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Effects of Asian dust events on atmospheric bacterial communities at different distances downwind of the source region

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ABSTRACT

Aeolian dust particles arising from arid and semiarid zones are known to carry microbes by air currents. The effect of wind-borne bacteria on atmospheric bacterial population at various downwind distances from the dust source regions must be clarified, but has not yet been reported. This study monitored the bacterial abundance and community composition in outdoor aerosol samples in Beijing, China, which is close to the Asian dust source regions, and compared them with the results obtained in a distant region (Osaka, Japan). The Asian dust collected in Beijing contained $(4 \pm 3) \times 10^4$ bacterial cells/m^3, approximately 4 times higher than in Osaka. On 15 April 2015, Beijing experienced severe Asian dust events with a 1000-fold increase in bacterial abundance, relative to non-Asian dust days. Dominant bacterial phyla and classes in Asian dust collected in Beijing were *Actinobacteria*, *Bacilli* and *Acidobacteria*, and the bacterial community composition varied more widely than in Osaka. The bacterial community compositions differed between the Beijing and Osaka dusts, even for the same Asian dust events. These results indicated that aerosol bacterial communities nearer the dust source are more affected by eolian dust than their distant counterparts.

Introduction

Aeolian dust is recognized as a main vehicle of intercontinental bacterial migration by atmospheric currents. Bacteria attached to aeolian dust particles were directly observed by bio-imaging (Yamaguchi et al., 2012), and bacteria transported from arid and semi-arid regions can impact on public health and ecosystems (Griffin et al., 2001, 2003; Griffin, 2007; Lim et al., 2011). Worldwide aeolian dust occurred 0.5–5.0 billion tons in every year (Perkins, 2001), and scattered around the world. Asian dust is one of the major aeolian dust, as Australian dust and African dust. Asian dusts affect not only East Asia (China, Korea and Japan), but can also reach North America, more than 15,000 km from the source region (Duce et al., 1980; Kellogg and Griffin, 2006; Smith et al., 2013). Several previous studies investigated the effects of Asian dust events on atmospheric microorganisms of downwind area. These studies carried out analyses of bacterial community composition (Jeon et al., 2011), abundance and viability estimates (Hara and Zhang, 2012), as well as investigations of atmospheric halotolerant bacterial communities (Maki et al., 2010). However,

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a microbiological comparative analysis of aeolian dust events at varying scales and source distances has not been reported. The Asian dust fallout is estimated as 180 g/(m^2·year) in Beijing, China (500–2500 km from the dust source regions) (Nishikawa et al., 2002) and 0.005–0.05 g/(m^2·year) in Osaka (3000–5000 km from the dust source regions) (Yoshinaga, 1998). In addition, because aeolian dust is lifted by updraft and transported by air current, its effect should depend on distance from the dust source region. The transport of aeolian dust is influenced by climatic conditions such as air pressure, wind direction and wind velocity, which depend on the ground conditions (Rosenfeld et al., 2001; Chkhetiani et al., 2012); moreover, larger dust particles are more difficult to transport over long distances. A previous report confirmed that 55% of the microbial cells detected on Asian dust particles were attached to larger particles (>5 μm), while 7% of them resided on smaller particles (1–2 μm) (Yamaguchi et al., 2012).

For these reasons, the microbial effect on downwind environments may depend on both the scale of the aeolian dust and the distance from the dust source regions. To assess the possible impacts by the bacteria attached to aeolian dust particles and the change of their community composition by the long-range movement, monitoring is required in downwind areas with different distance from Asian dust source regions.

The aim of study is assessment the effects of Asian dust events on atmospheric bacterial communities at different downwind distances from the source regions. To this end, we continuously monitored the bacterial abundance and community composition on various outdoor aerosol samples in Beijing collected during the 2015 Asian dust season (from April to June; on both Asian dust days and non-Asian dust days) for a general assessment of Asian dust events. We then compared the variations of bacterial abundances and community compositions in outdoor aerosol samples collected in Beijing and Osaka (which are close to and distant from the Asian dust source regions, respectively). Airborne bacterial abundances and community structures were determined by 16S rRNA gene-targeted quantitative PCR and amplicon sequencing (Yoo et al., 2017).

1. Materials and methods

1.1. Sample collection

In this study, for a general assessment of influence of bacteria transported by Asian dust event, we continuously monitored bacterial abundance and community composition of outdoor aerosols collected under different atmospheric conditions for three months in Asian dust season.

Fifty-seven outdoor aerosol samples were collected on a second-floor roof (height *ca.* 5 m) at the China Agricultural University in Beijing (latitude: 40°00′14.9″ N, longitude: 116°21′10.8″ E) using a high-volume air sampler (HV500R; SIBATA, Saitama, Japan), from 14 April to 19 June in 2015. Data in Osaka were quoted from our published data (Park et al., 2016) (Table S1). Air samples were collected onto 0.6-μm pore-size glass fiber filters at 500 L/min. During each sampling event (200 min), aerosol particles were captured from 100 m^3 of ambient air. The occurrence of atmospheric Asian dust was confirmed and their severities were assessed from the increased mass of the glass fiber filter after sampling and also the visibility at the sampling location (Fig. S1). Visibility data was obtained at China Air Dairy (http://www.chinaairdaily.com/). The Asian dust observed in Beijing on 15 April 2015 reached Osaka on 18 April 2015 (Fig. 1).

1.2. DNA extraction

Aerosol samples collected on the glass filter were pulverized by bead-beating (4800 r/min, 90 sec) with EZ-Beads (EZ, Tokyo, Japan). Genetic DNA (gDNA) was then extracted and purified as described by Tsai and Olson (1991). The extracted gDNA was subsequently purified using a Wizard DNA Clean-Up System kit (Promega, Madison, WI, USA) and eluted with 50 μL of TE buffer (10 mM Tris–HCl and 1 mM EDTA [pH 8.0]). No DNA contamination was detected on a blank filter by quantitative PCR and DNA gel electrophoresis.

1.3. Estimation of bacterial abundance

To determine the bacterial abundance, 16S rRNA gene was quantified by real-time PCR using a LightCycler (Roche Diagnostics, Mannheim, Germany). Real-time PCR was performed with eubacterial primer sets as described by Yamaguchi et al. (2012). To generate the standard curve for quantifying the 16S rRNA gene, PCR products of *E. coli* W3110 were used as the gDNA template (1×10^1 to 1×10^7 copies per reaction). As the copy number of the 16S rRNA gene differed among the bacterial phyla, the bacterial abundance was calibrated by a phylum-level analysis of the bacterial community composition (Park et al., 2016).

1.4. Analysis of bacterial community composition

The 16S rRNA gene was amplified for pyrosequencing by two-step PCR (Sutton et al., 2013). Two-step PCR increases the reproducibility and recovers higher genetic diversity during amplicon sequencing than one-step PCR (Berry et al., 2011; Ichijo et al., 2016). Using this approach, tags and adapters were added during a second round of PCR amplification. Second round of PCR amplification was performed with 968F (AACGCGAAGAACCTTAC) and 1401R (CGGTGTGTACAAGACCC) sets as described by Ichijo et al. (2016). Amplicons were sequenced using Ion PGM (Thermo Fisher Scientific KK, Yokohama, Japan) at the Center for Medical Research and Education, Osaka University (Osaka, Japan). The raw sequence data of the obtained amplicons were screened, trimmed, and filtered using the default settings of QIIME pipeline version 1.9.1 (http://qiime.org/). Over 278000 sequences were obtained across all samples (averaging 5100 sequences per sample). Total operational taxonomic units (OTUs), defined at the 97% nucleotide-sequence identity level using the UCLUST function of QIIME software (Caporaso et al., 2010), were identified in all sequences. On average, approximately 2800 OTUs per sample were recovered. The community composition differences among the samples were graphically assessed by principal coordinate analysis (PCoA) using the unweighted pair group method. Finally, the bacterial community composition was calibrated by the copy