Global dispersion of bacterial cells on Asian dust

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The atmospheric dispersion of bacteria over long distances is an important facet of microbial ecology. Certain groups of dispersed bacteria can adapt to their new location and affect established ecosystems. Aeolian dust particles are known to be carriers of microbes but further research is needed to expand our understanding of this field of microbiology. Here we showed the potential of aeolian dust to global migration of bacterial cells. We demonstrated the presence of microbial cells on dust particles directly by bio-imaging. Bacterial abundance on dust particles declined from $10^5$ to less than $10^3$ cells/m$^3$ as the dust event subsided. Taxonomically diverse bacteria were identified by 16S rRNA gene sequencing and some of these bacteria retained growth potential. Our results confirm that bacteria can attach to aeolian dust particles and they have the potential to migrate globally during dust events and thus can contribute to the diversity of downwind ecosystems.
fabricated dust sampler which adsorbs dust particles onto the surface of wet beads. The source of these dust particles was estimated to be the Gobi desert (Supplementary Fig. S1b).

Particle size distribution was determined by scanning electron microscopy. The proportion of dust particles exceeding 1 µm increased during the severe Asian dust event, while most particles were less than 1 µm as the event ended (Fig. 1b). The major components of particles 1 to 5 µm in diameter were determined by scanning electron microscopy with energy dispersive X-ray (SEM-EDX) analysis\(^ {19,20}\) to confirm that the particles were truly aeolian dust particles and not suspended particulate matter. From the SEM-EDX profile, silicon and aluminum were identified as the major components of collected dust particles. Significant quantities of iron, calcium and magnesium were also present, consistent with soil particle composition\(^ {19,20}\) (Fig. 1c).

These results suggest that a portion of Asian dust particles could be sufficiently large to be efficient carriers of bacteria and that those larger dust particles can reach down-wind regions several thousand kilometers away from their source in severe events.

Visualization of microbial cells on Asian dust particles and change in bacterial abundance on the particles during the dust event. Microbial cells on collected Asian dust particles were visualized using a laser scanning microscope equipped with a microspectrophotometer, following fluorescent nucleic acid staining to distinguish microbial cells from dust particles based on their fluorescence\(^ {21}\) (Supplementary Fig. S2, S3 and S4, Fig. 2a–c). Microbial cells were attached to particles exceeding 1 µm in size (Fig. 2c). Of 8,500 Asian dust particles collected on 12 Nov. 2010 at the start of the severe event, large particles (> 5 µm) harbored 55% of detected microbial cells, intermediate size particles (2–5 µm) supported 38% of the cells, while particles of 1–2 µm supported only 7% of the cells. No microbial cells were attached to small particles (< 1 µm) (Supplementary Fig. S5) while the detection limit of our laser scanning microscopy is 0.3 µm, and bacteria on the bottom side of particle could not be observed. We also investigated Asian dust particles collected on 16 Nov. 2010, immediately following the dust event, and found few microbial cells on any of the dust particles. The number of microbial cells occupying

Figure 1 | (a) Scanning electron micrograph of Asian dust particles collected on 12 Nov. 2010. (b) Size distribution of Asian dust particles collected on 12 Nov. 2010. Size of each dust particle was determined by image analysis of scanning electron micrographs and 20,000 to 100,000 particles were analyzed in each sample. (c) Elemental composition of Asian dust particles (size: 1 to 5 µm) determined by energy dispersive X-ray analysis. 100 dust particles collected on 12 Nov. 2010 were analyzed. Each bar represents one of the 100 dust particles.

Figure 2 | Selective detection of microbial cells on Asian dust particles (collected on 12 Nov. 2010) by laser scanning microscopy following fluorescent nucleic acid staining. (a) Microbial cells on Asian dust particles. (b) Asian dust particles. (c) Composite image of a) and b). (d) Microcolony of microbial cells formed on an Asian dust particle. Microbial cells fluoresce green/yellow, while particles fluoresce red.
Asian dust particles was inferred to decrease as the dust event weakened. Bacterial abundance on collected dust particles, determined by quantitative real-time PCR targeting eubacterial 16S rRNA gene sequences, declined dramatically to less than 1% of their starting values (from 10^4 – 10^5 to less than 10^3 cells/m^3) as the dust event subsided (Supplementary Table S1).

We determined bacterial abundance on Asian dust particles by two different methods: laser scanning microscopy and quantitative PCR, and the results obtained by these methods were similar (Supplementary Fig. S5 and Supplementary Table S1). These data support that Asian dust carries bacterial cells to down-wind areas.

**Diversity of bacteria carried with Asian dust.** The bacterial community structure on Asian dust particles was analyzed by sequencing nearly the full length of the 16S rRNA gene of 480 clones obtained from Asian dust particles collected on 12 Nov. 2010 and 2 May 2011. Rarefaction curves revealed class richness in the dust source soil and Asian dust (Fig. 3a). Sequences were affiliated with more than 20 bacterial classes, of which *Actinobacteria*, *Bacilli* and *Sphingobacteria* dominated (Fig. 3b). Previous studies reported that these bacteria were often found in African dust or Asian dust samples. Since little is known about the source bacterial community structure of Asian dust particles, the bacterial communities of the samples were compared with those of their putative source environments (Taklamakan and Gobi deserts and Loess plateau). A similar diversity of bacterial groups was found in the source regions as in the Asian dust particles (Fig. 3b) and other arid regions. These results indicate that the diversity of bacterial communities carried by Asian dust reflects the bacterial diversity in dust source regions.

**Growth potential of bacterial cells on Asian dust particles.** We incubated the dust particles in both nutrient-poor and nutrient-rich liquid media and phylogenetically analyzed the proliferated cells. This experiment revealed that *Bacilli* and *Sphingobacteria* maintain the potential for physiological activity during long-distance transportation on Asian dust particles (Fig. 4b and 4c). We also plated Asian dust particles on agar media and incubated them for 48 hours. Some microbes on Asian dust particles proliferated into microcolonies (Fig. 2d and Supplementary Fig. S6), suggesting that taxonomically diverse bacteria are not only transported long distances by Asian dust events, but that some groups retain potentially physiologically active.

**Discussion**

In this study, we used bio-imaging with laser scanning microscopy and quantitative PCR of bacterial 16S rRNA genes to demonstrate that bacterial cells can be transported several thousand kilometers on Asian dust particles. We also confirmed the phylogenetic diversity of these bacteria, and that some groups retain growth potential despite such long-distance transportation.

Bacterial populations on aeolian dust particles are considered likely to impact natural environments and human health. The abundance of bacteria moving with dust particles (10^4 – 10^5 cells/m^3) is equivalent to or less than bacterial abundance in the air of our typical non-dust environment (10^7 cells/m^3), and their diversity is similar to that of the soil in the dust source regions (Fig. 3). Dominant bacterial classes found on Asian dust particles are commonly found in natural environments. *Actinobacteria* inhabit extreme environments such as hypersaline lakes, thermal springs, and arid soils. *Bacilli* include spore-forming bacterial genera, while *Sphingobacteria* are commonly found in soil and aquatic environments. We may therefore conclude that the bacteria carried with Asian dust probably rarely affect human health, based on their abundance and phylogenetic composition, although they constitute a possible source of opportunistic infection, and their potential as allergens must also be considered.

On the other hand, these phylogenetically diverse bacteria could affect established ecosystems. Most bacteria transported by aeolian dust will be stressed by atmospheric transport (UV exposure, reduced nutrient availability, etc.). Some of them, however, will adapt to favorable environments, where they retain characteristics which may render them highly resilient to competition in their new habitat.
Transported bacteria are therefore a potential driving force of bacterial diversity maintenance or enhancement in these habitats.

Bacterial genes are recognized as important resources. Fair and equitable sharing of the benefits arising from international genetic resource use was proposed at the tenth meeting of the Conference of the Parties to the Convention on Biological Diversity (COP 10) held in 2010. Bacteria are transported worldwide during aeolian dust events and the genes of these transported bacteria may be transferred to indigenous bacteria, introducing new traits and expanding gene diversity within bacterial communities. Bacteria found in a given geographic area are likely to be found elsewhere, and aeolian dust has an important role in global bacterial inoculation.

Asian dust particles have been transported from deserts or arid regions in China and Mongolia to Korea, Japan, and the Pacific islands in geologic time scale, and they have carried phylogenetically and functionally diverse bacterial groups. Aeolian dust events may contribute to global migration of bacterial cells and their genes, and therefore can be important sources of bacterial diversity in the earth’s ecosystem.

Methods

Collection of Asian dust particles. The occurrence of atmospheric Asian dust and its drift were confirmed by LIDAR (Light Detection and Ranging) data from the Ministry of the Environment, Japan (http://www.gis5.nies.go.jp/eastasia/DustLider.php), Asian dust information from Japan Meteorological Agency (http://www.jma.go.jp/jp/kosa/index.html) and direct calling to aviation weather service centers. The geographic origin of Asian dust was determined by the backtrajectory analysis (http://ready.arl.noaa.gov/HYSPLIT.php; Supplementary Fig. S1b).

Asian dust particles were collected with a sampler set from a small airplane. Air outside of the airplane was collected in the sampler, which consisted of wet glass beads in a stainless can (volume: 2 liter) and a sterilized Teflon inlet tube. The inlet was inserted into the air intake, which opens at the leading edge of a small airplane, to avoid boundary effects. Asian dust particles in the air were adsorbed on the surface of the wet glass beads. To prepare the sampler, glass beads were washed with particle-free water (commercial distilled water for injection) in an ultrasonic washer and sterilized in dry oven (180°C, 2 hours twice). The stainless can was washed with particle-free water and sterilized in dry oven (180°C, 2 hours × twice). Teflon tubes were washed with particle-free water and sterilized by autoclaving.

Asian dust particles were collected on 12 November 2010 (severe conditions, visibility: less than 10 km), 13 November 2010 and 16 November 2010 (end of the event, visibility: 25 km). Asian dust particles were also collected on 2 May 2011 when a severe dust event occurred. The airplane took off from Yao Airport, Osaka, Japan and started collection of Asian dust particles in the air over the Japan Sea (10 km from coasts) at an altitude of 900 meters (latitude: N 35°42.004’, longitude: 134°52.134’). The airplane route was westbound to near Akasaki (latitude: N 35°34.187’, longitude: 133°41.870’), then eastbound and collected Asian dust particles along this out and inbound flight path. Asian dust particles from a total of more than 10,000 liters of the air were collected for this study.

During sampling, we recorded the time, altitude, longitude and latitude, visibility, aircraft heading and course, airspeed and ground speed, and we confirmed that there was no wind from land during sampling. In addition, the numbers of particles in the atmosphere were measured on-site with a particle counter (KR-12A, Rion, Tokyo, Japan).

Dust particles collected on wet beads were suspended in 2.5 L of particle-free water and used for the following experiments.

Collection of soil in dust source regions. Soil samples were collected in three dust source regions: (i) Gobi desert (site 1: latitude: N 40°21.259’, longitude:...
Determination of size distribution and elemental composition of collected Asian dust particles. Dust particles (50 ml of the 2.5 L suspension) were trapped onto a sterilized 0.4 μm-pore size polycarbonate membrane filter and sputter coated with evaporated gold.

Samples were observed under a scanning electron microscope (ISM-5600L; JEOL, Tokyo, Japan) using high vacuum mode. Electron micrographs were obtained at magnifications of ×1,000 using a backscatter electron detector.

Elemental composition of Asian dust particles were analyzed under a scanning electron microscope (ISM-5500LV; JEOL) equipped with EDX (JED-2200; JEOL) operating at 30 kV. For the particle size distribution, image processing was carried out by automated particle size distribution analysis software.

To determine the size distribution and elemental composition of collected dust particles accurately, particles of more than 0.5 μm were utilized and analyzed.

Visualization of microbial cells on Asian dust particles. Dust particles (100 ml of the 2.5 L suspension) were fixed with 4% (w/v) paraformaldehyde at 4°C for 16 h. After fixation, 10 ml-aliquots were filtered through a sterilized 0.4 μm-pore size polycarbonate membrane filter and rinsed twice with particle-free water. The filters were transferred to a microtube and dehydrated in an ethanol series (50%, 80%, 90%, and 100% ethanol for 3 min each) and dried under vacuum. The filters were stained with nucleic acid-staining dye, SYBR Green I (Invitrogen, Carlsbad, USA; 1/10,000 dilution of the supplied product) which can be used to distinguish cells from non-biological backgrounds, containing 2% Tween 20 for 10 min. After washing with particle-free water (<3×), the filters were mounted on a cover glass with immersion oil for observation by laser scanning microscopy (TCS-SP5, Leica Microsystems, Wetzlar, Germany).

To discriminate microbial cells from dust particles, a microscope with a micro-spectrophotometer was used. Escherichia coli W3110 cells inoculated in sterilized soil (collected in Loess Plateau, Shaanxi, China) as well as Asian dust particles collected in Beijing, China, were used to determine the protocol to distinguish microbial cells from dust particles based on their fluorescence (Supplementary Fig. S2 and S3).

All particles were observed under blue excitation (wavelength 480 nm) and particles with green fluorescence (wavelength 500 – 540 nm) were enumerated as microbial cells; those with yellow to red fluorescence (wavelength 550 – 680 nm) were enumerated as non-biological particles such as soil particles.

In this analysis, at least 200 different microscopic fields were observed for each sample; more than 6,000 particles were observed.

Visualization of microcolony-forming microbial cells on Asian dust particles. Dust particles (10 ml of the 2.5 L suspension) were filtered through a sterilized 0.4 μm-pore size polycarbonate membrane filter. The filter was then removed immediately from the funnel and placed on standard agar or R2A agar media.

Visualization of microcolony-forming microbial cells on Asian dust particles. Dust particles (10 ml of the 2.5 L suspension) were filtered through a sterilized 0.4 μm-pore size polycarbonate membrane filter and sputter coated with evaporated gold.

Samples were observed under a scanning electron microscope (ISM-5600L; JEOL, Tokyo, Japan) using high vacuum mode. Electron micrographs were obtained at magnifications of ×1,000 using a backscatter electron detector.

Elemental composition of Asian dust particles were analyzed under a scanning electron microscope (ISM-5500LV; JEOL) equipped with EDX (JED-2200; JEOL) operating at 30 kV. For the particle size distribution, image processing was carried out by automated particle size distribution analysis software.

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**Author contribution**

N. Y., T. I. and T. B. contributed equally to this work. M. N. conceived the study; N. Y., T. I. and T. B. collected samples; T. I. performed quantitative PCR and phylogenetic analysis; A. S. performed SEM–EDX analysis; T. B. performed scanning electron microscopy and laser scanning microscopy; N. Y. and M. N. wrote the paper; all authors discussed the results and commented on the manuscript.

**Additional information**

Nucleotide sequence accession numbers: Sequences were deposited with DNA Data Bank of Japan (DDBJ) and assigned accession numbers were AB636927 to AB637350 (samples without cultivation), AB637351 to AB637760 (samples incubated with PYG broth) and AB637761 to AB638212 (samples incubated with R2A broth) for samples collected on November 2010. Assigned accession numbers were AB695733 to AB696139 (samples collected on 2 May 2011), AB696140 to AB696280 (Gobi desert samples), AB696281 to AB696430 (Loess Plateau samples) and AB696431 to AB696579 (Taklamakan desert samples).

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Competing financial interests: The authors declare no competing financial interests.

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