An Investigation of the Time Dependency for the Staining Process of Microbes: the Behavior of Microbes in relation to Bio-aerosol Online Monitoring

日本海域研究，第49号，1-10ページ，2018
JAPAN SEA RESEARCH, vol.49, p.1-10, 2018

An Investigation of the Time Dependency for the Staining Process of Microbes: the Behavior of Microbes in relation to Bio-aerosol Online Monitoring

Suthida PIRIYAKARNSAKUL[1], Kaoru TAKARADA[1], Mitsuhiko HATA[2] and Masami FURUUCHI[2*]

Received 22 September 2017
Accepted 22 January 2018

Abstract

The staining of a bio-aerosol, or airborne biological particles that are suspended in the atmosphere in the form of an aerosol, represents a tool that can be used to evaluate the detailed characteristics, and the concentration, of such substances. This can be done online using available technology such as flow cytometry (Aerosol Flow Cytometry). Such an online tool could be useful for examining emission sources and the transport of bio-aerosols. Mixing a bio-aerosol and a mist containing fluorescence dye solution could be utilized as a possible "aerosol dying process". This is a process in which the time required to stain microbes with the dye solution would be a key factor both for staining efficiency and for the size of the instrument to be designed. In this study, the time dependency of the staining process was examined in relation to a combination of a typical type of microbe, or, yeast (*Saccharomyces cerevisiae*) suspended in water and a DAPI solution using a fluorescence spectrophotometer in conjunction with a dye concentration of 0.1-10 μg ml^{-1} and a yeast concentration 0.1-100 μg ml^{-1}. The study showed that the fluorescence intensity of a mixed solution (compared to that of the free dye) increased instantaneously after mixing under selected conditions and that 50 % of the maximum amount of stained yeast could be observed within less than 10 seconds. DAPI was confirmed to stain yeast cells in the order of seconds in the fluorescence spectrum and this could be observed as a red shift in the excitation and emission wavelengths of a mixed solution. By adjusting suitable conditions (e.g., DAPI 3 μg ml^{-1} and yeast ≥ 10 μg ml^{-1} in the present case), the staining processes (<10 sec. 50 % staining) was rapid and the product remained stable. This suggests that the aerosol staining process has the potential for use in this area.

Key Words: bio-aerosol, DAPI, fluorescence staining, time dependency, yeast

I. INTRODUCTION

The airborne biological particles, so-called bio-aerosols, include bacteria, viruses, fungi, plant and animal debris etc., which may exert various human health effects, including allergies and infectious diseases such as tuberculosis (Ryan *et al.*, 2014), influenza (O'Brien *et al.*, 2016), legionellosis (Diederen, 2008; Langer *et al.*, 2012; Wunderlich *et al.*, 2016) and food poisoning. Bio-aerosols and contaminated water can be distributed, suspended and transported long-ranges in the atmosphere by wind and storms. Some microbes can be

[1]Division of Environmental Design, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa, 920-1192 Japan
[2]Faculty of Environmental Design, Institute, of Science and Engineering, Kanazawa University, Kakuma-machi, Kanazawa, 920-1192 Japan
* Author for correspondence

combined with dust particles and cloud droplets (Creamean *et al.*, 2013) that can affect the ecosystem and result in dynamic and unnoticeable human health risks. Bio-aerosols studies have been reported by aerosol and microbial researchers (Ghosh *et al.*, 2015; Yao, 2017). Nevertheless, the evaluation of bio-aerosols requires complicated procedures, extensive skills and time; moreover, the real-time of emission sources cannot be analyzed.

Airborne microbe communities over the Sea of Japan were investigated in terms of their transport over a long distance over the Sea of Japan via Asian dust (KOSA) from the Gobi and Taklamakan desert regions of northern China. Some microbes from KOSA, are tolerant to extreme environmental situations, remain physiologically active and viable over long periods of time and can reach land masses in Japanese territories which may affect the microbial diversity and the ecosystem (Yamaguchi *et al.*, 2012, 2014), and human health risk (Kobayashi *et al.*, 2016). It is also noteworthy that some microbes can be transported from Japan marine areas to local land masses (Maki *et al.*, 2015) and also airborne microbes via KOSA can be transported to North America (Smith *et al.*, 2012).

The evaluation of airborne microbes based on culture methods requires special skills and is quite labor intensive, although the majority of microbes cannot be detected due to the incompatible of medium or the fact that they a difficult or impossible to culture (Fabian *et al.*, 2005; Heo *et al.*, 2017). To address this issue, technologies that are culture-independent such as fluorescence microscope, liquid flow cytometry (FCM), gene analysis and related methods have been developed. The detection sensitivity and accuracy of these technologies have been dramatically improved. In Japan, communities of airborne microbes during KOSA and non KOSA events were investigated by, for example, epifluorescence microscope (Hara and Zhang, 2012; Murata and Zhang, 2014), PCR-DGGE (Maki *et al.*, 2010, 2011) and real-time PCR (Yamaguchi *et al.*, 2012, 2014; Kobayashi *et al.*, 2016). In North America (Smith *et al.*, 2012) and Korea (Jeon *et al.*, 2013) the influence of these microbes during a KOSA event were examined using a combination of culture methods and PCR to evaluate characteristics and sources. Nevertheless, such analytical methods required to collect or transfer airborne microbes into liquid before analysis. Existing methods for detecting airborne microbes utilize the autofluorescence of microbes that directly detect the fluorescence emitted by airborne microbes (Hill *et al.*, 1999; Stanley *et al.*, 2011; Bhangar *et al.*, 2014). False positive/false negative of autofluorescence was rather large due to the fact that some microbes could not be detected and classified (Healy *et al.*, 2014; O'Connor *et al.*, 2015; Saari *et al.*, 2015); however, the fluorescent staining of airborne microbes has not been examined. On the other hand, techniques for detecting scattered light of aerosol particles in the air have been reported (Anselm *et al.*, 1987; Quinten *et al.*, 2000; Snider and Petters, 2007). The intensity of scattered light depends on the size, shape and refractive index of individual single particles. However, because some such particles derived from microbes are nonspherical in shape, it is more difficult to accurately detect them, since the scattered light of nonspherical particles is different from that of spheres. In addition, the fluorescent staining of cells in a liquid for use in fluorescence microscopy observations and in a pretreatment for FCM has not been yet performed in the air.

Since FCM technology is practical is used as a cell measurement device in liquids due to its capabilities of rapid cell counting and characteristic evaluation, it can be used to detect not only scattered but also absorbed and fluorescence emitted light from single stained cells (Caron *et al.*, 1998; Gaforio *et al.*, 2002; Raithatha and Stuart, 2009). FCM has had a large impact in medical engineering and has been widely used for the environmental analysis of microorganisms recently (Porter *et al.*, 1997; Lange *et al.*, 1997; Chen and Li, 2005, 2007; Galès *et al.*, 2015). On the other hand, methods for the detection and evaluation of microbes in an aerosol state, somewhat lacking and our understanding of the behavior of airborne microbes is less than in liquid. Staining of airborne microbes in aerosol state can provide a tool for evaluating the detailed characteristics and concentration on line by applying FCM technology as Aerosol Flow Cytometry.

Such an online tool would be useful for examining emission sources and transportation behaviors of bio-aerosols. Therefore, the mixing of airborne microbes and a mist of a fluorescence dye solution may be a possible "aerosol staining process". If aerosol microbial fluorescence staining technology could be achieved, a rapid and accurate in-situ measurement which has not existed so far will be realized. Accordingly, it will have a great benefit on fields such as infection risk in medical institutions, workplace environmental management, environmental monitoring, and real-time evaluation of bio-aerosols.

For the above proposed technology, time dependency for the microbe staining process in the order of seconds is critically important since it would be a key factor both for staining efficiency and size of the instrument to be designed, therefore, the time dependency of the staining process was discussed on a combination of a typical sort of microbes, or, yeast (*S. cerevisiae*) suspended in water and a DAPI solution using a fluorescence spectrophotometer which may propose a large plausibility of the aerosol staining process.

II. MATERIALS AND METHODS

DAPI (4',6-diamidino-2-phenylindole) (Nacalai Tesque, Inc., Japan) is generally known as a DNA binding fluorescence dye for DNA assays in flow cytometry. Since DAPI is a stable fluorescence dye and the time dependency was investigated in the order of seconds, DAPI was used to prepare dye solution in pure water without further purification. A dye solution was prepared in a 5 mm micro cuvette which was used, since it was necessary to mix the solution inside the cuvette extensively and reduce the path length to minimize inner filtering effects of solution. Dye was prepared in the micro cuvette and placed inside spectrofluorometer (Jasco FP8200, Jasco International Co., Ltd., Japan) before mixing with yeast solution. Commercial baker's yeast powder (*S. cerevisiae*) from Nippon Flour Mills Co., Ltd., Japan was suspended in pure water without further purification to prepare yeast solution. For a well suspended mixture of yeast and dye solution and instantaneous measurement after mixing was needed, the yeast solution was injected directly by means of a micro syringe (Hamilton 1750RN, USA) from outside spectrofluorometer through the injection hole above the closing cover into the cuvette that dye solution was already prepared and placed inside the spectrofluorometer. The total volume of the solutions were adjusted to 1 ml. DAPI at a final concentration of 0.1 and 1 $\mu g\ ml^{-1}$ were mixed with the yeast suspension which was adjusted to a final concentration of 1-100 $\mu g\ ml^{-1}$ to examine the consequences of yeast concentration. Yeast at a final concentration of 100 $\mu g\ ml^{-1}$ were mixed with a dye solution at a final concentration of 0.1-10 $\mu g\ ml^{-1}$ to study consequence of dye concentration on the staining process (Table 1).

Table 1 Conditions for the experiments.

Consequence of yeast concentration					
DAPI concentration ($\mu g\ ml^{-1}$)	Yeast concentration ($\mu g\ ml^{-1}$)				
0.1	1	5	10	50	100
1	1	5	10	50	100
Consequence of dye concentration					
Yeast concentration ($\mu g\ ml^{-1}$)	DAPI concentration ($\mu g\ ml^{-1}$)				
100	0.1	1	3	5	10

The change in fluorescence intensity with time and the excitation and emission spectrum of each condition were measured instantaneously after mixing at room temperature in three replications. An excitation wavelength of 360 nm, an emission wavelength of 456 nm, excitation and emission bandwidths of 5 nm were used in the measurement. Time dependency of each mixture was measured at each second after the mixing.

III. RESULTS AND DISCUSSION

Fluorescence emission intensity at an excitation wavelength 360 nm, emission wavelength 456 nm of free DAPI and mixtures of DAPI and yeast are shown in Fig. 1, the intensity of mixtures of 0.1 and 1 μg ml^{-1} DAPI concentration with 1-100 μg ml^{-1} yeast concentration increased instantaneously and gradually reached equilibrium except for a mixture of 0.1 μg ml^{-1} DAPI with 1 μg ml^{-1} yeast. Mixtures of 0.1 μg ml^{-1} DAPI with 50-100 μg ml^{-1} yeast and 1 μg ml^{-1} DAPI with ≥ 10 μg ml^{-1} yeast resulted a greater increase in intensity, a higher stability and a lower staining time due to the decrease in the time to reach equilibrium when yeast concentration was increased, and concentrations of yeast ≤ 1 μg ml^{-1} yeast resulted in inconsistent data. Furthermore the mixtures of 0.1 μg ml^{-1} DAPI needed longer time to reach equilibrium compared with the mixtures of 1 μg ml^{-1} DAPI. When the yeast concentration was increased the total intensity change in 0.1 μg ml^{-1} DAPI was decreased, since the concentration of dye was limited. On the other hand, Tijssen *et al.* (1982) reported an instantaneous decrease of fluorescence intensity after adding yeast (*Saccharomyces fragilis*) to a DAPI solution at a concentration of 0.2 μg ml^{-1} in 3 mM Tris-maleate at an emission wavelength 456 nm while the increase was instantaneous at 525 nm due to DAPI bound to peripherally-localized polyphosphates on the surface of yeast cells which could not observed in commercial baker's yeast in their study and after prolonged incubation the fluorescence intensity at 456 nm gradually increased since the DAPI was bound to and taken up by the yeast cells. Therefore our results suggest that the proper amount of yeast (*S. cerevisiae*) for staining should be ≥ 10 μg ml^{-1} since the staining of live cells requires a plasma membrane that is permeable to fluorochrome that stoichiometrically stains DNA and depends on cell type which some of which take up fluorochrome at different rates (Darzynkiewicz, 2011) thus, yeast type and concentration are important factors in the overall staining process.

Fluorescence intensity of 0.1-10 μg ml^{-1} DAPI concentration mixed with 100 μg ml^{-1} yeast concentration increased instantaneously from free DAPI. The mixtures of 0.1 μg ml^{-1} DAPI required a longest time to reach equilibrium and the time required decreased with increasing dye concentration as shown in Fig. 2; however, fluorescence intensity of the mixture of ≥ 3 μg ml^{-1} DAPI trended to decrease gradually with

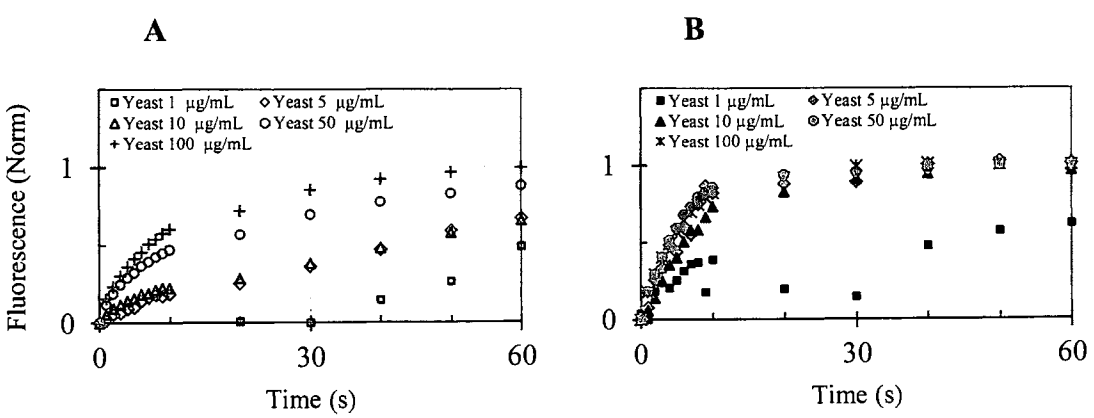

Fig. 1 Time dependency for fluorescence intensity change per 1 μg ml^{-1} yeast concentration (normalize). DAPI (A) 0.1 μg ml^{-1} and (B) 1 μg ml^{-1} concentration and yeast at a concentration of 1-100 μg ml^{-1}, excitation wavelength 360 nm and emission wavelength 456 nm.

time. The proper DAPI concentration for yeast (*S. cerevisiae*) staining should be < 10 μg ml^{-1}. A clear histogram from cells counted by fluorescent microscopy in glioma cell line U87 was demonstrated using the optimal DAPI concentration at DAPI 0.4-0.6 μg ml^{-1} (Sun *et al.*, 2017). A DAPI concentration at >2–10 mg L^{-1} could easily distinguished bacteria by microscopy when DAPI was used (Yu *et al.*, 1995). At high dye concentration, the contribution the fluorescence spectrum of DAPI bound to different polynucleotides could not be observed (Biancardi *et al.*, 2013), therefore the dye concentration was also a factor in the characteristics and stability of microbes in the staining process.

To achieve stability in staining focusing on time dependency at 50 % of the maximum amount of stained yeast, the proportion of microbes (microbe: dye ratio) is an important factor that can affect the staining results (Darzynkiewicz, 2011), while staining time is important for staining efficiency and the size of the instrument to be designed (Fig. 3). A staining time of 0.1 μg ml^{-1}, a DAPI concentration of 50 % of the maximum of stained yeast at 1, 5, 10, 50 and 100 μg ml^{-1} yeast concentration were 60, 40, 40, 10 and 7 sec, respectively. A 1 μg ml^{-1} DAPI concentration resulted in staining times of 50, 5, 6, 4 and 4 sec for 50 % of the maximum of stained yeast concentrations of 1, 5, 10, 50 and 100 μg ml^{-1} respectively. 0.1 μg ml^{-1} DAPI with ≥ 50 μg ml^{-1} yeast and 1 μg ml^{-1} DAPI with ≥ 5 μg ml^{-1} yeast required a staining time less than 10 sec while a longer time was required for the other concentrations. These data indicate that half of the yeast fraction could be stained within 10 sec using 0.1 and 1 μg ml^{-1} DAPI concentrations with a yeast concentration of using ≥ 10 μg ml^{-1}. For 100 μg ml^{-1} yeast concentration, 50 % of the maximum staining of yeast was achieved by using 0.1, 1, 3 and 5 μg ml^{-1} DAPI concentrations at 7, 4, 1 and 1 sec, respectively. Thus, ≥ 10 μg ml^{-1} yeast with DAPI 3-10 μg ml^{-1} should be used in staining airborne microbes in an aerosol state in order to achieve a high stability and lower total intensity change in staining because a shorter time for yeast staining is required using this condition.

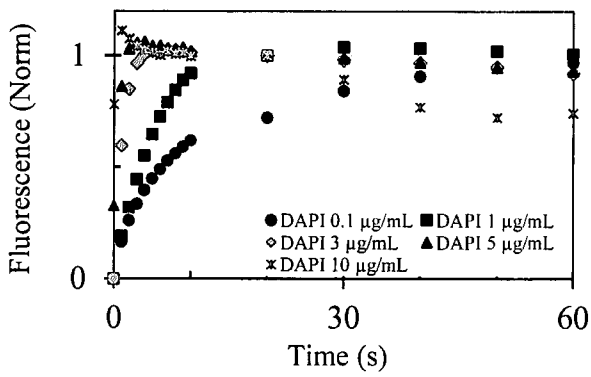

Fig. 2 Time dependency for fluorescence emission intensity change (normalize) of 0.1-10 μg ml^{-1} DAPI concentration and 100 μg ml^{-1} yeast concentration, excitation wavelength 360 nm and emission wavelength 456 nm.

Excitation and emission spectra of 0.1 and 1 μg ml^{-1} DAPI concentration and mixtures of 1-100 μg ml^{-1} yeast concentration were obtained immediately after mixing. The excitation fluorescence intensity could be enhanced

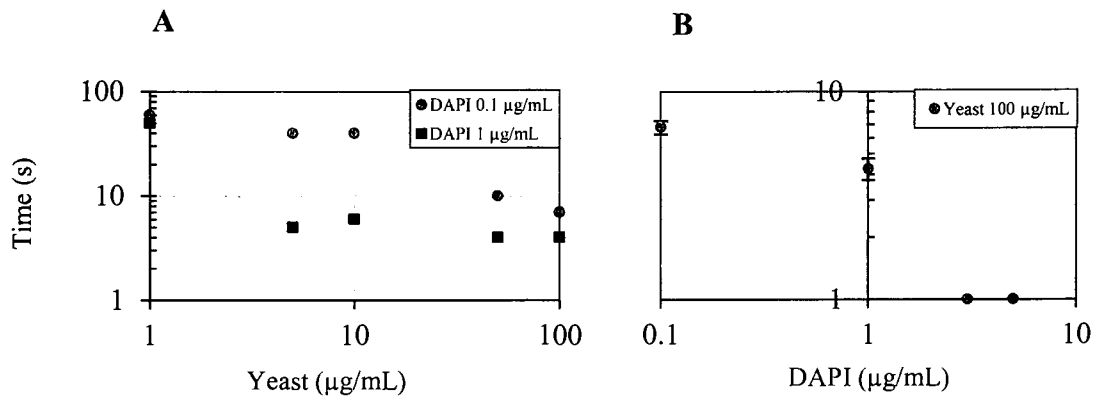

Fig. 3 Time dependency for staining half of the yeast cells (A) as a consequence of yeast concentrations in the range of 1-100 μg ml^{-1}, (B) consequences of dye concentration for 0.1-10 μg ml^{-1}, excitation wavelength 360 nm and emission wavelength 456 nm.

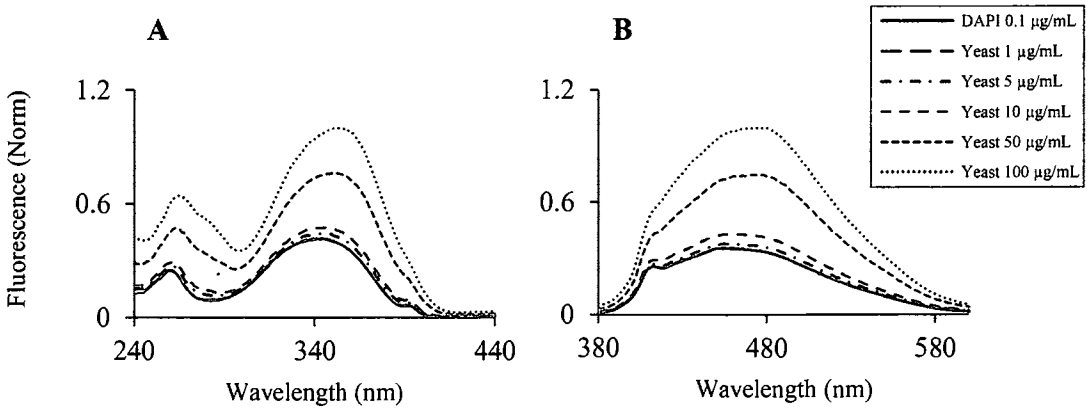

Fig. 4 Fluorescence spectrum of free DAPI at concentrations of 0.1 μg ml^{-1} and mixing with yeast at concentration of 1-100 μg ml^{-1}. (A) excitation spectrum (emission at 456 nm) and (B) emission spectrum (excitation at 360 nm).

by using 0.1 and 1 μg ml^{-1} DAPI mixed with 5-100 μg ml^{-1} yeast compared with DAPI mixed with < 5 μg ml^{-1} yeast or free DAPI (Fig. 4). When the yeast concentration was increased, the maximum excitation spectrum of 0.1 and 1 μg ml^{-1} free DAPI was shifted from 344 and 344 to 351 and 350 nm respectively. The above data are consistent with the absorbance red shift reported for DAPI bound to calf thymus DNA (Lin *et al.*, 1977), polyd(G-C)2 (Wilson *et al.*, 1989), poly(A) (Kapuscinski, 1990), poly(A)-poly(U) (Tanious *et al.*, 1992), single- and double-stranded DNA (Kapuscinski, 1995; Cosa *et al.*, 2001; Biancardi *et al.*, 2013). Analogous to mixing DAPI with calf thymus DNA, double-stranded DNA, poly(A) or poly(A)-poly(U), mixing DAPI with yeast was also found to enhance the fluorescence intensity in our study. When the yeast concentration was increased, the fluorescence intensity was also increased substantially. The maximum emission spectrum of 0.1 and 1 μg ml^{-1} free DAPI were shifted from 455 and 457 to 470 and 477 nm respectively when yeast at a concentration of 5-100 μg ml^{-1} was added. The enhancement in fluorescence intensity and the maximum emission spectrum red shift for the mixture of 0.1 and 1 μg ml^{-1} DAPI with 5-100 μg ml^{-1} yeast gave results that were the same as that for when DAPI bound to poly(A) and poly(A)-poly(U). Tijssen *et al.* (1982) observed a rapid red shift in the emission spectrum from 456 nm for free DAPI to 525 nm for DAPI stained yeast (*S. fragilis*) since a reaction of DAPI with peripherally-localized polyphosphates on the surface of yeast cells could not observed in commercial baker's yeast, indicating the absence of or a low concentration of peripherally-localized polyphosphates.

IV. CONCLUSION

The time dependency and spectra of the yeast staining process indicate that the yeast *S. cerevisiae* were stained instantaneously by DAPI in pure water at dye concentrations of 0.1-10 μg ml^{-1} and 50 % of the maximum amount of stained yeast could be observed in less than 10 seconds, while using optimal microbe and dye ratios resulted in an instantaneous increase of fluorescence intensity when yeast cells were added to a dye solution and spectral shifts demonstrated that the fluorescence intensity was increased and that spectral shifts were observed when DAPI became bound to and formed an intercalation complex in G-C regions in DNA, A-U regions in RNA or polynucleic acid inside the cells. The proportion of yeast and dye concentration effected both staining efficiency and time dependency, and, by adjusting the conditions (*e.g.*, DAPI 3 μg ml^{-1} and yeast ≥ 10 μg ml^{-1} for the present case), stable and rapid staining processes (<10 seconds 50 % staining) could be obtained. The above findings suggest that the use of an aerosol staining process represents a plausible approach to solving this problem.

REFERENCES

Anselm, A., Gebhart, J. and Heyder, J., 1987: Interpretation of Response Functions of Optical Particle Counters. *Journal of Aerosol Science*, **18**, 873-876.

Bhangar, S., Huffman, J. A. and Nazaroff, W. W., 2014: Size-resolved fluorescent biological aerosol particle concentrations and occupant emissions in a university classroom. *Indoor Air*, **24**, 604-617.

Biancardi, A., Biver, T., Secco, F. and Mennucci, B., 2013: An investigation of the photophysical properties of minor groove bound and intercalated DAPI through quantum-mechanical and spectroscopic tools. *Physical Chemistry Chemical Physics*, **15**, 4596-4603.

Caron, G. N., Stephens, P. and Badley, R. A., 1998: Assessment of Bacterial Viability Status by Flow Cytometry and Single Cell Sorting. *Journal of Applied Microbiology*, **84**, 988-998.

Chen, P. S. and Li, C. S., 2005: Bioaerosol characterization by flow cytometry with fluorochrome. *Journal of Environmental Monitoring*, **7**, 950-959.

Chen, P. S. and Li, C. S., 2007: Real-time monitoring for bioaerosols—flow cytometry. *Analyst*, **132**, 14-16.

Cosa, G., Focsaneanu, K. S., McLean, J. R. N., McNamee, J. P. and Scaiano, J. C., 2001: Photophysical properties of fluorescent DNA-dyes bound to single- and double-stranded DNA in aqueous buffered solution. *Photochemistry and Photobiology*, **73**, 585-599.

Creamean, J. M., Suski, K. J., Rosenfeld, D., Cazorla, A., DeMott, P. J., Sullivan, R. C., White, A. B., Ralph, F. M., Minnis, P., Comstock, J. M., Tomlinson, J. M. and Prather, K. A., 2013: Dust and biological aerosols from the Sahara and Asia influence precipitation in the western U.S.. *Science*, **339**, 1572-1578.

Darzynkiewicz, Z., 2011: Critical Aspects in Analysis of Cellular DNA Content. *Current Protocols in Cytometry*, doi:10.1002/0471142956.cy0702s52

Diederen, B. M. W., 2008: *Legionella* spp. and Legionnaires' disease. *Journal of Infection*, **56**, 1-12.

Fabiana, M. P., Millerb, S. L., Reponenc, T. and Hernandez, M. T., 2005: Ambient bioaerosol indices for indoor air quality assessments of flood reclamation. *Aerosol Science*, **36**, 763-783.

Gaforio, J. J., Serrano, M. J., Ortega, E., Algarra, I. and Cienfuegos, A., 2002: Use of sytox green dye in the flow cytometric analisis of bacterial phagocytosis. *Cytometry*, **48**, 93-96.

Galès, A., Bru-Adan, V., Godon, J. J., Delabre, K., Catala, P., Ponthieux, A., Chevallier, M., Birot, E., Steyer, J. P. and Wéry, N., 2015: Predominance of single bacterial cells in composting bioaerosols. *Atmospheric Environment*, **107**, 225-232.

Ghosh, B., Lal, H. and Srivastava, A., 2015: Review of bioaerosols in indoor environment with special reference to sampling, analysis and control mechanisms. *Environment International*, **85**, 254-272.

Hara, K. and Zhang, D., 2012: Bacterial abundance and viability in long-range transported dust. *Atmospheric Environment*, **47**, 20-25.

Healy, D. A., Huffman, J. A., O'Connor, D. J., Pöhlker, C., Pöschl, U. and Sodeau, J. R., 2014: Ambient measurements of biological aerosol particles near Killarney, Ireland: A comparison between real-time fluorescence and microscopy techniques. *Atmospheric Chemistry and Physics*, **14**, 8055-8069.

Heo, K. J., Lim, C. E., Kim, H. B. and Lee, B. U., 2017: Effects of human activities on concentrations of culturable bioaerosols in indoor air environments. *Journal of Aerosol Science*, **104**, 58-65.

Hill, S. C., Pinnick, R. G., Niles, S., Pan, Y. L., Holler, S., Chang, R. K., Bottinger, J., Chen, B. T., Orr, C. S. and Feather, G., 1999: Realtime measurement of fluorescence spectra from single airborne biological particles. *Field Analytical Chemistry and Technology*, **3**, 221-239.

Jeon, E. M., Kim, Y. P., Jeong, K., Kim, I. S., Eom, S. W., Choi, Y. Z. and Ka, J. O., 2013: Impacts of Asian dust events on atmospheric fungal communities. *Atmospheric Environment*, **81**, 39-50.

Kapuscinski, J., 1990: Interactions of nucleic acids with fluorescent dyes: spectral properties of condensed complexes. *Journal of Histochemistry and Cytochemistry*, **38**, 1323-1329.

Kapuscinski, J., 1995: DAPI: a DNA-specific fluorescent probe. *Biotechnic and Histochemistry*, **70**, 220-233.

Kobayashi, F., Iwata, K., Maki, T., Kakikawa, M., Higashi, T., Yamada, M., Ichinose, T. and Iwasaka, Y., 2016: Evaluation of the toxicity of a Kosa (Asian duststorm) event from view

of food poisoning: observation of Kosa cloud behavior and real-time PCR analyses of Kosa bioaerosols during May 2011 in Kanazawa, Japan. *Air Quality, Atmosphere, and Health*, **9**, 3-14.

Lange, J. L., Thorne, P. S. and Lynch, N., 1997: Application of flow cytometry and fluorescent in situ hybridization for assessment of exposures to airborne bacteria. *Applied and Environmental Microbiology*, **63**, 1557-1563.

Langer, V., Hartmann, G., Niessner, R. and Seidel, M., 2012: Rapid quantification of bioaerosols containing L. pneumophila by Coriolis smair sampler and chemiluminescence antibody microarrays. *Journal of Aerosol Science*, **48**, 46-55.

Lin, M. S., Comings, D. E. and Alfi, O. S., 1977: Optical studies of the interaction of 4'-6-Diamidino-2-Phenylindole with DNA and metaphase chromosomes. *Chromosom*, **60**, 15-25.

Maki, T., Susuki, S., Kobayashi, F., Kakikawa, M., Tobo, Y., Yamada, M., Higashi, T., Matsuki, A., Hong, C., Hasegawa, H. and Iwasaka, Y., 2010: Phylogenetic analysis of atmospheric halotolerant bacterial communities at high altitude in an Asian dust (KOSA) arrival region, Suzu City. *Science of the Total Environment*, **408**, 4556-4562.

Maki, T., Aoki, K., Kobayashi, F., Kakikawa, M., Tobo, Y., Matsuki, A., Hasegawa, H. and Iwasaka, Y., 2011: Communities in asian desert dust (KOSA) bioaerosol accumulated in layers of snow on Mount Tateyama, central Japan. *Aerobiologia (Bologna)*, **27**, 277-290.

Maki, T., Hara, K., Kobayashi, F., Kurosaki, Y., Kakikawa, M., Matsuki, A., Chen, B., Shi, G., Hasegawa, H. and Iwasaka, Y., 2015: Vertical distribution of airborne bacterial communities in an Asian-dust downwind area, Noto Peninsula. *Atmospheric Environment*, **119**, 282-293.

Murata, K. and Zhang, D., 2014: Transport of bacterial cells toward the Pacific in Northern Hemisphere westerly winds. *Atmospheric Environment*, **87**, 138-145.

O'Brien, K. M., Nonnenmann, M. W., Ellenbecker, M., Moure-Eraso, R., Sparer, J. and Vegso, S., 2016: Airborne influenza A is detected in the personal breathing zone of Swine Veterinarians. *PloS One*, **11**, 8 p.

O'Connor, D. J., Daly, S. M. and Sodeau, J. R., 2015: On-line monitoring of airborne bioaerosols released from a composting/green waste site. *Waste Management*, **42**, 23-30.

Porter, J., Pickup, R. and Edwards, C., 1997: Evaluation of flow cytometric methods for the detection and viability assessment of bacteria from soil. *Soil Biology and Biochemistry*, **29**, 91-100.

Quinten, M., Friehmelt, R. and Ebert, K. -F., 2000: Sizing of aggregates of spheres by a white-light optical particle counter with 90° scattering angle. *Aerosol Science*, **32**, 63-72.

Raithatha, S. A. and Stuart, D. T., 2009: A comparison of fluorescent DNA binding dyes for flow cytometric analysis of sporulating *Saccharomyces cerevisiae*. *Journal of Microbiological Methods*, **78**, 357-359.

Ryan, G. J., Shapiro, H. M. and Lenaerts, A. J., 2014: Improving acid-fast fluorescent staining for the detection of mycobacteria using a new nucleic acid staining approach. *Tuberculosis*, **94**, 511-518.

Saari, S., Mensah-Attipoe, J., Reponen, T., Veijalainen, A. M., Salmela, A., Pasanen, P. and Keskinen, J., 2015: Effects of fungal species, cultivation time, growth substrate, and air exposure velocity on the fluorescence properties of airborne fungal spores. *Indoor Air*, **25**, 653-661.

Smith, D. J., Jaffe, D. A., Birmele, M. N., Griffin, D. W., Schuerger, A. C., Hee, J. and Roberts, M. S., 2012: Free tropospheric transport of microorganisms from Asia to North America. *Microbial Ecology*, **64**, 973-985.

Snider, J. R. and Petters, M. D., 2007: Optical particle counter measurement of marine aerosol hygroscopic growth. *Atmospheric Chemistry and Physics. Discuss*, **7**, 12381-12415.

Stanley, W. R., Kaye, P. H., Foot, V. E., Barrington, S. J., Gallagher, M. and Gabey, A., 2011: Continuous bioaerosol monitoring in a tropical environment using a UV fluorescence particle spectrometer. *Atmospheric Science Letters*, **12**, 195-199.

Sun, J., Zhang, J., Yang, H., Wang, G., Li, Y., Zhang, X., Chen, Q. and Lang, M. F., 2017: Microfluidic cell cycle analysis of spread cells by DAPI staining. *Micromachines*, **8**, 1-8.

Tanious, F. A., Veal, J. M., Buczak, H., Ratmeyer, L. S. and Wilson, W. D., 1992: DAPI (4',6-Diamidino-2-phenylindole) binds differently to DNA and RNA: minor-groove binding at AT sites and intercalation at AU sites. *Biochemistry*, **31**, 3103-3112.

Tijssen, J. P. F., Beekes, H. W. and Van Steveninck, J., 1982: Localization of polyphosphates in *Sccharomyces fragilis*, as

revealed by 4',6-Diamidino-2-phenylindole Fluorescence. *Biochimica et Biophysica Acta*, **721**, 394-398.

Wilson, W. D., Tanious, F. A., Barton, H. J., Strekowski, L. and Boykin, D. W., 1989: Binding of 4',6-diamidino-2-phenylindole (DAPI) to GC and mixed sequences in DNA: intercalation of a classical groove-binding molecule. *Journal of the American Chemical Society*, **111**, 5008-5010.

Wunderlich, A., Torggler, C., Elsässer, D., Lück, C., Niessner, R. and Seidel, M., 2016: Rapid quantification method for Legionella pneumophila in surface water. *Analytical and Bioanalytical Chemistry*, **408**, 2203-2213.

Yamaguchi, N., Ichijo, T., Sakotani, A., Baba, T. and Nasu, M., 2012: Global dispersion of bacterial cells on Asian dust. *Scientific Report*, **2**, 525.

Yamaguchi, N., Ichijo, T., Baba, T. and Nasu, M., 2014: Long-range transportation of bacterial cells by Asian dust introduction: aeolian dust events and long-range transportation of bacterial cells. *Genes and Environment*, **36**, 145-151.

Yao, M., 2018: Bioaerosol: A bridge and opportunity for many scientific research fields. *Journal of Aerosol Science* **115**, 108-112.

Yu, W. E. I., Dodds, W. K., Banks, M. K. and Skalsky, J., 1995: Optimal staining and sample storage time for direct microscopic enumeration of total and active bacteria in soil with two fluorescent dyes. *Applied and Environmental Microbiology*, **61**, 3367-3372.

微生物染色特性の時間依存性に関する検討：バイオエアロゾルオンライン計測のための基礎特性

PIRIYAKARNSAKUL Suthida[1]・寶田　馨[1]・畑　光彦[2]・古内正美[2*]

2017年9月22日受付
2018年1月22日受理

要　旨

気中に浮遊する微生物（バイオエアロゾル）のDNAを浮遊状態で気中染色できれば，既存のフローサイトメトリー等の計測技術をエアロゾルに応用して（エアロゾルフローサイトメトリー），微生物の種類・濃度などの詳細な情報をオンライン計測できる可能性がある。こうしたオンライン計測の実現は，バイオエアロゾル発生源の特定や輸送変動を議論する上で有用なツールとなる。バイオエアロゾルと蛍光染料ミストとの混合は気中染色法の候補の一つであるが，染色液と接した菌のDNA染色に要する時間がエアロゾル保持時間・方法，装置寸法を決定する重要なファクターとなる。本研究では，イースト菌と代表的な蛍光物質であるDAPI溶液の組み合わせを例として，様々な菌・蛍光物質濃度で菌分散液とDAPI溶液の混合直後からの秒単位のDNA蛍光強度の変化特性を蛍光分光光度計で計測し，混合条件と染色時間の関係に検討を加えた。この結果，適切な濃度条件（例えばDAPI 3 μg ml^{-1}，イースト菌10 μg ml^{-1}）を設定することで再現性のある安定した時間特性が得られること，10秒以下の極めて短時間にDNAの50%以上が染色されることを示し，気中染色法の実現可能性を示した。

キーワード：バイオエアロゾル，DAPI，蛍光染色，時間依存性，イースト菌

[1]金沢大学大学院自然科学研究科環境デザイン学専攻　〒920-1192 石川県金沢市角間町
[2]金沢大学理工研究域環境デザイン学系　〒920-1192 石川県金沢市角間町
*連絡著者