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Assessment of composition and origin of airborne bacteria in the free troposphere over Japan

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HIGHLIGHTS

- Aerosol samples were collected at high altitudes using an aircraft and a balloon.
- During four sampling periods, the air masses came from the Gobi Desert and North Asia.
- Airborne bacterial species were investigated using a 16S rDNA clone library technique.
- Bacterial species at high altitudes varied by the direction of free tropospheric winds.

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ABSTRACT

Long-range transport of airborne microorganisms through the free troposphere significantly impacts biological ecosystems, human life, and atmospheric processes in downwind areas. However, microbial communities in the free troposphere have rarely been investigated because the direct collection of microbial cells at high altitudes requires sophisticated sampling techniques. In this study, tropospheric air sampling was performed using a balloon and an aircraft at 800 m and 3000 m, respectively, over the Noto Peninsula in Japan (37.5°N, 137.4°E) where free tropospheric winds carry aerosols from continental areas. The air samples were collected during four different sampling periods when air masses came from desert regions of Asian continent (west samples) and from Siberia of Russia North Asia (north samples). The west samples contained higher levels of aerosols, and bacteria from the west samples grew in culture media containing up to 15% NaCl. In contrast, bacteria from the north samples could not be cultured in the same media. All isolates obtained from the NaCl-amended cultures were similar to *Bacillus subtilis* and classified as *Firmicutes*. A 16S rDNA clone library prepared from the west samples was mainly composed of one phylotype of *Firmicutes* that corresponded to the cultured *B. subtilis* sequence. A clone library prepared from the north samples consisted primarily of two phyla, i.e., *Actinobacteria* and *Proteobacteria*, which are known to dominantly inhabit low-temperature environments of North Asia. Our results suggest that airborne bacterial communities at high altitudes include several species that vary by the direction and interaction of free tropospheric winds.

1. Introduction

Bioaerosols, which include bacteria, fungi, and viruses, are transported from marine and terrestrial environments to the free troposphere and are significantly abundant in the organic carbon fraction of atmospheric aerosols (Prospero et al., 2005). Airborne microorganisms increase allergen burden causing increased incidence of asthma (Ichinose et al., 2005) and contribute to dispersion of diseases such as Kawasaki disease in humans (Rodó et al., 2011) and rust diseases in plants (Brown and Hovmøller, 2002). Moreover, bioaerosols are thought to influence atmospheric processes by participating in atmospheric chemical reactions and cloud particle formation (Pratt et al., 2009).

The bacterial species composition of the atmosphere should be investigated for understanding the characteristics of bacterial communities that are transported to long distances and influence

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downwind ecosystems and climates. Aerosol sampling at altitudes of 200–800 m above the ground level has demonstrated that bioaerosols are composed of several species of bacteria (Li et al., 2010). The atmosphere is a heterogeneous environment, and meteorological shifts can alter the bacterial species composition of bioaerosols. The airborne bacterial abundance and species composition at ground level in Asia (Hara and Zhang, 2012) and at 2700 m above sea level on North American mountains (Smith et al., 2012) change significantly depending on Asian dust events. However, few reports have directly investigated bacterial dynamics at high altitudes, such as the free troposphere, where long-range transported bioaerosols are abundant (Griffin, 2004).

Halotolerant bacteria are tolerant to high salinity and resistant to stressors, such as high pH, extreme temperatures, and desiccation (Lippert and Galinski, 1992). Indeed, using NaCl-amendment culture techniques, viable halotolerant bacteria have been detected from bioaerosols collected at high altitudes (Maki et al., 2008). Halotolerant bacterial communities are typically common to bioaerosols transported hundreds or thousands of kilometers (Echigo et al., 2005). Some halotolerant bacteria isolated from sand dunes in the Gobi Desert were identical to bacterial species isolated in Higashi-Hiroshima, Japan, suggesting their long-range transport (Hua et al., 2007). An experimental design facilitating the isolation and identification of halotolerant bacteria at high altitudes is expected to be useful for analyzing transported bacteria through the free troposphere.

To investigate bacterial composition dynamics and the different air mass sources in the free troposphere, we collected air samples at altitudes of 800 m and 3000 m above the ground level over the edge of the Noto Peninsula, Japan. In this region, the air masses moving from continental areas to Japan can be monitored while avoiding aerosol contamination from local areas. We observed the amount of aerosols in air samples microscopically, and estimated the trajectories of air masses during the sampling periods. The viabilities of halotolerant microbial communities in air samples were evaluated using culture media amended with various NaCl concentrations. The bacterial species composition of the air samples was analyzed using clone library analysis targeting bacterial 16S rRNA genes.

2. Materials and methods

2.1. Sampling

Aerosol samplings were performed over Suzu City (37.5°N, 137.4°E) during four sampling periods. Suzu City is located on the northern coast of the Noto Peninsula, Japan and is the arrival site for aerosols from continental areas. A balloon was used for sampling over Suzu City from 11:00 to 12:00 on May 8, 2008 and from 10:50 to 11:50 on April 29, 2009. An aircraft was used for sampling from 14:50 to 16:50 on March 27, 2010 and from 11:50 to 13:50 on May 15, 2010. On March 27, 2010, the aircraft traveled westward from Suzu City to a distance of 150 km single way and back (Fig. 1). On May 15, 2010, from Suzu City, the aircraft traveled a distance of 150 km toward northwest, returned to Suzu City, and traveled a distance of 150 km toward northeast. The conditions of the four sampling periods are summarized in Table 1. The four samples collected on May 8, 2008; April 29, 2009; March 27, 2010; and May 15, 2010 were named A, B, C, and D, respectively.

During the sampling periods on May 8, 2008 and April 29, 2009, the air samples were collected at 800 m above the ground level using a tethered balloon (Maki et al., 2008). An air pump with a sterilized filter holder was carried by the balloon and was switched on at a specific altitude by a signal transmitted from the ground. An air sample (700 l) was collected on a sterilized polycarbonate filter (0.22-μm pore-size; Whatman, Tokyo, Japan) for 1 h. After sampling for an hour, the battery for the air pump failed at 800 m in the atmosphere.

On April 29, 2009 and May 15, 2010, aerosol samplings were performed at 3000 m above the ground level using an aircraft with a 25-mm-diameter hole at the top. A sterilized sampling tube (1.5 m in length) was inserted into the hole with one end of the tube

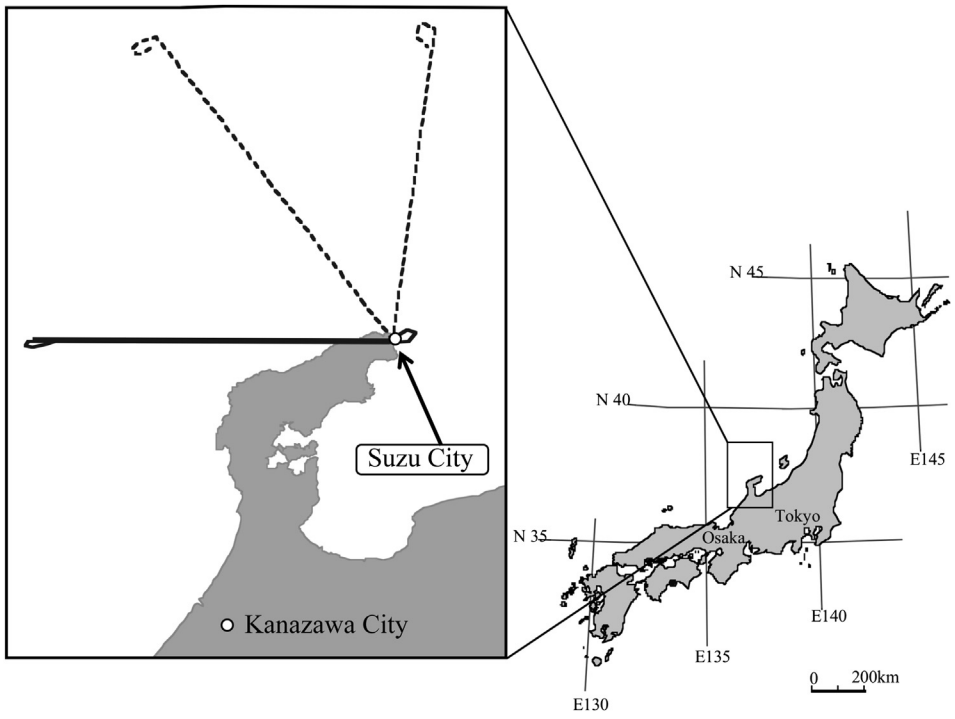

Fig. 1. Aircraft flight routes during the sampling periods: 14:50–16:50 on March 27, 2010 (solid line) and 11:50–13:50 on May 15, 2010 (dotted line).