Development of Rapid Assessment Method to Determine Bacterial Viability Based on Ultraviolet and Visible (UV-Vis) Spectroscopy Analysis Including Application to Bioaerosols

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ABSTRACT

We evaluated a method for the assessment of bacterial viability that is based on ultraviolet and visible (UV-Vis) spectroscopy analysis. The quantities of intracellular materials inside a cell vary depending on change of bacterial viability by disruption of the membrane integrity. Therefore, normalized optical density in the range of 200–290 nm was analyzed to determine if it varied in samples containing different proportions of live bacteria. Our results indicate that samples containing higher proportions of live bacteria such as *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus epidermidis* had higher optical densities. In addition, the optical density at 230 nm divided by the optical density at 670 nm was found to have a strong linear correlation with bacterial viability (the R^2 values of *E. coli*, *B. subtilis*, and *S. epidermidis* are 0.9964, 0.9118, and 0.9861, respectively). Our proposed rapid assessment method takes less than three minutes and only requires optical measurements at 230 and 670 nm; therefore, it is simpler and faster than colony counting, fluorochromasia, or the dye-exclusion test. Moreover, our method was applied to bioaerosols, which are currently important issues in public health, microbiology, aerosol science, and other fields. In our study, the bacteria (*E. coli*) were dispersed into the air using a Collison-type atomizer, and were sampled in sterilized deionized water using an impinger with a pump. According to our method, the viability of *E. coli* was approximately 55.2%, which was similar to 52.5 ± 4.7% determined from the LIVE/DEAD BacLight bacterial viability assay.

Keywords: Bacterial viability; Ultraviolet and visible spectroscopy; Optical density; Bioaerosols.

INTRODUCTION

Antimicrobial tests require an efficient method for the assessment of bacterial viability, which is also important in several areas of microbiology, environmental research, and industrial applications (Diaper *et al*., 1992; Lebaron *et al*., 1998). The colony count method is the standard and conventional method for the assessment of culturability (Bunthof *et al*., 1999; Auty *et al*., 2001). However, the method requires several days for colony formation and this time delay can be one of the most serious defects of the conventional method, and the method is applicable only to culturable bacteria which can occur cell division at a sufficient rate to form colonies, which would cause underestimation of cells due to VBNC (viable but non-culturable) cells which can proliferate under suitable conditions. The staining material is often used in the colony count method for successfully separating many types of bacteria, but the staining material is harmful and human counting errors can occur. Moreover, it is difficult to obtain reproducible results using the colony counting method because the results are very sensitive to culture conditions such as temperature, media, and the duration of incubation (Boulos *et al*., 1999; Auty *et al*., 2001).

Molecular methods can detect low concentrations of microorganisms, are reliable and culture-independent (Alvarez *et al*., 1995; Landete *et al*., 2007). However, molecular methods require expensive and specialized apparatus such as a polymerase chain reaction (PCR) system and an electrophoresis system. Moreover, molecular methods need a technical skill for DNA extraction, primer design, and PCR amplification. One of the main disadvantages of PCR is that PCR only permits the detection of total cells including those dead cells. Currently, there is an increased interest in coupling ethidium monoazide (EMA) with PCR to differently detecting viable cells and dead cells, each other (Rawsthorne and Phister, 2009).

The adenosine triphosphate (ATP) method has been

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shown to be a robust and repeatable alternative to traditional microscopic counting and culture-based counting. Even though ATP-based bioluminescence assays can easily and rapidly detect cells, the method cannot detect dead cells (Seshadri *et al*., 2009). Although the fluorochromasia method and dye-exclusion test allow live and dead cells to be easily distinguished, these methods require a step to stain cells, and an additional step to analyze the stained cells such as fluorescence spectroscopy, fluorescence microscopy, or the use of fluorescence microplate readers and flow cytometry (Molecular Probes Inc., 2004; Perfetto *et al*., 2006; Barbosa *et al*., 2008). Yang *et al*. (2006) developed a flow cytometer system incorporated with an easily operated microfabricated flow cytometer chip. Operation of a traditional flow cytometer still relies on the need for specialized personnel and complicated sample pretreatment procedures.

Ultraviolet and visible (UV-Vis) spectroscopy, which measures the amount of light that is attenuated by a solution or a suspension of particles, is a quantitative, reliable, and rapid analytical tool that can be immediately applied as a biosensor for the detection, identification, and enumeration of cells (An and Friedman, 1997; Alupoaei *et al*., 2004; Alupoaei and García-Rubio, 2005). UV-Vis spectroscopy of cells has been used to estimate the number of cells in a solution and their chemical composition, including nucleic acid and protein concentrations (Leštan *et al*., 1993; Wang *et al*., 1995; Piereira *et al*., 2000; Alupoaei, 2001; Salard *et al*., 2001; Alupoaei and García-Rubio, 2005). Based on the current uses of UV-Vis spectroscopy, it is feasible that a detection scheme could be developed to distinguish live and dead cells based on UV-Vis spectroscopy.

Bioaerosols are currently important issues in public health, microbiology, aerosol science, and other fields. The monitoring of bioaerosols is essential for controlling air quality, assessing exposure in health risk evaluation studies, identifying bioaerosol emission sources, and estimating the performance of air cleaning devices. Exposure to bioaerosols can affect human health in variety of ways including infectious diseases, acute toxic reactions, and allergies (Terzieva *et al*., 1996; Lee *et al*., 2004; Rule *et al*., 2007; Blatny *et al*., 2008). The Severe Acute Respiratory Syndrome (SARS) and the threat of avian flu are natural examples illustrating profound, daily impacts of bioaerosols on public health (Ratnesar-Shumate *et al*., 2008). With the increasing concern on biological contamination of indoor environments, the importance of rapid monitoring of bioaerosols increases. Previous studies reported the feasibility for rapid detection of bioaerosols using modified Raman facility (Sengupta *et al*., 2005) or laser light source and fluorescence measurement system (Nachman *et al*., 1996; Hairston *et al*., 1997). The system developed by Hairston *et al.* (1997) was improved into UV-APS (ultraviolet aerodynamic particle sizer spectrometer) which is a commercial instrument for real-time, continuous monitoring of airborne particles and viable airborne microorganisms. Although these sophisticated instruments are effective in scientific analyses or for military purposes, they are not affordable for practical purposes, such as monitoring the indoor air quality, estimating

performance of bioaerosol control devices, and so on.

In this paper, we propose a rapid assessment method (requiring less than 3 min) to determine bacterial viability based on UV-Vis spectroscopy analysis of prokaryotic cells. Since airborne dead cells can result in toxigenic or allergenic effects (Shahamat *et al*., 1997), an important factor for assessing health risks is not solely the concentration of live cells but also the concentration of dead cells. The proposed method is simple and less expensive than the fluorochromasia and dye-exclusion tests because it is not necessary to stain the bacterial cells and the method uses only a UV-Vis spectrophotometer, which is relatively inexpensive and is widely available in every branch of microbiology. For application to bioaerosols in this study, bacteria (*Escherichia coli*) were dispersed into the air using a Collison-type atomizer, and were sampled in sterilized deionized (DI) water using an impinger equipped with a pump. Then, the bacterial viability was assessed using our proposed method.

METHODS

Preparation of Samples

Our experimental procedure for the preparation of samples is shown in Fig. 1(a). *Escherichia coli* (ATCC #11775), *Bacillus subtilis* (ATCC #6633)*,* and *Staphylococcus epidermidis* (ATCC #14990) cells were obtained from the Korean Culture Center of Microorganisms, Seoul, Korea. For each species, a bacterial suspension was prepared by culturing 0.1 mL of an overnight culture inoculated in 15 mL of nutrient broth for 18 h. The nutrient broth was prepared by dissolving 5 g of peptone and 3 g of meat extract in 1000 mL of sterilized DI water and then sterilizing the mixture in an autoclave. The tubes containing the suspension were centrifuged at 11,000 g for 10 min using a centrifuge (VS-15000N, Vision Scientific Co., Ltd., Korea), and the supernatant was carefully removed. The remaining pellets were then resuspended in sterilized DI water and vortexed for a few seconds using a vortex mixer (KMC-1300V, Vision Scientific CO., Ltd., Korea). This washing process was repeated three times, after which the pellets were resuspended in sterilized DI water. Six mL of the suspension were then mixed with 24 mL of 99% isopropyl alcohol (Daejung Chemicals & Metals Co., Ltd., Korea), which was used as a bactericidal agent to form a dead bacterial suspension. The remainder of the suspension was untreated and used as a live bacterial suspension. The dead bacterial suspension was placed at room temperature (25 ± 1°C) for 50 min and then washed with sterilized DI water three times. Both the dead bacterial suspension and the live bacterial suspension were diluted with sterilized DI water to obtain base suspensions with optical densities of 0.06 (for *E. coli* and *B. subtilis*) and 0.30 (for *S. epidermidis*) at a wavelength of 670 nm. In these optical densities, both live and dead cell concentrations were similar to each other for each species at the wavelength of 670nm (Molecular Probes Inc., 2004). The diluted live and dead bacterial suspensions were quantitatively mixed to obtain five different fractions of the bacterial suspensions (0%, 20%, 50%, 80%, and 100% live bacteria). The temperature and humidity of
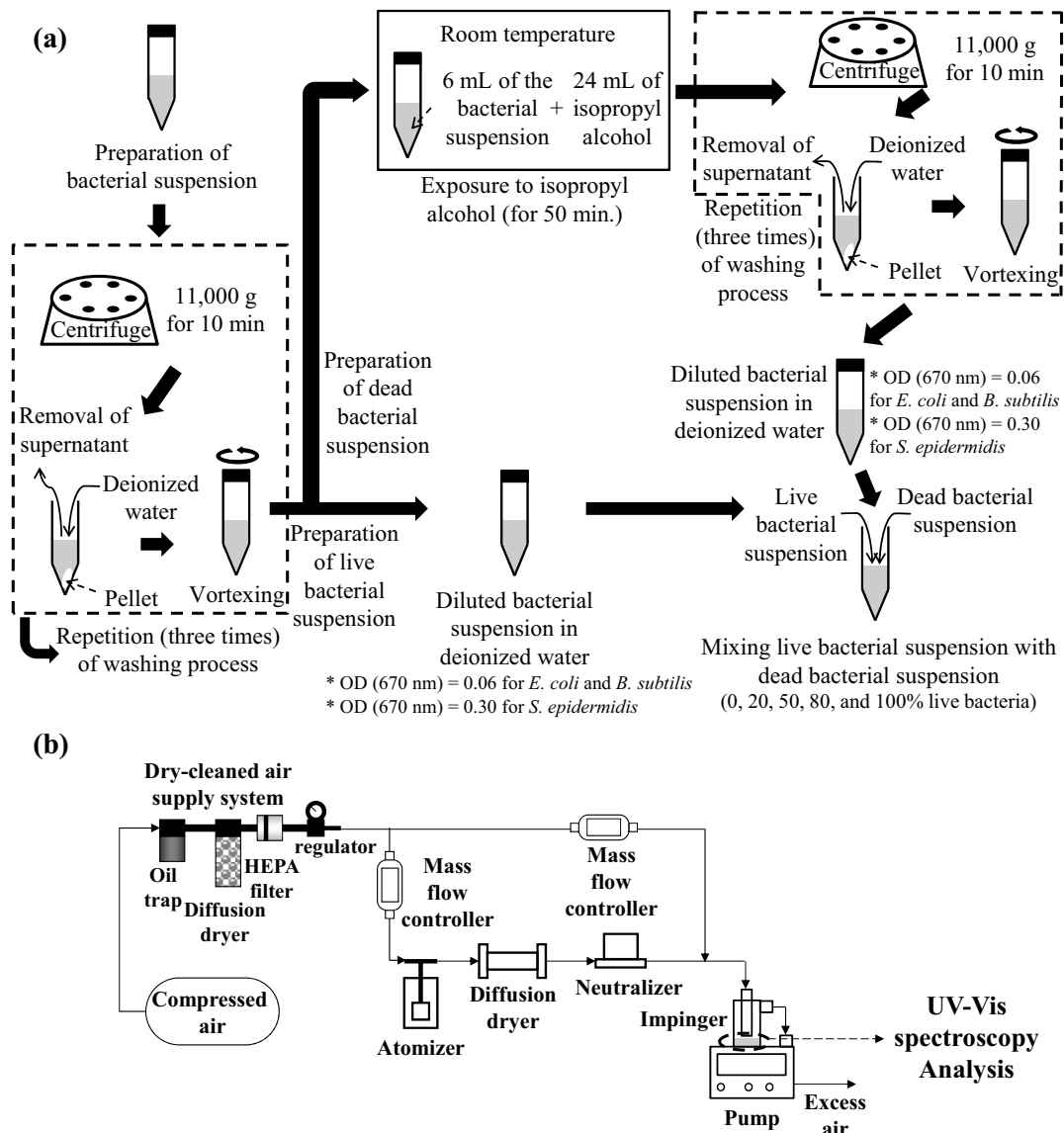
(a)

(b)

Fig. 1. Schematic diagram of (a) preparation of samples, and (b) viability assessment for airborne bacteria.

the laboratory were 25 ± 1°C and 20 ± 3% RH (relative humidity), respectively, when samples were prepared.

Viability Determined from ATP-based Bioluminescence Assay

ATP-based bioluminescence assay was used for confirming the viability of each bacterial sample. 0.1 mL of each bacterial sample was placed on a plastic Petri dish (diameter 5 cm), and the level of biological contamination on the plastic Petri dish was measured using an ATP detector consisting of a swab stick (LuciPac W, Kikkoman, Japan) and measuring instrument (Lumitester PD10-N, Kikkoman, Japan). The surface of the contaminated plastic Petri dish was wiped, and then the swab was then placed into the swab stick, which contained an ATP releasing reagent and a luminescence reagent for the light-generating reaction. The swab stick was shaken several times and inserted into the measuring instrument. The instrument displayed the intensity of light generated from the reaction

between ATP and the luminescence reagent as the relative luminescent units (RLU). For control check, the surface of a clean Petri dish was measured using ATP detector with 0.1 mL of sterilized DI water. The average RLU of the result was 54 (background RLU). This method is similar to that of Yoon *et al.* (2010).

The viability is defined as follows:

$$\text{Viability} = \frac{\Delta\text{RLU}_{\text{from a sample}}}{\Delta\text{RLU}_{\text{from 100\% live bacteria sample}}} \times 100 \ (\%) \qquad (1)$$

where the numerator and denominator represent the difference between the RLU from each bacterial sample and the background RLU, and the difference between the RLU from a 100% live bacterial sample and the background RLU, respectively. Table 1 summarizes the results for the viability of each bacterial sample determined using the ATP-based bioluminescence assay.

Table 1. Viability and culturability of each bacterial suspension estimated as ΔRLU ratio and colony ratio, respectively.

Ratio of live to dead bacteria		*E. coli*	*B. subtilis*	*S. epidermidis*
0 : 100	ΔRLU ratio	0.001 ± 0.001	0.003 ± 0.003	0.002 ± 0.001
(0% live bacteria)	Colony ratio	0	0	0
20 : 80	ΔRLU ratio	0.182 ± 0.019	0.177 ± 0.013	0.184 ± 0.005
(20% live bacteria)	Colony ratio	0.202 ± 0.003	0.195 ± 0.014	0.208 ± 0.002
50 : 50	ΔRLU ratio	0.469 ± 0.042	0.472 ± 0.013	0.508 ± 0.020
(50% live bacteria)	Colony ratio	0.488 ± 0.014	0.505 ± 0.007	0.523 ± 0.003
80 : 20	ΔRLU ratio	0.792 ± 0.043	0.758 ± 0.054	0.770 ± 0.018
(80% live bacteria)	Colony ratio	0.825 ± 0.003	0.767 ± 0.014	0.821 ± 0.012
100 : 0	ΔRLU ratio	1	1	1
(100% live bacteria)	Colony ratio	1	1	1

Moreover, we used conventional colony counting for determining culturability of each sample, and the colony ratios are also presented in Table 1. It is interesting to note that there is a negligible difference between the colony ratio and ΔRLU ratio for each sample. Kell *et al.* (1998) reported that culturability and viability are synonymous for readily culturable organisms, and several studies (Barer *et al.*, 2000; Kort *et al.*, 2008; Falk *et al.*, 2010) reported that *E. coli*, *B. subtilis*, and *Staphylococcus* sp. are readily culturable organisms.

Spectroscopy

The UV-Vis spectra at wavelengths ranging from 200 to 900 nm were measured using a UV-Vis spectrophotometer (Libra S12, Biochrom Ltd., UK) with a 1 cm path length quartz cuvette (40 00 9104, Biochrom Ltd., UK). The scan speed and bandwidth of the spectrophotometer were 500 nm/min and 1.0 nm, respectively. Prior to recording the spectrum of each sample, the spectrophotometer was zeroed to account for any stray light. To avoid the effect of inhomogeneities in the suspending medium, the background spectrum was determined using the corresponding suspending media from the batch used to prepare the original sample. The temperature and humidity of the laboratory were 25 ± 1°C and 20 ± 3% RH (relative humidity), respectively, when spectroscopic measurements were carried out.

Structure of Bacterial Cells

The complex structure of the selected prokaryotic cells was divided into two main substructures for analysis (or modeling) of the spectra of the cells: a macrostructure and an internal structure. The definition of "macrostructure" is the body of the cell and its characteristic dimensions, and the definition of "internal structure" implies identifiable structures such as ribosomes or inclusions, dense bodies, and any other intracellular materials that could absorb or scatter light (Alupoaei, 2001).

Multi-wavelength transmission measurements are related to turbidimetric techniques. The degree of transmission is expressed using transmittance, which is defined (Kim *et al.*, 2004) as

$$T(\lambda) = \frac{I(\lambda)}{I_0(\lambda)} \tag{2}$$

where T is the transmittance, I_0 is the intensity of the light source of wavelength λ, and I is the intensity of the beam passed through the cell at wavelength λ. For suspensions, the reduction in light intensity is related to turbidity τ (Kim *et al.*, 2004) as follows:

$$T(\lambda) = \exp[-\tau(\lambda)l] = \exp[-OD(\lambda)] \tag{3}$$

where l is the path length and OD is the optical density.

Alupoaei *et al.* (2004) reported that spectra of bacteria are divided into spectra of macrostructure and spectra of internal structure, and suggested the following equation that expresses turbidity $\tau(\lambda)$ measured at a given wavelength λ:

$$\tau(\lambda) = \tau_1(\lambda) + \tau_2(\lambda) \tag{4}$$

where subscripts 1 and 2 correspond to characteristics of the macrostructure and internal structure, respectively.

Scanning Electron Microscopy Analysis

A scanning electron microscope (SEM) study of isopropyl alcohol with non-treated (live) and treated (dead) bacterial cells was performed to observe the morphology of the bacterial cells. The bacterial samples were primarily fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer solution (pH 7.2), washed with 0.05 M sodium cacodylate buffer solution (pH 7.2), and post-fixed with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer solution (pH 7.2). The samples were dehydrated using sequential ethanol concentrations ranging from 30 to 100% with 10 min of exposure per concentration, and the ethanol was replaced by tetramethylsilane. After dehydration, each sample was spread on double-sided conducting adhesive tape pasted onto a metallic stub and coated with gold in a sputter coating unit for 5 min. The samples were observed using a SEM (JSM-5410LV, JEOL, Japan).

Application to Bioaerosols

Fig. 1(b) shows the schematic diagram of viability assessment for airborne bacteria. 100% live *E. coli* was selected as the test bacterium. A stream of particle-free air (temperature: 22.5 ± 3°C, relative humidity: 10 ± 5%) from a dry-cleaned air supply system consisting of an oil trap, a

diffusion dryer, and a high efficiency particulate air (HEPA) filter was delivered to a mass flow controller, and then delivered to a Collison-type atomizer (model 9302, TSI Inc., USA). The flow rate was regulated and monitored by the mass flow controller prior to its introduction to the atomizer. The bacteria were dispersed into the air by an atomizer. The dry-cleaned air at a flow rate of 2 L/min formed a high velocity jet through a 100 μm-diameter orifice in the atomizer. The pressure drop from this jet drew the bacterial suspension up through a narrow tube. The bacterial suspension was then broken into droplets by the high velocity air jet. The resultant larger droplets impinged on an impaction plate or a ball, while the smaller droplets made no contact and formed an aerosol that exited through an outlet. After passing through a diffusion dryer to remove the residual moisture, the flow, including airborne bacteria, entered an aerosol neutralizer (Soft X-ray charger 4530, HCT Co. Ltd., Korea) to eliminate charges induced during the atomization process. The airborne bacteria were mixed with particle-free air (1 L/min; 22.5 ± 3°C and RH 10 ± 5%) delivered through a dry-cleaned air supply system and then sampled in sterilized DI water by using an impinger equipped with a pump (Air Sampler 4640, HCT Co. Ltd., Korea). The sampling time and sampling flow rate for each test run were 15 min and 3 L/min, respectively.

The LIVE/DEAD BacLight bacterial viability assay, which has routinely been used to investigate the viability of bacterial cells, was also tried in this study. After the bioaerosols were sampled in sterilized DI water, 1 mL of sterilized DI water including sampled bioaerosols was mixed thoroughly with a fresh working solution of stain, which was a mixture of 1.5 μL of solution A (SYTO9 dye) and 1.5 μL of solution B (propidium iodide). The solution with bacteria was incubated in darkness at room temperature for 15 min. After extracting a drop (about 5 μL) of the stained bacterial suspension and locating the drop between a slide and a cover glass (having the area of 18 mm^2), we observed the stained bacteria using a fluorescence microscopy (IX71, Olympus, Japan) with fluorescein and Texas red bandpass filters, and then the viability was determined by image analysis counts (Ivanova *et al.*, 2010).

RESULTS AND DISCUSSION

Fig. 2 shows the spectra of normalized optical density for various suspensions of *E. coli*, *B. subtilis*, and *S. epidermidis*. The optical density at wavelength λ is defined as Eqs. (2–3), and the optical density spectra were normalized with respect to their corresponding average optical densities to eliminate any concentration effect, thereby allowing direct comparison of the spectral features (Alupoaei, 2001). Each spectrum represents an average of ten repeated measurements, and the error range of each spectrum was less than 2%. We observed similar spectral absorption behavior for *E. coli* (Fig. 2(a)) and *B. subtilis* (Fig. 2(b)), most likely because the size and water content of these bacteria are similar (Ross and Billing, 1957; Alupoaei *et al.*, 2004). Moreover, the morphology and membrane surface roughness of both bacteria are similar to
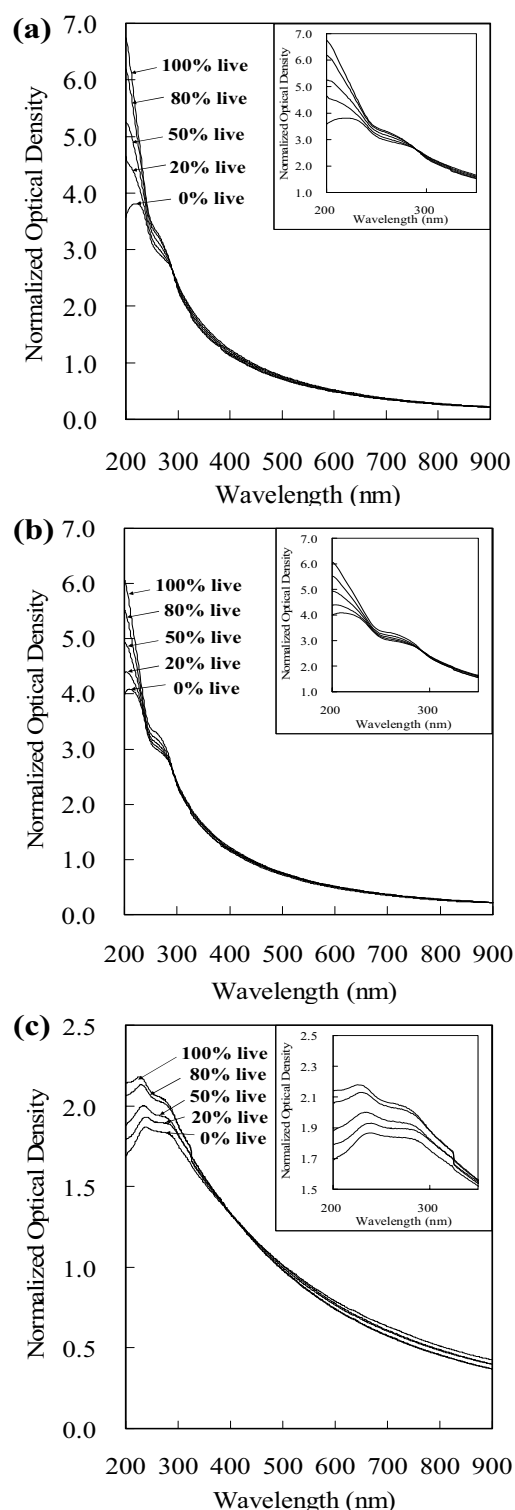

Fig. 2. Spectra of normalized optical density versus the ratio of live bacteria: (a) *E. coli*, (b) *B. subtilis*, and (c) *S. epidermidis*.

each other (Liu *et al.*, 2010; Nikiyan *et al.*, 2010), which would be one of the reasons for similar spectral absorption behavior of both bacteria. In the range of 200 to 350 nm, the spectra were enlarged as shown in the inset of Fig. 2. This result indicates that the normalized optical density in

the range of 200 nm to approximately 290 nm increased as the ratio of live bacteria increased, but that the normalized optical density measured at a wavelength of longer than approximately 290 nm is not correlated with the ratio of live bacteria. Spectral peaks at about 230 nm (Fig. 2) were generally attributed to the absorption of DNA (one of the intracellular materials); therefore, the amplitude of the peak was influenced by the ratio of live bacteria in the sample (Liu and Curtiss, 2009).

The morphologies of live bacteria and dead bacteria were confirmed through SEM studies (Figs. 3(a) and (b)). The live *E. coli* cells in the absence of isopropyl alcohol showed a regular smooth surface (Fig. 3(a)), and the dead *E. coli* cells exposed to isopropyl alcohol revealed membrane damage consistent with disruption of the membrane integrity (Fig. 3(b)). The bacterial membranes became compromised during a biocidal challenge of exposure to isopropyl alcohol, and intracellular materials could be released from the bacteria. Small ions such as potassium and phosphate tend to leach out first, followed by large molecules such as DNA and other materials (Chen and Cooper, 2002).

The 100% live *E. coli* suspension was treated with isopropyl alcohol in order to measure the optical density of the released intracellular materials. Fig. 3(c) shows the spectrum of the optical density for released intracellular materials. For wavelengths larger than approximately 290

nm, the optical density was nearly zero (< 0.005), and the optical density decreased with increasing wavelength within 200 to 290 nm. In a previous study (Fekete *et al.*, 2004), the optical density for nucleic acids which are a kind of intracellular material (internal structure components) was nearly zero at wavelengths larger than 290 nm.

Fig. 4(a) shows the optical density for the macrostructure and internal structure of *E. coli*. The inset of Fig. 4(a) shows the effect of wavelength (λ) on the ratio between turbidities of the internal structure (τ_2) and of the macrostructure (τ_1) of *E. coli*. The results shown in the inset of Fig. 4(a) were obtained from Eqs. (2–4) as follows:

$$\frac{\tau_2(\lambda)}{\tau_1(\lambda)} = \frac{OD_2(\lambda)}{OD_1(\lambda)} \tag{5}$$

For Fig. 4(a), we assumed that the measured optical densities (or turbidities) for dead *E. coli* and the released intracellular materials from *E. coli* were equal to optical densities (or turbidities) corresponding to macrostructure and internal structure, respectively ($\tau = \tau_1$ for dead *E. coli*, and $\tau = \tau_1$ for released intracellular materials from *E. coli*). In the range of 200 to 900 nm, the effect of macrostructure on optical density is greater than the effect of internal structure ($\tau_1 > \tau_2$). To verify the bacterial viability using
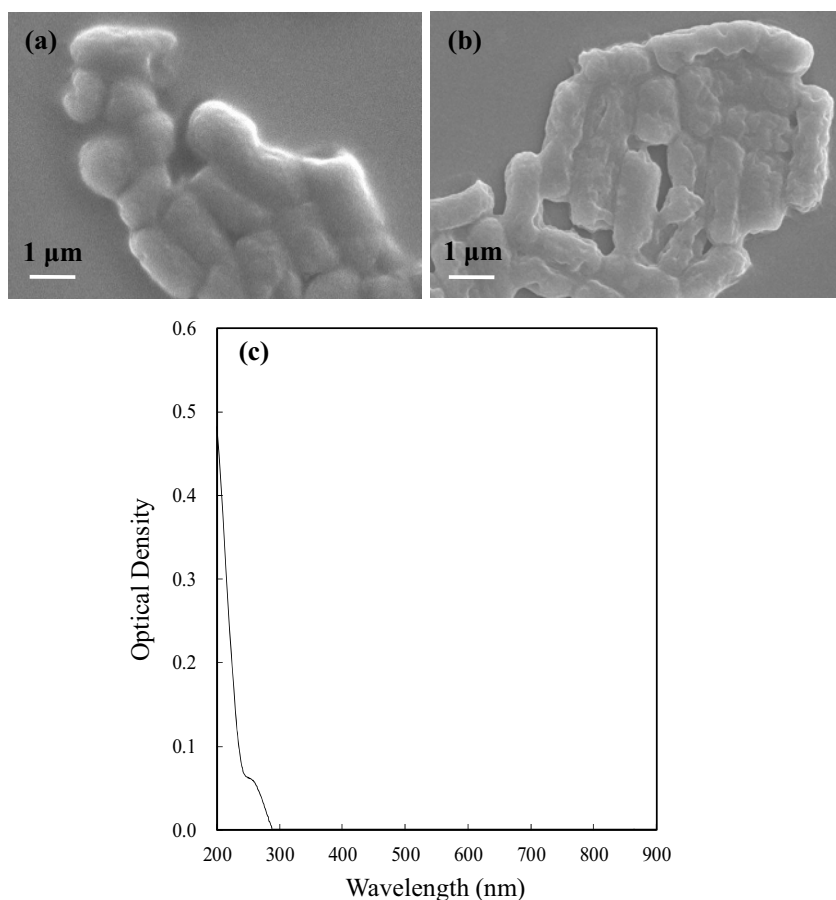

Fig. 3. Scanning electron micrographs of (a) live *E. coli* cells and (b) dead *E. coli* cells exposed to isopropyl alcohol. (c) Optical densities for released intracellular materials.
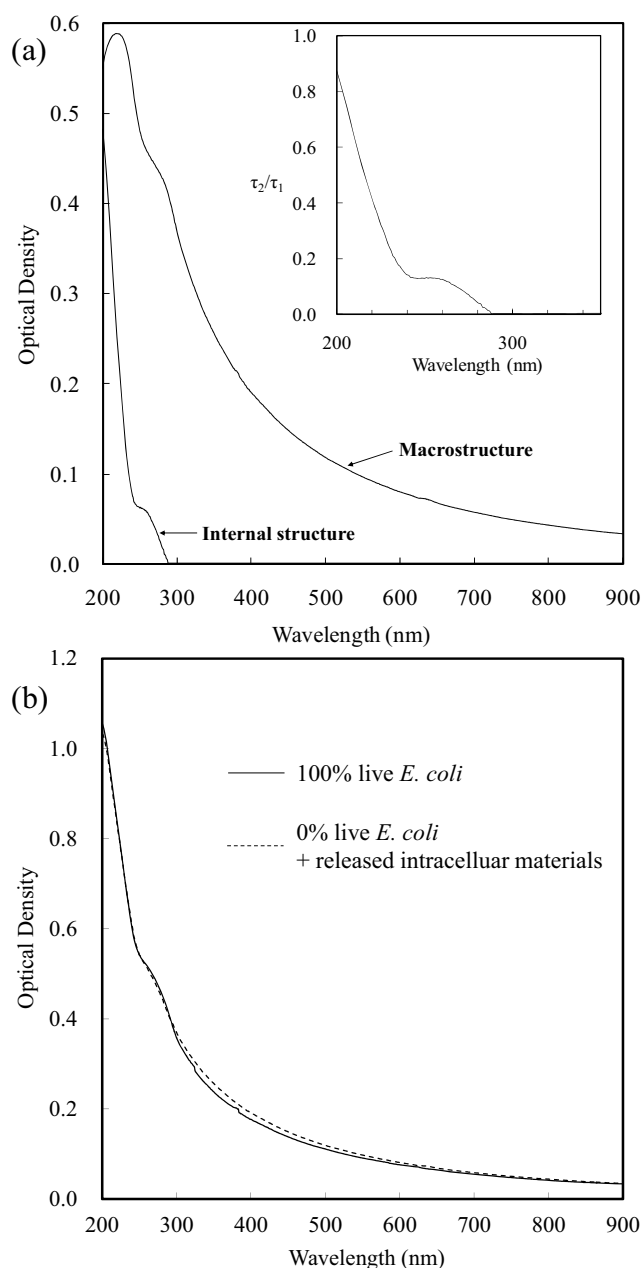
Fig. 4. Optical densities (a) for macrostructure and internal structure of *E. coli*, and the ratio of turbidities corresponding to the macrostructure (τ_1) and internal structure (τ_2) of *E. coli* (inset); and (b) for 100% live *E. coli* and the sum of 0% live *E. coli* and released intracellular materials.

spectroscopy, the multi-wavelength UV-Vis spectrum of the released intracellular materials from *E. coli* was measured and then added to the spectrum generated by samples containing 0% live *E. coli*, which produced results that were consistent with those obtained from an analysis of samples comprised of 100% live *E. coli* (Fig. 4(b)). These results can be explained using Eq. (4), and they suggest that our method for the assessment of bacterial viability was valid for this case. In addition, the results shown in Fig. 4(b) indicate that the difference between the

spectra generated by 0% live *E. coli* samples and 100% live *E. coli* samples shown in Fig. 2(a) was caused by differences in the quantities of intracellular materials that remained in the cells. In Fig. 3(c) and Fig. 4, each spectrum represents an average of more than three repeated measurements, and the error range of each spectrum was less than 10%.

As shown in Fig. 5, the ratios of the optical density at 230 nm to the optical density at 670 nm were clearly linear with respect to bacterial viability (the R^2 of *E. coli*, *B. subtilis*, and *S. epidermidis* are 0.9964, 0.9118, and 0.9861, respectively). At OD (230 nm) and OD (670 nm), the DNA have strong UV absorption, and the live and dead cell concentrations are same, respectively. For our method, the viability is related to the ratio of OD (230 nm) to OD (670 nm) as follows:

$$\text{Viability} = \alpha \times \frac{\text{OD (230 nm)}}{\text{OD (670 nm)}} - \beta \quad (\%) \qquad (6)$$

The viabilities measured by colony counting (Eq. (1)) were used in Eq. (6). In Fig. 5, the error range of each graph was less than 2%.

Table 2 shows the value of constants α and β in the equation related to viability and optical density. Taken together, these results indicate that the viability of any selected bacteria in our study can be rapidly and simply assessed by optical measurement at OD (230 nm) and OD (670 nm).

Fig. 6 shows the spectra for suspensions before atomizing the bacteria and after sampling the airborne bacteria, respectively. Each spectrum represents an average of three repeated measurements, and the error range of each spectrum was less than 9%. According to our method, the viability of *E. coli* was measured approximately 55.2%. The investigation of viability for bioaerosols was also studied with a LIVE/DEAD BacLight bacterial viability assay kit. Dead cells develop a breach in the continuity of the membrane that allows a cell-impermeable dye (propidium iodide component) to enter cells where it binds to DNA and makes cells fluoresce red. Fig. 7 shows the fluorescence microscopy images with the LIVE/DEAD BacLight bacterial viability kit. For live bacteria, the bacteria are represented by green color since their cell membranes can be stained with SYTO9 dye. Among dead bacteria, the bacteria whose cell membranes are disrupted will be represented by red color since their cells can be stained with propidium iodide component. Fig. 7(a) and 7(b) show images of live bacterial cells (green color) and dead bacterial cells (red color), respectively, resulting in the viability of 55.8% via image analysis counts. From repeated experiments with the LIVE/DEAD BacLight bacterial viability kit, the viability was 52.5 ± 4.7%, which was similar to 55.2% obtained using the UV-Vis spectroscopy (see Fig. 6). The viabilities of airborne bacteria sampled were decreased because the stress during atomization of the bacteria and the violent motion in the liquid after sampling may affect the viable bacteria (Dimmick *et al.*, 1969; Stewart *et al.*, 1995; Terzieva *et al.*, 1996).
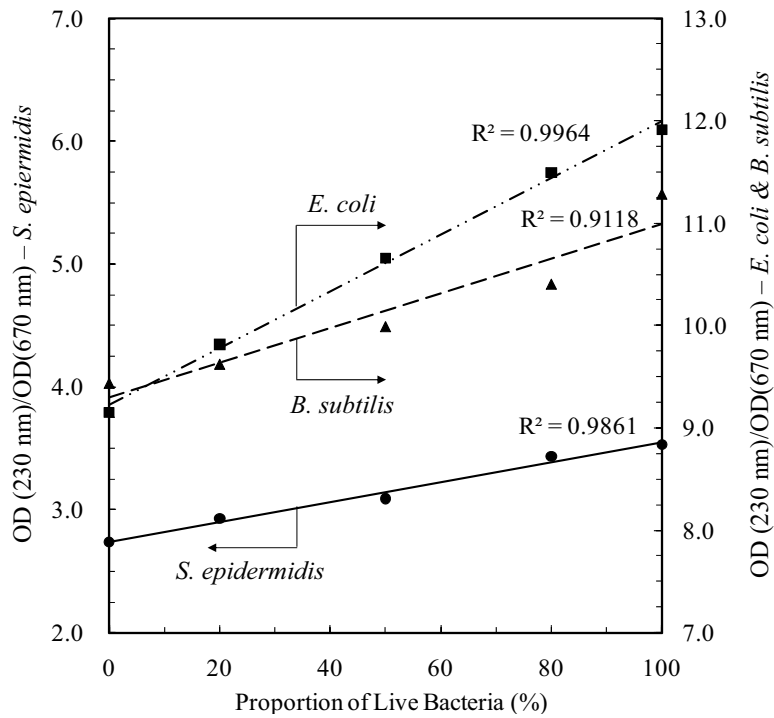
Fig. 5. Linear correlations between bacterial viability and the ratio of optical densities at 230 nm (OD_{230nm}) and 670 nm (OD_{670nm}).

Table 2. Constants α and β in the viability and optical density equation.

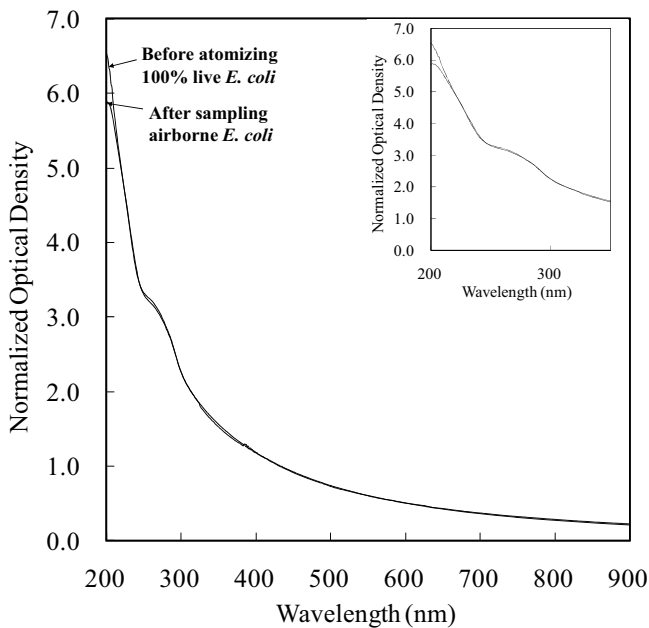| Bacteria	α	β
E coli	36.10	332.84
B. subtilis	58.82	546.99
S. epidermidis	123.46	338.49

Fig. 6. Spectra of normalized optical density for suspensions before atomizing 100% live *E. coli* and after sampling airborne 100% live *E. coli* versus the ratio of live bacteria.
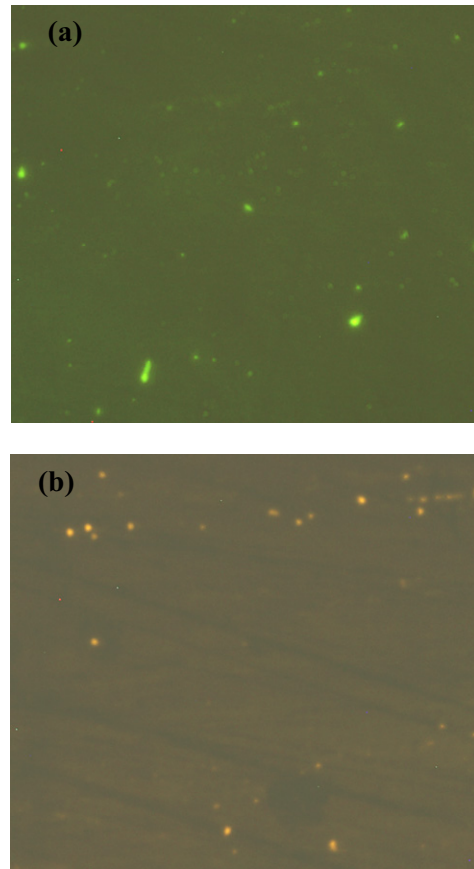

Fig. 7. Fluorescence microscopy images with a LIVE/ DEAD BacLight bacterial viability kit: (a) live bacterial cells (*E. coli*) and (b) dead bacterial cells (*E. coli*).

CONCLUSIONS

We proposed a method of UV-Vis spectroscopy analysis that allows the assessment of bacterial viability. In this study, normalized optical densities between 200–290 nm were found to be higher for samples that contained a higher proportion of live bacteria such as *E. coli*, *B. subtilis*, and *S. epidermidis*. This occurred due to the different quantities of intracellular materials, especially DNA, that were present inside the live and dead cells. In addition, we evaluated the ratios of OD (230 nm) to OD (670 nm) with respect to bacterial viability and found that they were linearly correlated (the R^2 of *E. coli*, *B. subtilis*, and *S. epidermidis* are 0.9964, 0.9118, and 0.9861, respectively). Taken together, our results indicate that the viability of the selected bacteria can be rapidly and simply assessed by optical measurements at 230 nm and 670 nm.

Even though the use of our method is limited since the method cannot be applied to samples with unknown bacteria or mixtures of different bacteria, the method has potential for the analysis of bacterial viability. Moreover, our method can be applied to bioaerosols, which are currently important issues in areas such as public health, microbiology, and aerosol science. In our study, the bacteria (*E. coli*) were dispersed into the air by using a Collison-type atomizer and sampled in sterilized DI water by using an impinger with a pump. According to our method, the viability of *E. coli* was approximately 55.2%, which was similar to 52.5 ± 4.7% determined from the LIVE/DEAD BacLight bacterial viability assay.

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REFERENCES

Alupoaei, C.E. (2001). *Modeling of the Transmission Spectra of Microorganisms*, Master of Science Thesis, University of South Florida.

Alupoaei, C.E. and García-Rubio, L.H. (2005). An Interpretation Model for the UV-Vis Spectra of Microorganisms. *Chem. Eng. Commun.* 192: 198–218.

Alupoaei, C.E., Olivares, J.A. and García-Rubio, L.H. (2004). Quantitative Spectroscopy Analysis of Prokaryotic Cells: Vegetative Cells and Spores. *Biosens. Bioelectron.* 19: 893–903.

Alvarez, A.J., Buttner, M.P. and Stetzenbach, L.D. (1995). PCR for Bioaerosol Monitoring: Sensitivity and Environmental Interference. *Appl. Environ. Microbiol.* 61: 3639–3644.

An, Y.H. and Friedman, R.J. (1997). Laboratory Methods for Studies of Bacterial Adhesion. *J. Microbiol. Methods* 30: 141–152.

Auty, M.A.E., Gardiner, G.E., Mcbrearty, S.J., O'sullivan, E.O., Mulvihill, D.M., Collins, J.K., Fitzgerald, G.F., Stanton, C. and Ross, R.P. (2001). Direct in situ Viability Assessment of Bacteria in Probiotic Dairy Products Using Viability Staining in Conjunction with Confocal Scanning Laser Microscopy. *Appl. Environ. Microbiol.* 67: 420–425.

Barbosa, J., Costa-de-Oliveira, S., Rodrigues, A.G. and Pina-Vaz, C. (2008). Optimization of a Flow Cytometry Protocol for Detection and Viability Assessment of *Giardia Lamblia*. *Travel Med. Infect. Dis.* 6: 234–239.

Barer, M.R., Smith, R.J., Cooney, R.P. and Kimmitt, P.T. (2000). Relationships between Culturability, Activity and Virulence in Pathogenic Bacteria. *J. Infect. Chemother.* 6: 108–111.

Blatny, J.M., Reif, B.A.P., Skogan, G., Andreassen, O., Høiby, E.A., Ask, E., Waagen, V., Aanonsen, D., Aaberge, I.S. and Caugant, D.A. (2008). Tracking Airborne Legionella and Legionella Pneumophila at a Biological Treatment Plant. *Environ. Sci. Technol.* 42: 7360–7367.

Boulos, L., Prévost, M., Barbeau, B., Coallier, J. and Desjardins, R. (1999). Live/Dead® BacLightTM : Application of a New Rapid Staining Method for Direct Enumeration of Viable and Total Bacteria in Drinking Water. *J. Microbiol. Methods* 37: 77–86.

Bunthof, C.J., van den Braak, S., Breeuwer, P., Rombouts, F.M. and Abee, T. (1999). Rapid Fluorescence Assessment of the Viability of Stressed *Lactococcus lactis*. *Appl. Environ. Microbiol.* 65: 3681–3689.

Chen, C.Z. and Cooper, S.L. (2002). Interactions between Dendrimer Biocides and Bacterial Membranes. *Biomaterials* 23: 3359–3368.

Diaper, J.P., Tither, K. and Edwards, C. (1992). Rapid Assessment of Bacterial Viability by Flow Cytometry. *Appl. Microbiol. Biotechnol.* 38: 268–272.

Dimmick, R.L., Akers, A.B., Heckly, R.J. and Wolochow, H. (1969). *An Introduction to Experimental Aerobiology*, Wiley-Interscience, New York.

Falk, S., Liu, B. and Braker, G. (2010). Isolation, Genetic and Functional Characterization of Novel Soil *nirK*-type Denitrifiers. *Syst. Appl. Microbiol.* 33: 337–347.

Fekete, A., Rontó, G., Hegedüs, M., Módos, K., Bérces, A., Kovács, G., Lammer, H. and Panitz, C. (2004). Simulation Experiments of the Effect of Space Environment on Bacteriophage and DNA Thin Films. *Adv. Space Res.* 33: 1306–1310.

Hairston, P.P., Ho, J. and Quant, F.R. (1997). Design of an Instrument for Real-Time Detection of Bioaerosols Using Simultaneous Measurement of Particle Aerodynamic Size and Intrinsic Fluorescence. *J. Aerosol. Sci.* 28: 471–482.

Ivanova, I.A., Kambarev, S., Popova, R.A., Naumovska, E.G., Markoska, K.B. and Dushkin, C.D. (2010). Determination of *Pseudomonas Putida* Live Cells with Classic Cultivation and Staining with "LIVE/DEAD BacLight Bacterial Viability Kit". *Biotechnol. Biotechnol. Equip.* 24: 567–570.

Kell, D.B., Kaprelyants, A.S., Weichart, D.H., Harwood, C.R. and Barer, M.R. (1998). Viability and Activity in Readily Culturable Bacteria: A Review and Discussion of the Practical Issues. *Anton. Van Lee.* 73: 169–187.

Kim, I.S., Kim, Y. and Lim, H.B. (2004). Turbidimetric Measurement for On-line Monitoring of SiO_2 Particles. *Bull. Korean Chem. Soc.* 25: 801–805.

Kort, R., Keijser, B.J., Caspers, M.P., Schuren, F.H. and Montijn, R. (2008). Transcriptional Activity around Bacterial Cell Death Reveals Molecular Biomarkers for Cell Viability. *BMC Genomics* 9: 590.

Landete, J.M., De las Rivas, B., Marcobal, A. and Muñoz, R. (2007). Molecular Methods for the Detection of Biogenic Amine-producing Bacteria on Foods. *Int. J. Food Microbiol.* 117: 258–269.

Lebaron, P., Catala, P. and Parthuisot, N. (1998). Effectiveness of SYTOX Green Stain for Bacterial Viability Assessment. *Appl. Environ. Microbiol.* 64: 2697–2700.

Leštan, D., Podgornik, H. and Perdih, A. (1993). Analysis of Fungal Pellets by UV-Visible Spectrum Diffuse Reflectance Spectroscopy. *Appl. Environ. Microbiol.* 59: 4253–4260.

Lee, A.K.Y., Chan, C.K., Fang, M. and Lau, A. P. S. (2004). The 3-hydroxy Fatty Acids as Biomarkers for Quantification and Characterization of Endotoxins and Gram-negative Bacteria in Atmospheric Aerosols in Hong Kong. *Atmos. Environ.* 38: 6307–6317.

Liu, S., Ng, A.K., Xu, R., Wei, J., Tan, C.M., Yang, Y. and Chen, Y. (2010). Antibacterial Action of Dispersed Single-Walled Carbon Nanotubes on *Escherichia Coli* and *Bacillus Subtilis* Investigated by Atomic Force Microscopy. *Nanoscale* 2: 2744–2750.

Liu, X. and Curtiss, R. (2009). Nickel-inducible Lysis System in *Synechocystis* sp. PCC 6803. *Proc. Nat. Acad. Sci. U.S.A.* 106: 21550–21554.

Molecular Probes Inc. (2004). *Live/Dead® BacLight™ Bacterial Viability Kits*, Leiden, Netherlands.

Nachman, P. Chen, G., Pinnick, R.G., Hill, S.C., Chang, R.K., Mayo, M.W. and Fernandez, G.L. (1996). Conditional-Sampling Spectrograph Detection System for Fluorescence Measurements of Individual Airborne Biological Particles. *Appl. Opt.* 35: 1069–1076.

Nikiyan, H., Vasilchenko, A. and Deryabin, D. (2010). In *Microscopy: Science, Technology, Applications and Education*, Vol. 1, Méndez-Vilas, A. and Díaz, J. (Eds.), Formatex Research Center, Badajoz, Spain, p. 523–529.

Perfetto, S.P., Chattopadhyay, P.K., Lamoreaux, L., Nguyen, R., Ambrozak, D., Koup, R.A. and Roederer, M. (2006). Amine Reactive Dyes: An Effective Tool to Discriminate Live and Dead Cells in Polychromatic Flow Cytometry. *J. Immunol. Methods* 313: 199–208.

Piereira, I.A.C., LeGall, J., Xavier, A.V. and Teixeira, M. (2000). Characterization of a Heme c Nitrite Reductase from a Non-ammonifying Microorganism, *Desulfovibrio vulgaris* Hildenborough. *Biochim. Biophys. Acta* 1481: 119–130.

Ratnesar-Shumate, S.R., Wu, C.Y., Wander, J., Lundgren, D., Farrah, S., Lee, J.H., Wanakule, P., Blackburn, M. and Lan, M.F. (2008). Evaluation of Physical Capture Efficiency and Disinfection Capability of an Iodinated Biocidal Filter Medium. *Aerosol Air Qual. Res.* 8: 1–18.

Rawsthorne, H. and Phister, T. (2009). Detection of Viable *Zygosaccharomyces Bailii* in Fruit Juices Using Ethidium Monoazide Bromide and Real-Time PCR. *Int. J. Food Microbiol.* 131: 246–250.

Ross, K.F.A. and Billing, E. (1957). The Water and Solid Content of Living Bacterial Spores and Vegetative Cells as Indicated by Refractive Index Measurements. *J. Gen. Microbiol.* 16: 418–425.

Rule, A.M., Kesavan, J., Sshwab, K.J. and Buckley, T.J. (2007). Application of Flow Cytometry for the Assessment of Preservation and Recovery Efficiency of Bioaerosol Samplers Spiked with *Pantoea agglomerans. Environ. Sci. Technol.* 41: 2467–2472.

Salard, I., Mercey, E., Rekka, E., Boucher, J.L., Nioche, P., Mikula, I., Martasek, P., Raman, C.S. and Mansuy, D. (2001). Analogies and Surprising Differences between Recombinant Nitric Oxide Synthase-like Proteins from *Staphylococcus aureus* and *Bacillus anthracis* in Their Interactions with L-arginine Analogs and Iron Ligands. *J. Inorg. Biochem.* 100: 2024–2033.

Sengupta, A., Laucks, M.L., Dildine, N., Drapala, E. and Davis, E.J. (2005). Bioaerosol Characterization by Surface-Enhanced Raman Spectroscopy (SERS). *J. Aerosol. Sci.* 36: 651–664.

Seshadri, S., Han, T., Krumins, V., Fennell, D.E. and Mainelis, G. (2009). Application of ATP Bioluminescence Method to Characterize Performance of Bioaerosol Sampling Devices. *J. Aerosol. Sci.* 40: 113–121.

Shahamat M., Levin, M., Rahman, I., Grim, C., Heidelberg, J., Stelma, G. and Colwell, R. (1997). Evaluation of Media for Recovery of Aerosolized Bacteria. *Aerobiologia* 13: 219–226.

Stewart, S.L., Grinshpun, S.A., Willeke, K., Terzieva, S., Ulevicius, V. and Donnelly, J. (1995). Effect of Impact Stress on Microbial Recovery on an Agar Surface. *Appl. Environ. Microbiol.* 61: 1232–1239.

Terzieva, S., Donnelly, J., Ulevicius, V., Grinshpun, S.A., Willeke, K., Stelma, G.N. and Brenner, K.P. (1996). Comparison of Methods for Detection and Enumeration of Airborne Microorganisms collected by Liquid Impingement. *Appl. Environ. Microbiol.* 62: 2264–2272.

Wang, Z.Y., Marx, G., Umetsu, M., Kobayashi, M., Mimuro, M. and Nozawa, T. (1995). Light Scattering and Absorption Caused by Bacterial Activity in Water. *Biochim. Biophys. Acta* 1232: 187–196.

Yang, S.Y., Hsiung, S.K., Hung, Y.C., Chang, C.M., Liao, T.L. and Lee, G.B. (2006). A Cell Counting/Sorting System Incorporated with a Microfabricated Flow Cytometer Chip. *Meas. Sci. Technol.* 17: 2001–2009.

Yoon, K.Y., Park, C.W., Byeon, J.H. and Hwang, J. (2010). Design and Application of an Inertial Impactor in Combination with an ATP Bioluminescence Detector for in Situ Rapid Estimation of the Efficacies of Air Controlling Devices on Removal of Bioaerosols. *Environ. Sci. Technol.* 44: 1742–1746.