emission of yellow-green light in the wavelength range of 550–570 nm, which was normally measured using a luminometer [28,29]. However, this biochemical method has several limitations owing to the activity of luciferase, which significantly reduced with increasing time in the solution phase, and the requirement of several hours to prepare the luciferase/D-luciferin solution. Numerous bacterial detection methods have been previously suggested; however, real-time airborne bacterial detection and enumeration from the air remains challenging.

In this study, we suggest techniques capable of rapid detection and enumeration of airborne bacteria using paper discs immobilized with a mixture of luciferase/D-luciferin (co-immobilization). The most commercially available ATP assay kits use the liquid-based reaction mixture and the essential enzyme, luciferase, should be kept in cold (−20 °C to 4 °C) before using it for maintenance of enzyme activity. These conditions are the bottleneck to use the ATP assay kit for a field test. Our approach can reduce the detection time using ready-made paper discs for the luciferase/D-luciferin reaction, wherein the activity of luciferase enzyme is well maintained. Following the immobilization process, paper discs are stored stably at room temperature and used directly without any pretreatment rather than solution-based reaction. They are ready-to-use for the heat-lysis of samples collected from the air. We optimized the immobilization procedure for the mixture of luciferase and D-luciferin using paper discs. We also investigated storage stability of the paper discs immobilized and the appropriate lysis methods for the reaction with the paper discs. Using Collison nebulizer, the present study simulated airborne bacteria status of *Escherichia coli* (*E. coli*) as a model case, and determined the detectable concentration of *E. coli* using the paper discs immobilized with luciferase/D-luciferin.

2. Material and methods

2.1. Reagents, instruments, chemicals, and bacterial cells

We used luciferase, D-luciferin, dilution buffer, lysis buffer, and ATP standard directly, which were included in the "Roche ATP Bioluminescence Assay Kit HSII" (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The luciferase/D-luciferin mixture was prepared from the kit components by adding 2.5 mL of supported dilution buffer into a luciferase vial which contains D-luciferin. The luciferase/D-luciferin solution which contains the traces of Mg^{2+} was then aliquoted to 200 μL each and frozen at −20 °C in a refrigerator until use. ATP standard solution (6.5 mM) was also prepared using dilution buffer supported by the kit. ATP dilutions were made by sequential dilution of ATP standard solution using dilution buffer, which was supplied in the kit. Whatman cellulose filter paper (Grade 5, thickness: 200 μm, pore size: 2.5 μm, diameter: 70 mm) was purchased from the Sigma-Aldrich (Merck KGaA). It was cut into small disc-shapes with 0.5 cm diameters that fit the wells of the Corning® 96 well-plate, which was used to evaluate bioluminescence intensity in the luminometer (spectramax-M2, Molecular Devices Corp., Sunnyvale, CA, USA). Dust standard powder, A2 fine test dust ISO 12103-1, was purchased from powder technology INC (Arden Hills, MN, USA). *E. coli* (KCTC 2571) was purchased from the Korean Collection for Type Culture (KCTC, Jeongeup, Korea). Nutrient broth and nutrient agar were purchased from Becton & Dickinson, Co. (Franklin Lakes, NJ, USA).

2.2. Cultivation of bacterial cells

E. coli is a model bacterium used in the present study. For the pre-cultivation of *E. coli* cells, the colony of *E. coli* in nutrient agar was collected and then grown in nutrient broth medium (5.0 g of peptone and 3.0 g of beef extract in 1 L of distilled water) overnight at 37 °C with agitation (150 rpm). The *E. coli* cells were subsequently cultured in nutrient broth medium with a 1:100 inoculum-to-medium ratio at the same conditions. The cultured *E. coli* cells (~10^8 CFU/mL) were recovered by centrifugation at 13,000 rpm for 10 min, washed three times with deionized water (DI water), and suspended again with DI water or DI water supplemented dust for the lysis step.

2.3. Optimization of the lysis process

To identify a straightforward and efficient cell lysis method, we tested four different methods: 1) Incubating in lysis buffer at room temperature (no agitation or heating); 2) agitation in lysis buffer at room temperature; 3) heating (95 °C) in lysis buffer (no agitation); 4) heating (95 °C)-only (in DI water with no agitation). All the *E. coli* samples (~10^8 CFU/mL) were washed with DI water and resuspended with DI water prior to lysis. For the incubation or agitation in lysis buffer method, 50 μL of *E. coli* suspension with DI water was mixed with 50 μL lysis buffer supplied from the kit, and then the mixture was incubated or agitated (1400 rpm in the thermomixer (Eppendorf, Hamburg, Germany) for 10 min at room temperature. For the heating (95 °C) in lysis buffer method, 50 μL *E. coli* suspension with DI water was mixed with 50 μL of lysis buffer supplied by the kit, and then the mixture was heated in a heating block for 10 min at 95 °C (Wise Therm, Daihan, Seoul, Korea) without agitation. The heating (95 °C)-only method was performed for 10 min at 95 °C using the heating block with 50 μL of *E. coli* suspension with DI water was mixed with 50 μL DI water. The lysis samples in this step were reacted with luciferase/D-luciferin cocktails solution. The bioluminescence was determined according to the "Roche ATP Bioluminescence Assay Kit HSII" manufacturer's instructions. For the further heating (95 °C)-only method, heating was performed from 0 to 10 min to determine the efficiency of the heating time.

2.4. Immobilization of luciferase/D-luciferin in paper discs

The filter paper was cut into disc-shapes with 0.5 cm diameters and then autoclaved. The mixture of luciferase/D-luciferin with reaction buffer was prepared as dissolving one vial of lyophilized luciferase with 2.5 mL of reaction buffer that contains D-luciferin and other ingredients for the reaction in the kit. Subsequently, the mixture of luciferase/D-luciferin solution (10 μL) from the kit was dropped directly onto the paper disc. Following dropping the mixture solution, the paper discs were dried 2 h in a drying box containing silica gel. This drop/dry process was repeated several times sequentially to optimize the number of drops for the immobilization of luciferase/D-luciferin (Fig. 1). Finally, the immobilized paper discs were dried additionally for one day to remove any residual liquid entirely in the drying box at room temperature. The paper discs immobilized with luciferase/D-luciferin were stored in a dry box containing silica gel at room temperature until further use.

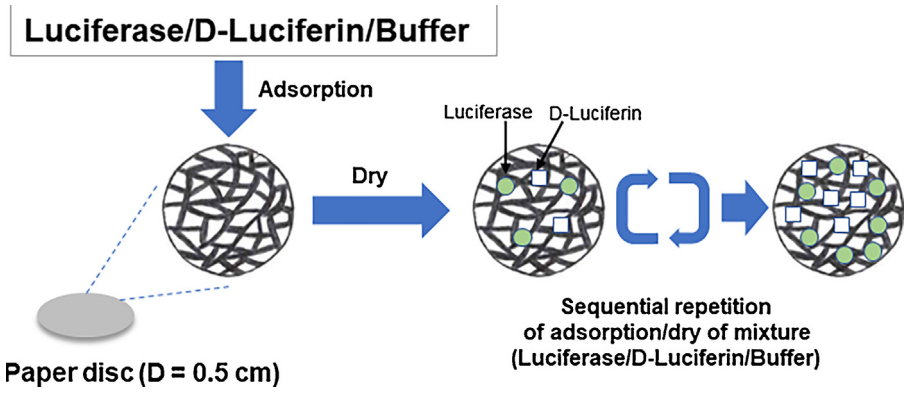

Fig. 1. The schematic procedure of luciferase/D-luciferin immobilization in paper disc.

2.5. Evaluation of bioluminescence using the paper discs immobilized with luciferase/D-luciferin

The paper discs immobilized with luciferase/D-luciferin were placed on the bottom of the 96-well plate to determine the bioluminescence intensity. The *E. coli* cells were cultivated up to ~10^8 CFU/mL in a nutrient medium. Subsequently, cell cultures were washed and resuspended using DI water or DI water supplemented with dust to the desired cell concentration. The resuspended cell stocks (100 μL) were then heated, and final 50 μL heat lysis samples were added to the well containing the paper discs immobilized with luciferase/D-luciferin. The bioluminescence of each well was evaluated directly following the addition of lysis samples to the 96-well plate, using a luminometer within around 5 min. The bioluminescence (BL) intensity is the maximum intensity during the measurement as time goes on. For the storage stability investigation, the paper discs immobilized with luciferase/D-luciferin were prepared at the same time and stored in a dry box containing silica gel at room temperature. The paper discs were removed from the dry box to determine the bioluminescence intensity of the ATP solution or *E. coli* sample after 1, 5, 7, 9, 11, 15, 19, 25, 30, 40, 50, and 60 days of storage. For the investigation of dust effect during lysis, *E. coli* samples containing various concentrations of dust were also lysed, and their bioluminescence (BL) intensities were evaluated. Washed *E. coli* samples were refilled using 100 μL of the various concentrations of dust solutions (0, 0.01, 0.02, 0.1, 0.2, 1, 2, 10, and 20 mg/mL in DI water). For these cases, all samples were lysed at 95 °C for 10 min, and then the bioluminescence intensity was determined using paper discs immobilized with luciferase/D-luciferin.

2.6. Aerosolization of bacterial samples

To generate the airborne status of *E. coli*, we prepared bacterial samples that were aerosolized through the Collison nebulizer (Fig. 2). *E. coli* cells were grown up to ~10^8 CFU/mL in nutrient broth with a total volume of 200 mL. Subsequently, the *E. coli* cells were washed with DI water three times to remove all nutrient broth. Finally, *E. coli* was suspended in 40 mL of DI water and then added to a Collison nebulizer bottle (Fig. 2). *E. coli* samples were also aerosolized with DI water supplemented with dust where *E. coli* cells suspensions were refilled with 40 mL of DI water containing dust (1 mg/mL) and poured into a Collison nebulizer bottle. The airborne *E. coli* cells were prepared by aerosolizing from a Collison nebulizer bottle through the clean airflow (12.5 L/min). Following 2 h of the aerosolization, airborne *E. coli* was captured in the biosampler bottle containing 20 mL of DI water. The *E. coli* solution obtained (20 mL) was concentrated by centrifugation and resuspended in 2 mL of DI water. The number of *E. coli* cells from the captured-solution was determined by the plate counting method, and subsequently, 100 μL of concentrated *E. coli* samples from 20 mL were heated at 95 °C for the lysis using a heating block. Following 10 min of lysis, 50 μL of the lysed samples were dropped on the paper discs immobilized with luciferase/D-luciferin to evaluate the bioluminescence intensity.

3. Results and discussion

3.1. Optimization of lysis method

To prepare the lysates of airborne bacteria, we aimed to identify the most convenient lysis method for the samples captured from the air, considering the automated bioaerosol monitoring system, which is our final goal (not in this study). Lysis is the key contributor for the successful detection of ATP extracted from the bacteria captured from the air, and the method should be simple with a high efficiency. The lysis process starts to break down the cellular membrane of bacterial cells and extracts ATP, which is an initiator of the oxidization of D-luciferin to oxyluciferin via a biocatalytic reac-

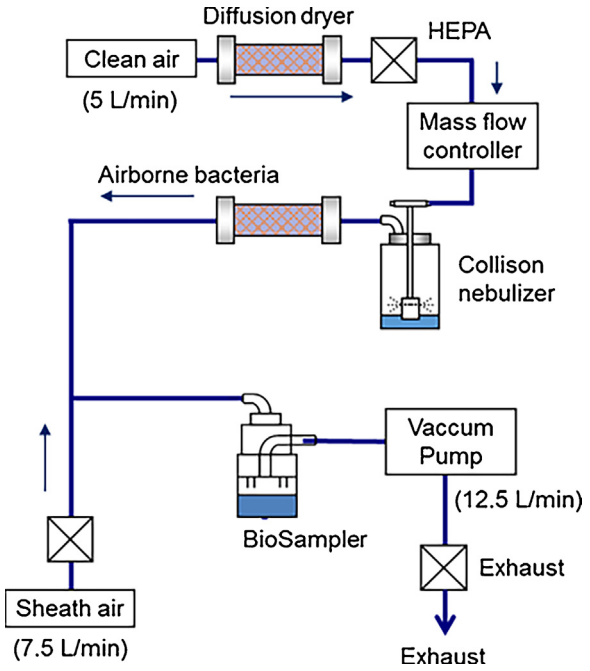

Fig. 2. The experimental setup used to aerosolize and collect airborne bacteria (spray system).

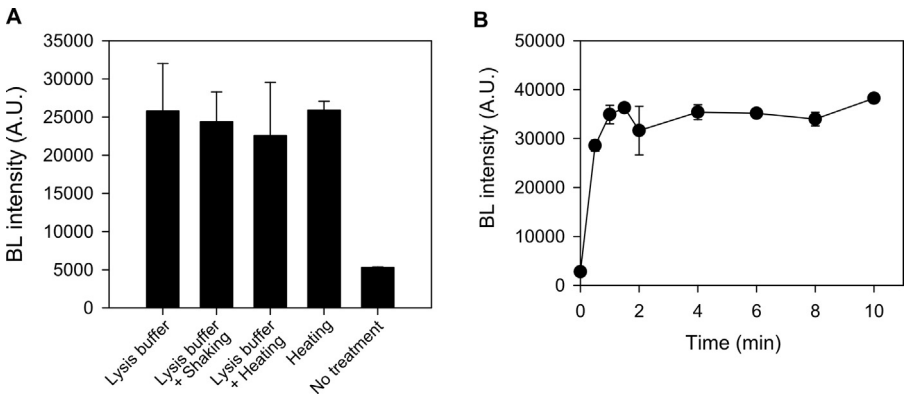

Fig. 3. The optimization of lysis method. (A) Investigation of different lysis methods, and (B) the investigation of different heating time.

tion of luciferase enzyme. This resulted in readable bioluminescent signals representing ATP expression levels and consequently indicating the number of bacterial cells in the air environment [30,31]. Therefore, the key process of lysis is the disruption of the cellular membrane of bacteria. The kit we used already has a supported lysis buffer and was suggested for use in efficient lysis. Although the use of lysis buffer is efficient for ATP extraction, the lysis buffer component should always be prepared and supplied to detect ATP extracted from bacteria. The protocol of the kit also suggested comparing the efficiency of the lysis buffer to that of heat lysis (heating the captured samples up to ~100 °C). Heating lysis also studied by some researchers [31–33]. If the heat lysis has comparable efficiency to lysis buffer treatment for lysis, it would be the simplest way to extract ATP from the samples collected. Therefore, we compared four different lysis methods to lyse bacteria using the solution-based ATP test: 1) Incubating in lysis buffer at room temperature (no agitation or heating); 2) agitation in lysis buffer at room temperature; 3) heating (95 °C) in lysis buffer (no agitation); 4) heating (95 °C) only (in DI water with no agitation). As presented in Fig. 3A, although the 'heating (95 °C)-only in DI water' method did not provide the highest bioluminescence intensity, it was comparable to the other methods using lysis buffer. Considering the operational convenience and cost of the lysis process, simple heating using DI water would be the best option compared with using lysis buffer for the lysis process. As the intensity value of 'heating (95 °C) only in DI water' method was comparable to the other methods using lysis buffer, we used the heating only lysis method for further experiments in this the present study.

In the next step, we investigated the effect of heating time for the 'heating (95 °C) only in DI water' method. The heating time is also critical for determining the time for the detection of airborne bacteria in the air environment. As presented in Fig. 3B, the bioluminescence intensity triggered by the ATP extracted from *E. coli* cells via the heating-only method was increased only for 30 s heating. The bioluminescence intensity was almost saturated after 2 min and slightly increased at 10 min heating. This result suggested that 2 min heating was sufficient for the extraction of ATP from a high concentration of bacterial cells (10^7 cells/mL of *E. coli*). However, we selected 10 min heating in further experiments in this study for the efficient extraction of ATP. Although ATP molecule has 48 min half-life at 95 °C in water [34,35], Fig. 3B shows no significant reduction of bioluminescence intensity during 10 min heat lysis. It may be enough to use 2 min heating instead of using 10 min heating to accomplish fast detection of ATP after lysis. Considering 2 min heat lysis and 5 min bioluminescence measurement it will take around 7 min after bacterial sampling from the air.

3.2. Immobilization of mixture of luciferase/D-luciferin in paper discs

Next, we investigated the immobilization procedure of the mixture of luciferase/D-luciferin. Firstly, we determined the immobilized quantity of the mixture of luciferase/D-luciferin and optimized its quantity. Subsequently, using the optimized quantity we analyzed the performance stability of the paper discs immobilized with the mixture of luciferase/D-luciferin. We used the luciferase/D-luciferin mixture contained in the ATP assay kit (Roche, HS-II). The kit did not mention the exact concentration of luciferase or luciferin and buffer composition; therefore, we cannot provide the concentration information at this stage. However, if we use the same volume of the mixture for each immobilization process and if the kit guarantees the quality control, we can expect the consistent performance of the immobilization process. We selected common filter paper as an immobilization matrix for luciferase and D-luciferin because it is easy to purchase and has large pores to capture the enzyme and its substrate. The filter paper was cut to 0.5-cm diameter disc shapes and autoclaved prior to use. To ensure that no luciferase/D-luciferin escaped over the surface of paper discs, but was gradually absorbed into paper's pore, 10 μL of the mixture solution of luciferase/D-luciferin supplied in the kit was dropped on the paper discs. The paper discs were dried in silica gel-chambers for 2 h and then 10 μL of the mixture solution of luciferase/D-luciferin was dropped again on the paper disc and dried for an additional 2 h in the same chamber. Following this procedure, we repeated the adsorption/dry loop. The final drying was performed in the silica gel-chamber overnight to remove all aqueous components (Fig. 1). We investigated the number of adsorption/dry loops by preparing each paper disc with different numbers of adsorption/dry loop (0, 1, 2, 3, 4, and 5 times). Fig. 4 presents the variation of bioluminescence intensity according to the different times of adsorption/dry loops performed. Following three repeats (total 30 μL of mixture was immobilized) of adsorption/dry loops, the bioluminescence intensity achieved the highest value (the performance of each paper disc was confirmed by *E. coli* (~10^7 cells) extract by heat-only lysis). As the luciferase/D-luciferin solution was introduced to the paper surface, it would be trapped in small pores, thereby keeping the spatial structure of luciferase and luciferin stable. When more luciferase/D-luciferin and other ingredients of reaction buffer were packed fully into the pores of paper discs in dried form, the excessive luciferase/D-luciferin and ingredients in reaction buffer was not localized or immobilized inside the pores of the paper. Luciferase and D-luciferin may cover the paper surface and not the paper pores, which may have caused the reduc-

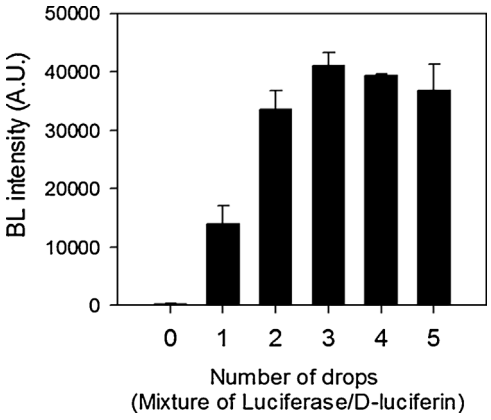

Fig. 4. The investigation of different repetition of sequential adsorption/dry luciferase/D-luciferin in paper discs.

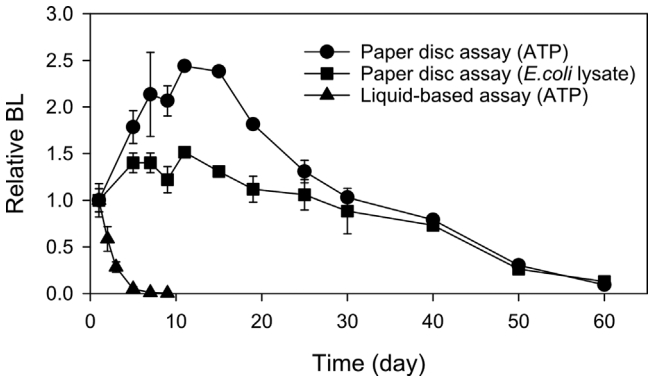

Fig. 5. The comparison of storage stability of the paper discs immobilized with Luciferase/D-luciferin and liquid-based assay. The luminescence intensity of 20 μM of ATP and the luminescence intensity of 1.17×10^7 CFU/mL of *E. coli* heat lysate were measured using different days of storage of paper discs or reaction mixture (luciferin and luciferase with reaction buffer in liquid solution) after immobilized with Luciferase/D-luciferin or preparation of the liquid solution, respectively (Relative BL = BL intensity of other days/BL intensity of day 1).

tion of the immobilized amount of luciferase and luciferin inside the pore; therefore, we suspected that this attributes to saturation of luciferase activity after following three-repeats of adsorption/dry loops. For further experiments using luciferase/D-luciferin immobilized paper discs, we used three-repeats of adsorption/dry loops.

In the following step, the storage stability of the paper discs immobilized with luciferase/D-luciferin and liquid solution-based assay (a mixture of luciferase, D-luciferin, and reaction buffer) was examined and compared at room temperature. The paper discs after immobilization with luciferase/D-luciferin were stored in a dry box containing silica gel during the time of stability test to ensure that the enzyme immobilized paper disc was completely dry. In the dried phase, the enzyme was trapped stably in paper pores resulting in a stable spatial structure. The results in Fig. 5 demonstrates that relative bioluminescence intensities (BL intensity of other days/BL intensity of day 1) were relatively maintained (not decreased below the intensity of day 1) for one month and gradually declined in the following days in both paper discs-based tests using ATP and *E. coli* heat lysate, while the relative BL of the liquid solution-based assay was decreased abruptly after the first measurement. After two months, the intensity of the paper disc assay was half the bioluminescence intensity obtained on the first day. Also, the assay activity of the paper discs showed better performance compared to liquid solution-based assay regarding maximum bioluminescence response (Fig. S1 in Supplementary information). These results revealed that the paper discs are a simple and efficient material for immobilizing luciferase enzyme and keeping its activity stable for at least one month without keeping it cold.

3.3. The evaluation of ATP extracted from heat-lysed E. coli using the paper discs immobilized with luciferase/D-luciferin

Following preparation of the paper discs immobilized, we investigated the performance of the paper discs using a different concentration of ATP or heat-lysed *E. coil* and compared this to the solution-based luciferase/D-luciferin reaction in the protocol of the commercial kit. Fig. 6 demonstrates the comparison for the detection of ATP or heat-lysed *E. coli* extract in solution or paper disc-based test. The intensity of bioluminescence using the paper discs exhibited a similar response compared with the solution-based test in ATP or heat-lysed *E. coli* analysis. The intensity of bioluminescence was revealed to increase by increasing the concentration of ATP or *E. coli* as expected. The ATP expression level analyses demonstrated that the intensities of bioluminescence using the paper discs were always higher compared with that of the solution-based test. We calculated the minimum detectable concentration of ATP or *E. coli* based on the value of $BL_{blank} + 3SD_{blank}$. Then, the minimum detectable concentration of ATP or *E. coli* was re-calculated based on the calibration curve obtained from the correlation of ATP or *E. coli* concentration to BL intensity. The minimum detectable concentration was 2 nM ATP for the paper disc or 10 nM for the solution-based test (Fig. 6A). The minimum detectable concentration of *E. coli* lysed by the heat-only method was 1.17×10^3 CFU/mL in the paper disc and 6.03×10^2 CFU/mL in the solution-based test (Fig. 6B). Therefore, we concluded that the paper discs

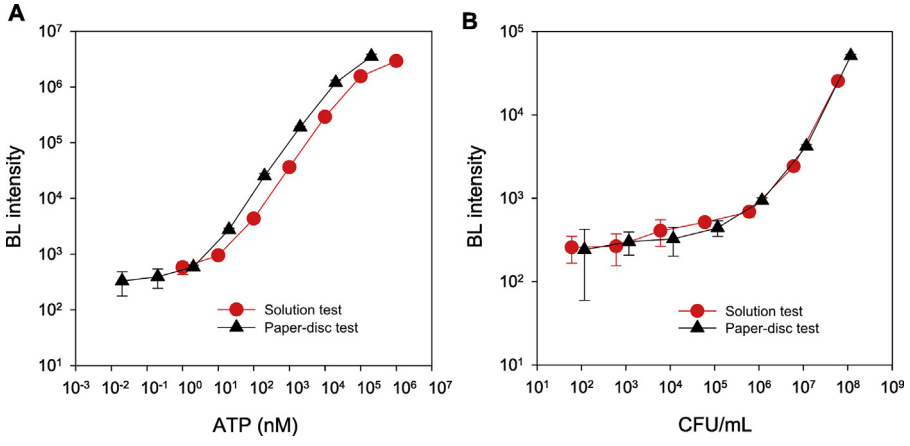

Fig. 6. The bioluminescence intensity of (A) ATP standard or (B) *E. coli* samples in paper disc-based or solution-based luciferase/D-luciferin reaction.

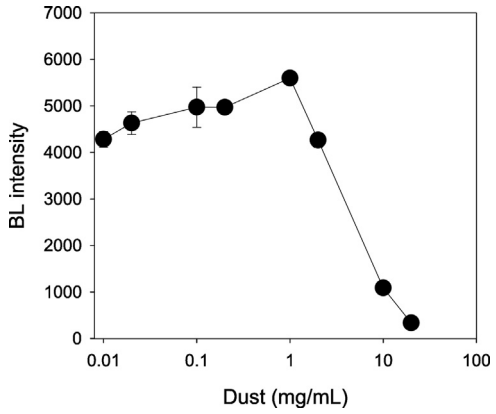

Fig. 7. The impacts of dust on bioluminescent intensity of *E. coli* detection (1.32×10^7 CFU/mL) using paper discs immobilized with luciferase/D-luciferin reagents.

immobilized with luciferase/D-luciferin has a comparable performance to the solution-based test.

3.4. Applicability of the paper discs immobilized with luciferase/D-luciferin in detection of airborne bacteria

The paper discs immobilized with luciferase/D-luciferin were applied to the *E. coli* samples containing dust during heat-only lysis to stimulate air environmental conditions. In the air, bacteria are usually in the form of small particles, such as aerosol, and bind to dust in the air [36–39]. Therefore, the effect of the concentration of dust with a fixed concentration of *E. coli* sample in heat-only lysis method was investigated using the paper discs immobilized with luciferase/D-luciferin. The intensity of bioluminescence in *E. coli* samples with a fixed concentration of 1.32×10^7 CFU/mL containing various concentrations of dust solutions in a range of 0.01 mg/mL and 20 mg/mL are presented in Fig. 7. With the concentration of dust solution below 1 mg/mL, the intensity of bioluminescence was negligibly altered; however, the signal was decreased significantly with dust concentrations over 1 mg/mL. The reduction of bioluminescence intensity in samples over 1 mg/mL dust may be due to the highly condensed dust in unit volume that inhibits the efficient heat-lysis. As a result, this will affect to the evaluation of intrinsic ATP quantity in the samples captured. However, the concentration of dust 1 mg/mL (being equally to 10^9 μg/m^3) is much higher than air quality standards of fine dust PM_{10} in Korea, United State, or Europe (the air quality standard for fine dust PM_{10} for 24 h are 100 μg/m^3, 150 μg/m^3, or 50 μg/m^3, respectively) [40–42]. This suggests that paper discs immobilized with luciferase/D-luciferin in accordance with heat lysis methods can be applied without serious concerns about inhibition of heat lysis when air captured samples containing highly concentrated dust (up to 10^9 μg/m^3).

The intensity of bioluminescence of *E. coli* detection (1.32×10^7 CFU/mL) was not markedly affected below a dust concentration of 1 mg/mL which is very high compared to the air quality standards in any other countries; therefore, the intensities of bioluminescence of various concentrations of *E. coli* samples containing dust (1 mg/mL) were investigated. The results are presented in Fig. 8 where the bioluminescence intensities of *E. coli* samples containing dust (1 mg/mL) exhibited a trend similar to that of *E. coli* samples without dust, by using immobilized paper discs (Fig. 6A). The minimum detectable concentration of *E. coli* samples containing 1 mg/mL dust was 2.03×10^3 (CFU/mL), which is higher compared with that of *E. coli* samples without dust in Fig. 6A. The higher value of the minimum detectable concentration of *E. coli* with dust can be explained by heat lysis efficiency. The presence of dust particles

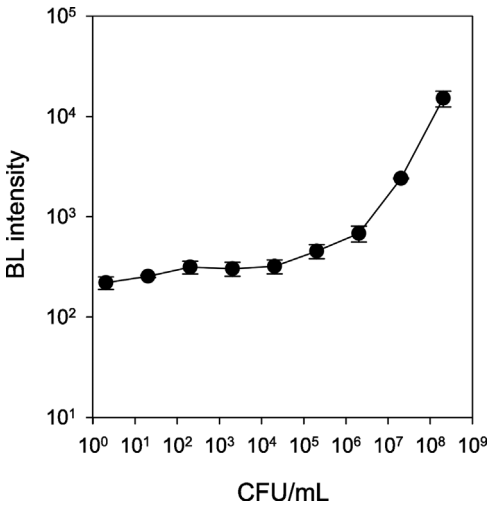

Fig. 8. The bioluminescent intensities of *E. coli* samples containing 1 mg/mL dust.

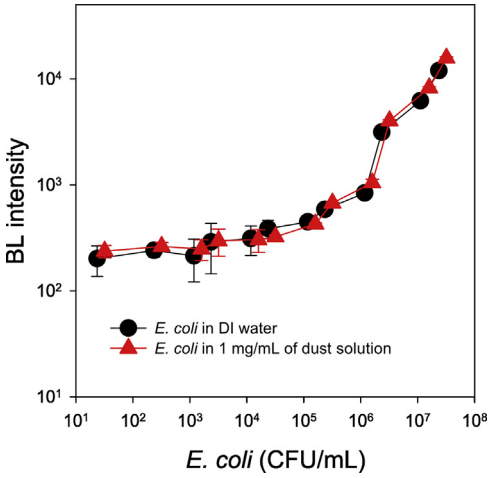

Fig. 9. The bioluminescence intensity of mimic airborne bacteria samples containing dust (1 mg/mL) and no dust.

in samples may reduce the heat lysis efficiency by preventing heat transfer to the cells in solution-based sampling and resulting in a lower extraction of ATP.

In the next step, in order to generate airborne bacteria, *E. coli* samples with or without dust was aerosolized by Collison nebulizer system (Fig. 2). The intensity of bioluminescence of various concentrations of airborne bacteria containing 1 mg/mL dust solution supported the conclusion that immobilized paper discs with luciferase/D-luciferin have high applicability in real air samples. The results are presented in Fig. 9. The curve of bioluminescent intensities of aerosolized *E. coli* samples containing 1 mg/mL dust had a similar pattern compared with aerosolized *E. coli* samples without dust. Therefore, it can be concluded that the immobilization of luciferase/D-luciferin in paper discs can be used to detect bacteria in the air, and the detection was not affected by dust with a concentration below 1 mg/mL. The minimum detectable concentration of *E. coli* samples without dust after aerosolization was 1.17×10^3 (CFU/mL), whereas that of *E. coli* samples aerosolized with dust to form airborne bacteria was 2.32×10^3 (CFU/mL).

4. Conclusions

We proposed that paper discs immobilized with luciferase/D-luciferin can be used to detect ATP extracted from bacterial samples

collected from the air. The paper discs following immobilization could be stored stably for over 30 days at room temperature, and be used to detect bacterial ATP immediately after simple heat lysis of the bacterial samples. The detection time for bacterial samples after collected from the air was less than 7 min included 2 min of heat lysis and 5 min of bioluminescence measurement. Furthermore, it can be used to detect airborne bacteria with the presence of dust up to 1 mg/mL with a minimum detectable *E. coli* concentration of 2.32×10^3 CFU/mL. Therefore, it is highly applicable for the biosensor of bacterial detection in the air with on-time measurement using a paper type matrix immobilized with luciferase and D-luciferin.

Acknowledgments

This work was finally supported by the Korea Institute of Science and Technology (KIST) Institutional Research Program and in part by the Korea Ministry of Environment (MOE) as "Public Technology Program based on Environmental Policy (No. 2016000160008).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.snb.2018.01.009.

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