Review

Long-range Transportation of Bacterial Cells by Asian Dust

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Received April 16, 2014; Revised May 23, 2014; Accepted May 26, 2014
J-STAGE Advance published date: May 31, 2014

Relocation of bacteria over long distances is a key issue in global bacterial inoculation. Certain of mobile bacteria can adapt to their new location and affect the established ecosystem. Aeolian dust particles are thought to be carriers of microbes but definitive research is lacking. The contribution of aeolian dust to global migration of bacterial cells and their genes was therefore examined by culture-independent approaches. Asian dust particles were collected over the Japan Sea (10 km from coasts) at an altitude of 900 m to avoid contamination of soil particles lifted from ground, on 12 November 2010 (midst of the event, visibility: less than 10 km), 13 Nov. 2010, 16 Nov. 2010 (end of the event, visibility: 25 km) and 2 May 2011 (midst of the event, visibility: less than 10 km), with a sampler set in a small airplane. Microbial cells on dust particles were directly visualized by bio-imaging with laser scanning microscopy equipped with a microspectrophotometer, based on their specific fluorescence. 16S rRNA gene was directly extracted and it was then confirmed by quantitative PCR that bacterial abundance on collected dust particles drastically declined from \(10^5\) cells/m\(^3\) to less than 1/100 as the dust event subsided. Taxonomically diverse bacteria were found by sequencing of 16S rRNA genes of clones obtained from each collected Asian dust sample, such as Actinobacteria, Bacilli and Sphingobacteria. Some of these bacteria retained growth potential despite the long-range transportation. These results demonstrate that bacteria attach to aeolian dust particles and migrate globally during dust events thus may contribute to the diversity of the bacterial gene pool.

Key words: aeolian dust, global bacterial inoculation, bioaerosol, airborne bacteria, microbial diversity

Introduction: Aeolian Dust Events and Long-range Transportation of Bacterial Cells

A fundamental paradigm in microbial ecology states that “Everything is everywhere, but the environment selects” (1,2). Several data support this paradigm with development of high-throughput sequencing techniques (3). Relocation of bacteria over long distances is a key issue in global bacterial inoculation, which is promoted by ocean currents and atmospheric events. When a wind-sand stream occurs in arid and semi-arid regions, microorganisms on dust particles can be lifted and transported over long distances by air currents. Major aeolian dust events arise from the Sahara and Sahel deserts (African dust), Australian deserts (Australian dust), and the Taklamakan desert, Gobi desert and Loess Plateau (Asian dust). Bacteria on these dust particles may impact cloud development, precipitation, atmospheric chemistry as well as microbial biogeography (4,5). Bacterial abundance and community compositions in these aeolian dusts have therefore been reported to clarify their possible impacts on ecosystems and also on public health (6–10).

Approximately 4,000,000 tons of Asian dust particles are estimated to fall on Japan annually (11), 3,000–5,000 km from their source regions (China and Mongolia). It is well known that Asian desert dust particles are transported long distances (12,13), even reaching the North American Continent (more than 15,000 km away) (14,15). Moreover, oceanic deposition has been shown to enhance phytoplankton growth in the North Pacific Ocean via natural iron fertilization (16). Asian dust particles can sometimes be transported globally in 13 days (17) and have been found in ice and snow cores of Greenland (18) and the French Alps (19). These dust particles have been considered as carriers of microbes, however presence of microbial cells on dust particles has not been confirmed.

Sampling of Asian Dust Particles by a Small Airplane

To directly demonstrate that Asian dust particles carry microbial cells, we thought that Asian dust particles should be collected without contamination of soil particles lifted from ground. We therefore collected Asian dust particles during a severe dust event (from 12 Nov. 2010 [midst of the event; visibility less than 10 km]
Table 1. Sampling date and altitude, event severity, sampling volume, and bacterial abundance on Asian dust particles determined by quantitative PCR targeting 16S rRNA gene

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Sampling date</th>
<th>Sampling altitude (m)</th>
<th>Severity of Asian dust(^a)</th>
<th>Sampling volume (liter)</th>
<th>16S rRNA gene (copies/m(^3))</th>
<th>Estimated bacterial number (cells/m(^3))(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan Sea</td>
<td>12 Nov., 2010</td>
<td>900</td>
<td>++</td>
<td>15,000</td>
<td>(2 \times 10^5)</td>
<td>(1 \times 10^4 - 2 \times 10^5)</td>
</tr>
<tr>
<td></td>
<td>13 Nov., 2010</td>
<td>900</td>
<td>+</td>
<td>12,000</td>
<td>(8 \times 10^4)</td>
<td>(5 \times 10^3 - 8 \times 10^4)</td>
</tr>
<tr>
<td></td>
<td>16 Nov., 2010</td>
<td>900</td>
<td>-</td>
<td>10,000</td>
<td>\textless (2 \times 10^3)</td>
<td>\textless (2 \times 10^3)</td>
</tr>
</tbody>
</table>

\(^a\)Severity of Asian dust was determined by METAR, SYNOP, LIDAR and pilot observation at sampling altitude. \(^b\)Bacterial cells carry 1 to 15 copies of the 16S rRNA gene in their genome.

Fig. 1. Source region of Asian dust storm commencing 12 Nov. 2010 and 2 May 2011 in Japan, estimated by backward trajectory analysis.

through 16 Nov. 2010 [the event was terminated by rain on 15 Nov. 2010; visibility 25 km] over the Japan Sea (10 km from coasts) at an altitude of 900 m, using a newly fabricated dust sampler which adsorbs dust particles onto the surface of wet beads (20). Severe Asian dust event occurred on 2 May 2011 and we collected dust particles as well. During two hours flight, we collected more than 10,000 to 15,000 L of air to obtain reliable results (Table 1). The source of these dust particles was estimated to be the Gobi desert by backward trajectory analysis (Fig. 1). The major components of collected dust particles were determined to be the Gobi desert by backward trajectory analysis (Fig. 1). The major components of collected dust particles were determined by scanning electron microscopy with energy dispersive X-ray (SEM-EDX) analysis to confirm that the collected particles were truly aeolian dust particles and not suspended particulate matter (13,20), and actually silicon and aluminum were identified as the major components of collected dust particles.

Size Distribution of Asian Dust Particles Determined by Digital Image Analysis

We determined the size distribution of collected Asian dust particles by processing a scanning electron microscopy (SEM) images of the particles. This image analysis software was programmed using computer language C based on our original image analysis software (21,22) and named as PaSDAS (Particle Size Distribution Analysis Software). The flow chart of PaSDAS is shown in Fig. 2(A). This program determines the particle size distribution after observation of samples under SEM in seven steps: (i) acquisition and binarization of the SEM image; (ii) seeking the first white pixel (Fig. 2(A)-a); (iii) labeling the first white pixel and seeking the next white pixel (Fig. 2(A)-b); (iv) if a labeled pixel exists in an 8-neighbourhood, labeling the sought pixel with the same number (e.g., ‘‘1’’), and if not, labeling the sought pixel with the next number (e.g., ‘‘2’’, ‘‘3’’), performing this process until the final pixel (Fig. 2(A)-c); (v) again, seeking the first white pixel, replacing the
Fig. 2. Algorithm of the labeling performed by the image analysis software (PaSDAS) for the particle size distribution analysis of aeolian dust particles (A) and correlation of particle sizes certified by the manufacturers and those determined by PaSDAS (B). Total more than 1,200 particles were analyzed.
number with the lowest number in the 8-neighbourhood pixels, and performing this process until the final pixel (Fig. 2(A)-d); (vi) repeating this replacement process (step v) 300 times (Fig. 2(A)-e); and (vii) calculating and recording the square measure of each particle (Fig. 2(A)-f). The accuracy of the particle size distribution determined using this software was evaluated by analyzing commercially available particles (particle size: 0.3–10.7 μm), and there was strong correlation between the certified size and size determined with this software (Fig. 2(B); $r^2 = 1.00$). By the image analyses of collected dust particles by this software, we confirmed 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 Asian dust events.

**Visualization of Microbial Cells on Asian Dust Particles by Bio-imaging**

To demonstrate the presence of microbial cells on Asian dust particles, we visualized microbial cells on collected Asian dust particles using a laser scanning microscope equipped with a microspectrophotometer (20), following fluorescent nucleic acid staining to distinguish microbial cells from dust particles based on their fluorescence (Fig. 3). Microbial cells were attached to particles exceeding 1 μm in size. More than 8000 of Asian dust particles collected on 12 Nov. 2010 at the midst of the severe event were observed, and 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) (Fig. 4). We also investigated Asian dust particles collected on 16 Nov. 2010 (end of the event), and found few microbial cells on any of the dust particles. The size of bacterial cells in natural environment is generally more than 0.5 μm and we think that small particles (less than 1 μm) are not adequate for these cells to attach. In other words, rather large particles must be suitable to attach for them. The number of microbial cells occupying Asian dust particles was inferred to decrease as the dust event weakened.

**Growth Potential of Bacterial Cells on Asian Dust Particles**

We collected unfractionated Asian dust particles on membrane filters (pore size: 0.4 μm) and plated them on standard method agar (peptone 5 g, yeast extract 2.5 g, glucose 1 g, agar 15 g per 1 L water). These filters were incubated at 25°C for 48 h, and microbial cells were fluorescently stained as described above. We found microbial microcolonies on Asian dust particles by observation of more than 500 of dust particles under laser scanning microscopy (Fig. 5), suggesting that some of long-range transported microbial cells remain physiologically active.

**Change in Airborne Bacterial Abundance during Asian Dust Event**

We determined bacterial abundance on collected dust particles by quantitative real-time PCR targeting eubacterial 16S rRNA gene sequences, and it declined dramatically to less than 1% of their starting values as the dust event subsided (Table 1). The abundance of bacteria moving with dust particles ($10^7$ to $10^9$ cells/m$^3$) is equivalent to those in outdoor environment (rooftop of a building; ca. 20 m in height) during severe Asian dust events ($10^5$ cells/m$^3$) (23).

We determined bacterial abundance on Asian dust particles by two different approaches: not only laser scanning microscopy (targeting single cell) but also quantitative PCR (targeting DNA), and the results obtained by these methods were similar (Fig. 4 and Table 1). These data support that Asian dust carries bacterial cells to down-wind areas and abundance of long-range transported bacteria increases by severe dust events.

**Phylogenetic Diversity of Bacteria Carried by Asian Dust Events**

We analyzed the bacterial community structure on Asian dust particles by sequencing nearly the full length of the 16S rRNA gene of ca. 500 clones obtained from each Asian dust sample collected on 12 Nov. 2010 and 2 May 2011 (severe dust days). Sequences were affiliated with more than 20 bacterial classes, of which Actinobacteria, Bacilli and Sphingobacteria dominated (Fig. 6). These bacteria 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 with high environmental stress tolerance, while Sphingobacteria are commonly found in soil and aquatic environments. These bacterial genera were also found in outdoor environments (rooftop of a building) on both Asian dust and no Asian dust days (23).

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 (Fig. 6) could affect established ecosystems of downwind areas. Most bacteria transported by aeolian dust will be stressed by atmospheric transport (UV exposure, reduced nutrient availability, etc.), however, some of them remain viability (Fig. 5) and they will adapt favorable environments, where they retain characteristics which may render them highly resilient to competition in their new habitat.
Fig. 3. Selective detection of microbial cells on an Asian dust particles by laser scanning microscopy. Microbial cells show yellow fluorescence while dust particles show red fluorescence under excitation at 488 nm.

Fig. 4. Bacterial number and size distribution of Asian dust particles collected on 12 Nov. 2011. Bacterial number was determined by laser scanning microscopy.

Transported bacteria can be a potential driving force of bacterial diversity maintenance or enhancement in these habitats. Aeolian dust events therefore may contribute to global migration of bacterial cells and their genes, and can be important sources of bacterial diversity in the earth’s ecosystem.

Acknowledgements: This research was supported by the Environment Research and Technology Development Fund of the Ministry of the Environment, Japan.
Fig. 5. Selective detection of microbial microcolonies formed on Asian dust particles (collected on 12 Nov. 2010) by laser scanning microscopy. Microcolonies were formed by incubation on standard method agar at 25°C for 48 h.

Fig. 6. Bacterial community composition of Asian dust particles determined by bacterial DNA analysis.

Conflicts of interests: The authors declare no conflicts of interest.

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