Journal of Bioscience and Bioengineering
VOL. 119 No. 5, 570–579, 2015

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Bioprocess of Kosa bioaerosols: Effect of ultraviolet radiation on airborne bacteria within Kosa (Asian dust)

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Received 10 April 2014; accepted 22 October 2014
Available online 24 February 2015

Kosa (Asian dust) is a well-known weather phenomenon in which aerosols are carried by the westerly winds from inland China to East Asia. Recently, the frequency of this phenomenon and the extent of damage caused have been increasing. The airborne bacteria within Kosa are called Kosa bioaerosols. Kosa bioaerosols have affected ecosystems, human health and agricultural productivity in downwind areas. In order to develop a new and useful bacterial source and to identify the source region of Kosa bioaerosols, sampling, isolation, identification, measurement of ultraviolet (UV) radiation tolerance and experimental simulation of UV radiation conditions were performed during Kosa bioaerosol transportation. We sampled these bioaerosols using a Cessna 404 airplane and a bioaerosol sampler at an altitude of approximately 2900 m over the Noto Peninsula on March 27, 2010. The bioaerosol particles were isolated and identified as *Bacillus* sp. BASZHR 1001. The results of the UV irradiation experiment showed that the UV radiation tolerance of Kosa bioaerosol bacteria was very high compared with that of a soil bacterium. Moreover, the UV radiation tolerance of Kosa bioaerosol spores was higher than that of soil bacterial spores. This suggested that Kosa bioaerosols are transported across the atmosphere as living spores. Similarly, by the experimental simulation of UV radiation conditions, the limited source region of this Kosa bioaerosol was found to be southern Russia and there was a possibility of transport from the Kosa source area.

[**Key words:** Bioaerosol; Airborne bacteria; Bioprocess; Kosa; Asian dust; Sampling using airplane; UV radiation; *Bacillus* sp.]

Mineral or soil particles that are blown into the atmosphere by winds in arid and semi-arid areas such as the Taklamakan Desert, Gobi Desert and Loess Plateau in inland China are transported by the westerly winds and often reach Japan (1). The phenomenon called Kosa describes the entrainment of mineral and soil particles from the atmosphere, diffusion on a global and/or regional scale and their deposition in a distant area. This phenomenon, also referred to as dust or sandstorm in more general terms, is often observed in March and April or sometimes in November in Japan. Kosa has been a well-known weather phenomenon since ancient times. Recently, however, the frequency and the associated damage have been increasing (2).

In our previous study, we pioneered the investigation of microorganisms within Kosa particles by sampling and bioanalysis using a tethered balloon in Dunhuang City, western China, which was the source region of Kosa in 2006 (3). Since then, we have investigated Kosa bioaerosols in the atmosphere using a tethered balloon and an airplane in Japan and China and have detected many kinds of microorganisms (4–8).

The long-range transport of Kosa bioaerosols plays an important role in microbial dispersal and considerably affects ecosystems, human health and agricultural productivity in downwind areas (8–10). Mankinds successful development and progress of research related to cellular activities has resulted in many health, social, environmental and economic effects on past and contemporary human civilizations (11). Many useful microorganisms have been isolated from the soil, river or sea, and have been used to treat wastewater and produce ethanol, glycerol and fermented foods, such as cheese, bread and yogurt. However, few investigations have used isolates from aerosols for biochemical engineering purposes. Because the environmental conditions of high-altitude atmosphere include high ultraviolet (UV) radiation, low pressure, dryness and low temperature, living bioaerosols that survive such long-range transportation must exhibit a high tolerance to these conditions and are likely to be extremophiles. In the future, isolates from high-altitude aerosols should be investigated as a source of novel and useful microorganisms.

Determination of the origin of Kosa bioaerosols is not only interesting but also important. To understand the phylogeography

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1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2014.10.015

of these bioaerosols, DNA analysis has been commonly used. Hua et al. (12) investigated, in detail, desert-originated bacteria that were transported to Japan by Kosa storms. Bacteria isolated from dust in Higashi-Hiroshima, Japan, during a Kosa event were compared with those from the sand dunes in Dunhuang City, China revealing high sequence similarities in not only 16S rRNAs but also in the universal housekeeping genes, *gyrB* and *ParE* (12). This study provides evidence of microbial transport by a Kosa event in northeast Asia. However, definitive identification of long-range bacterial transport based only on DNA information is difficult, particularly for cosmopolitan bacteria.

In this study, we investigated microbial transport during a Kosa event using a bioprocess approach and tried to estimate the limited source region of these bioaerosols. In addition, we tried to clarify the effect of solar UV radiation on microbe during long-range transport. First, we sampled microbes using an airplane and a bioaerosol sampler. The cultured isolates were subsequently identified. Second, to evaluate the utility of airborne isolates, we examined their tolerance to UV radiation experimentally. Finally, the limited source region of these bioaerosols was estimated by a UV irradiation experiment that simulated the atmospheric conditions, and for the first time, the effect of solar UV radiation on bioaerosols during long-range transport was assessed.

MATERIALS AND METHODS

Bioaerosol sample collection We focused primarily on preventing contamination from factors such as researchers, observers, airplanes and other microorganisms. We sampled these bioaerosols using a Cessna 404 airplane (Nakanihon Air Service Co., Ltd., Toyoyama, Aichi, Japan), and this collection was sampled using the apparatus as shown in Fig. 1. A hole of 20 mm in diameter was present on the aircraft roof. Before sampling, this hole was sealed and during, sampling, it was opened, and a sterilized inlet was inserted (Fig. 1). The inlet was connected to a sterilized tube. The inlet and tube used for sampling were made of conductive polytetrafluoroethylene (PTFE) (Asone Co., Ltd., Osaka, Japan) and conductive silicon (Sibata Scientific Technology Co., Ltd., Kusaka, Saitama, Japan), respectively. Because both tubes are highly conductive and can be sterilized by steam, they are suitable for sampling these bioaerosols. By calculating streamlines of the air around the airplane body, we found that it had a little influence on an inlet length of $\leq$10 mm. We thus used a 15 mm long inlet. This tube was connected to the bioaerosol sampler system developed by us (Fig. 1), which was used on the tethered balloon in our previous experiment (3–8). These bioaerosols were collected on a membrane filter with 0.45 μm pores that was set into a filter holder (in-line filter holder, 47 mm; Merck Millipore Co., Ltd., Tokyo, Japan) on the sampler under sterile conditions after autoclaving (Fig. 1, c). The volume of the air sampled was estimated to be 1.0 m^3 over a 60 min sampling time with a flow rate of 17.0 L min^{-1} and was regulated by a light-weighted, small and high-performing air pump (Fig. 1, e). To avoid contamination during nonsampling periods, the inlet and outlet of the filter holder were closed by the shutter (Fig. 1, a).

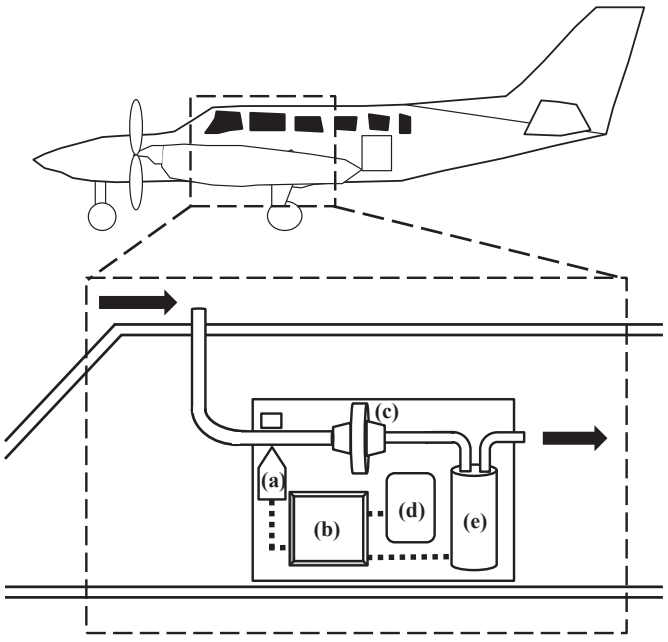

FIG. 1. Schematic diagram of the atmospheric bioaerosol sampling apparatus used with the airplane; (a) air shutter to protect from contamination by bioaerosols from different altitudes; (b) signal receiver and controller; (c) membrane filter unit; (d) high discharge capacity lithium battery; (e) small air rotary pump.

Sampling data The sampling of these bioaerosols over the Noto Peninsula on March 27, 2010 was performed using the airplane. Because the Noto Peninsula protrudes into the Sea of Japan, the collection of the sample while crossing this peninsula is not affected by microorganisms that may originate in the soil from the Japanese island (Fig. 2A). The numbers in Fig. 2B are landmarks during the flight indicated as Global Positioning System locations. The weather was cloudy on the date of sampling. Fig. 2C shows the altitude of flight during sampling and the horizontal axis indicates the same landmarks as in Fig. 2B. The atmospheric bioaerosols in this study were collected from an altitude of approximately 2900 m over the Noto Peninsula.

To estimate the origin of atmospheric bioaerosols, we calculated the backward trajectories of air masses using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model (http://ready.arl.noaa.gov/HYSPLIT.php) from the Air Resources Laboratory of the U.S. National Oceanic and Atmospheric Administration (13). In this study, the sampling was performed by wide-field circling of the aircraft over the Noto Peninsula and backward trajectories were calculated on the basis of the trajectory ensembles as a trend. For search of weather condition on the sampling, the data of mass concentration, relative humidity, temperature, wind direction, wind speed, the attenuated backscatter coefficient (532 and 1054 nm) and depolarization ratio (532 nm) in Toyama was obtained from Lidar Observation of Toyama Prefectural Environmental Science Research Center (Lidar observation in Toyama: http://www-lidar.nies.go.jp/Toyama/archives/100320-100324-met.png; http://www-lidar.nies.go.jp/Toyama/archives/100325-100329- met.png; http://www-lidar.nies.go.jp/Toyama/archives/100325-100329-abc-bl.png, Lidar observation in Nagasaki: http://www-lidar.nies.go.jp/Nagasaki2/archives/100325-100329-abc-bl.png).

Cultivation and identification The bioaerosols were inoculated on a simple clean booth with fan filter units (TOP Co. Ltd., Tokyo, Japan) and bacterial lamp immediately after collection. The membrane filter containing the sample was placed on an R2A agar plate (Oxoid Co. Ltd., Hampshire, UK). Genomic DNA was extracted from an isolate on this agar plate using a cell-wall lytic enzyme, lysozyme, and proteinase K (Sigma–Aldrich Co. Ltd., St. Louis, MO, USA). The genomic DNA was purified by phenol-chloroform extraction, chloroform extraction and ethanol precipitation. The prokaryotic 16S rRNA was amplified by polymerase chain reaction (PCR) (14). Oligonucleotide primers used in this study were 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-GGY TAC CTT GTT ACG ACT T-3′). The PCR reaction mixture (20 μL) comprised the following: 4 μL of 5× buffer; 1.6 μL of 10× dNTP (2.5 mM each dATP, dCTP, dGTP and dTTP); 0.2 μL of each primer (20 mM); 12.8 μL of sterile deionized H_2O; 1 U of Primes STAR DNA polymerase (Takara Bio Inc. Co. Ltd., Otsu, Shiga, Japan) and 1 μL of DNA extract (approximately 30 ng). A thermal cycler (GeneAmp PCR system 9700, Applied Biosystems Co., Ltd., Carlsbad, CA, USA) was used under the following conditions for amplification: an initial 3 min denaturation at 94°C; 35 cycles of 15 s denaturation at 94°C; 30 s annealing at 50°C; a 2 min extension at 72°C and a final 7 min extension at 72°C.

The DNA sequencing of cloned rDNA was determined using a genetic analyzer (AB3001, Applied Biosystems), and the related strains of isolates were searched using the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLAST) against DNA databases (such as GenBank/EmBL/DDBJ). A phylogenetic tree including all sequences was constructed according to the neighbor-joining algorithm using TreeView PPC (15).

UV irradiation experiment The UV irradiation experiment was performed using a thermostatic incubator (BR-30 L, Taitec Co. Ltd., Nagoya, Aichi, Japan) at a temperature of 25°C with a UV lamp. Emissions of the sun are in the lowest UV bands, i.e., the UV-A (400–320 nm), UV-B (320–290 nm) and UV-C bands (290–200 nm). The ozone layer is particularly important in blocking the UV-C and a part of the UV-B radiations. Percentages of UV-A and UV-B of the total UV radiation at the altitude of the Kosa transportation are 96.7% and 3.3%, respectively (16). The black light (UV-A) and UV-B radiation were employed using UV lamps. It was necessary and important for the experimental conditions to be the same as that during Kosa transportation. Hu et al. (17) reported that the total UV radiation of a single day at the city of Lhasa in Tibet at an altitude above 3000 m (the Kosa bioaerosol sampling altitude was 2900 m) was 0.87 MJ m^{-2}. Assuming that sunlight lasts for 12 h a day, the UV radiance of the experimental condition was adjusted to 20.1 ± 0.1 Wm^{-2}. UV radiances of UV-A (96.7%) and UV-B (3.3%) were approximately 19.4 ± 0.1 and 0.60 ± 0.1 Wm^{-2}, respectively, while adjusting the direction and distance of black light (UV-A) and the UV-B lamp. The UV radiance was measured by a digital UV intensity meter (UV-340, Custom Co. Ltd., Tokyo, Japan). Culture inoculum was smeared on the prepared specimens. We irradiated these prepared specimens present using UV radiation on the apparatus described above for the specified times, and measured their survival rates. The survival rate and measurement of living and/or dead cells were evaluated with the LIVE/