Aerosol bacteria over the Southern Ocean during ACE-1

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Abstract

Individual bacterial cells occur in many samples that were collected at Cape Grim, Tasmania and during the Lagrangian “B” experiment of the Aerosol Characterization Experiment 1 (ACE-1) campaign that was conducted above the Southern Ocean. They are present in samples from altitudes as high as 5.4 km. Morphologically, almost all bacteria are rod-shaped, about 1 \( \mu \)m long or smaller, have one polar flagellum, and contain inclusions that are rich in P and K. Their morphological features suggest that these bacteria are motile, marine species. It seems likely that the cells became airborne by the same bubble-bursting mechanism that ejects sea-salt aerosol particles into the atmosphere; however, the bacteria and sea-salt particles are typically not aggregated with one another. The estimated number ratio of bacteria and the dominant aerosol species, sea salt, varies in the samples and averages about 1%. The aerosol bacteria seem to represent an important atmospheric reservoir of P and organic compounds; on the other hand, since they are externally mixed with sea salt, they are unlikely to be effective as cloud condensation nuclei.

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1. Introduction

The Aerosol Characterization Experiment 1 (ACE-1) was designed with the aim of studying aerosol formation and evolution in the essentially unpolluted, remote troposphere.
of the Southern Ocean (Bates et al., 1998a). In order to examine the mixing states, chemistry, and structure of individual particles, we used transmission electron microscopy (TEM) to study this clean marine aerosol. The major particle types are sea salt (Murphy et al., 1998), sulfates, and soot (Pósfai et al., 1999). In addition, individual cells of bacteria occur in almost every sample that we studied. In this report, we describe the morphological and compositional characteristics of these bacteria and discuss their potential significance in atmospheric processes.

Bacteria and other primary biological particles can be released into the atmosphere from water surfaces (Blanchard and Syzdek, 1970, 1982; Baylor et al., 1977), vegetation (Artaxo and Hansson, 1995; Lindemann and Upper, 1985), and by anthropogenic sources (Sawyer et al., 1996). Biological constituents of atmospheric aerosols have long been recognized, and large numbers of bacteria were found even at high altitudes and in remote oceanic and polar regions (reviewed by Gregory, 1973; Cox, 1987). However, knowledge of the relative concentrations, global distributions, and atmospheric importance of airborne bacterial cells is still very limited.

The presence of bacteria in the atmosphere is significant for several reasons: they may spread diseases (Cox, 1989; Griffin et al., 2001), play a role in ice nucleation in the atmosphere (Schnell and Vali, 1976; Vali et al., 1976), and could nucleate cloud and fog droplets (Saxena, 1983; Fuzzi et al., 1997). Certain species of bacteria are known to be effective ice nuclei at temperatures as high as $-1^\circ\text{C}$. Such bacteria were found living on plant leaves (Maki and Willoughby, 1978) and in the surface layer of the ocean (Schnell, 1977), places from which they can be easily transported into the atmosphere. Although mineral dust particles are believed to be the major ice-nucleating agents in supercooled clouds, there has been speculation about the significance of biological particles, primarily bacteria, in atmospheric ice nucleation processes (Vali, 1985). Organic aerosol particles can form the majority of cloud condensation nuclei in certain regions (Novakov and Penner, 1993), but it is not known what fraction, if any, of these particles are microorganisms. Some bacteria and their attachments (like pili, flagella) are known to be effective nucleation sites for minerals (Fortin et al., 1997), as well as for water droplets (Sattler et al., 2001), and so they have the potential to be effective cloud nucleating agents in the atmosphere. It was even found that bacterial populations live and grow in cloud and fog droplets (Sattler et al., 2001; Fuzzi et al., 1997).

There are two general methods for sampling bacterial aerosols: (1) collecting cells on culture media and then counting the colonies that form, and (2) collecting aerosol particles on filters or on impactor plates, staining the samples with a dye that specifically binds to DNA, and then counting the individual cells using epifluorescent microscopy (Griffin et al., 2001). The first approach can be applied to obtain the number of viable cells in the atmosphere and to learn details about the physiology, taxonomy, and other characteristics of the collected, viable bacteria. However, many species or strains are unable to grow on the selected media, and so they remain undetected. In addition, this method does not provide information on non-viable organisms, which might still have as large a role in cloud droplet and ice nucleation as living cells do. The second approach is useful for obtaining the total number of bacterial cells in the atmosphere, and thus can potentially yield more useful information regarding the role of bacteria in atmospheric processes.
Since our experiments during ACE-1 were designed with the purpose of studying sea salt, sulfate, and other, mostly inorganic aerosol particles, and we only encountered bacteria by chance, our experimental setup was not optimal for collecting biological particles. Our approach was similar to the direct-count method, except that we did not stain the samples, and instead of using epifluorescent microscopy, we observed individual cells using transmission electron microscopy. Bacteria were distinguished from other aerosol particles on the basis of their morphologies and compositions.

2. Experimental

We collected two types of aerosol samples for TEM analysis during the December, 1995 ACE-1 campaign, conducted above the Southern Ocean, near Tasmania (Bates et al., 1998a); one set was collected at Cape Grim, Tasmania and the other from a C-130 aircraft. Also collected were filter samples for analysis by automated scanning electron microscope; results are described by Murphy et al. (1998) and Sievering et al. (1999). For TEM, particles were impacted directly onto 3-mm grids covered by carbon-coated Formvar films. Most ACE-1 samples are representative of clean marine conditions.

At the Cape Grim Baseline Air Pollution Station, particles were collected on the 30-m level of a tower located on a 94-m steep bluff overlooking the Southern Ocean. The TEM grids were placed on the 2nd, 3rd, and 4th stages of a Casella cascade impactor; these stages collect particles with nominal aerodynamic diameters in the ranges of >2, 2–0.7, 0.7–0.2 μm, respectively. In this study, we used samples that were collected on December 1, 3, 4, and 10 (Table 1), days that were characterized by clean oceanic conditions, with winds out of the west and air mass back-trajectories indicating no or very little land contact for 96 h prior to arrival at the sampling site (Whittlestone et al., 1998).

During the Lagrangian “B” experiment of ACE-1, we sampled aerosols with a Programmable Streaker Sampler (PIXE International) configured as a one-stage impactor on the NCAR C-130 aircraft and obtained TEM samples in clear air at altitudes ranging from 30 to 5400 m on December 7, 8, and 9 (flights #24, 25, and 26). The community

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date</th>
<th>Impactor stage number</th>
<th>Number of bacterial cells</th>
<th>Number of sea-salt particles</th>
<th>Bacteria/total particle number (%)</th>
</tr>
</thead>
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<tr>
<td>A4</td>
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<td>3</td>
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<td>12/01/95</td>
<td>3</td>
<td>5</td>
<td>926</td>
<td>0.5</td>
</tr>
<tr>
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<td>12/03/95</td>
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<td>1</td>
<td>663</td>
<td>0.2</td>
</tr>
<tr>
<td>B9</td>
<td>12/04/95</td>
<td>2</td>
<td>6</td>
<td>155</td>
<td>4</td>
</tr>
<tr>
<td>C3</td>
<td>12/10/95</td>
<td>3</td>
<td>8</td>
<td>286</td>
<td>3</td>
</tr>
<tr>
<td>C4</td>
<td>12/10/95</td>
<td>4</td>
<td>8</td>
<td>24</td>
<td>25</td>
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<tr>
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<td></td>
<td></td>
<td>31</td>
<td>3052</td>
<td>1</td>
</tr>
</tbody>
</table>
aerosol inlet was used; it is described by Bates et al. (1998a). The impactor had a nominal lower cut size of 0.2 μm.

For TEM analysis we used a JEOL 2000FX electron microscope operated at a 200-kV accelerating voltage. Compositions were determined by energy-dispersive X-ray spectrometry (EDS) using an attached ultra-thin-window KEVEX detector that allowed the detection of C and heavier elements (for details see Põsfai et al., 1999).

A legitimate concern is whether the bacteria are contaminants, particularly because we did not use sterile equipment. However, we are certain that the bacteria were present in the atmospheric aerosol and are not a result of contamination. After sample collection, the TEM grids were stored in grid boxes that were kept in a desiccator. The specimens were handled in the ambient laboratory air for a few minutes when they were removed from the grid box and inserted into the vacuum of the TEM; the chances of accidentally depositing bacteria from the laboratory air were thus minimal. We have handled hundreds of mineral and aerosol specimens in a similar fashion and only rarely observed bacteria in any other samples from other regions; for instance, we observed only a single cell from a set of samples from the North Atlantic. In addition, blank grids that were stored and handled in the same way as the bacterium-bearing ones but were not exposed to the atmospheric air on the C-130 aircraft did not contain bacteria or sea-salt particles.

Fig. 1. Electron micrograph showing a single bacterial cell (arrowed) and four sea-salt particles. Within sea-salt particles, the dark, square-shaped crystals are NaCl, and the lath-shaped crystals are sulfates (Cape Grim).
3. Results

A few bacteria typically occur in all of the samples examined with TEM; based on their morphologies, they are easily distinguished from the abundant sea-salt particles (Fig. 1). The Cape Grim samples were obtained at 120 m above sea level. The samples from the Lagrangian experiment were obtained from various heights, ranging from 30 m above sea level to an altitude of 5.4 km. We did not observe any obvious differences in the relative numbers of bacteria in the samples obtained at different altitudes.

Almost all bacteria have the same morphological features (Fig. 2): they are rod-shaped (a and c), have a cell-wall structure that suggests they are Gram-negative (see the white arrow in a), possess one polar flagellum (a, b, c), and typically contain several dark inclusions that are rich in P and K. In some bacteria features are preserved that indicate the process of cell division: the cell wall is incised and there are dark regions, presumably genetic material, at both ends of the cell (c). The remarkable uniformity of cell morphologies suggests that these bacteria share a common source. In addition to the typical rod-shaped bacteria, only a few other cell types occur, including some curved rods (b) and the microorganism shown in Fig. 3.

Fig. 2. Typical bacteria from the Southern Ocean aerosol; they possess polar flagella and phosphatic granules (the dark spots in the cells). The arrows in (b) point to soot particles. In (c), the black arrows point to filaments associated with the bacterium, and white arrows mark dark areas that likely contain DNA. The diagonal white line in panel (a) is a break in the substrate (a and c) are from Cape Grim, and (b) is from south of Tasmania, collected in “Lagrangian Experiment B”, at 2.1 km altitude).
The number of bacteria relative to other aerosol species is difficult to determine. In the impactor that we used on the C-130 aircraft, the TEM grid was placed under the orifice, producing a radial distribution of particles with regard to aerodynamic diameter, with the largest particles closest to the center. This uneven distribution of particles makes it impossible to calculate the concentration ratio of bacteria to sea salt except for particles of equal aerodynamic diameters. Since the density of bacteria is lower than that of sea salt, the equivalent geometric diameter of bacteria is greater than that of corresponding sea salt. Rod-shaped bacteria are aligned on each grid with their long axes parallel to one another. This orientation likely results from the characteristic shapes of the cells.

We compared the numbers of bacterial cells and sea-salt particles in six samples from Cape Grim by counting all particles within 6 to 8 randomly selected meshes (all about 90 × 90 μm squares) (Table 1). Bacterial cells comprised about 1% of the total particle number. There is no evident relationship between the total aerosol loading on the grids and the number of bacteria. Four samples were collected on the 3rd stage of the Casella impactor, and one sample each on the 2nd and 4th stages; the only 4th stage specimen stands out among the samples for its highest relative concentration of bacteria (25% of all particles). We interpret this change of relative cell concentrations between the 3rd- and 4th-stage samples that were collected at the same time (samples C3 and C4, Table 1) as an indication that sea-salt particles were more efficiently trapped on the upper stages than the bacteria.
4. Discussion

The most likely source of the observed bacteria is the ocean water, since the aerosol samples were obtained from air masses that arrived from the undisturbed Southern Ocean atmosphere and the dominant aerosol type is sea salt. Also, the bacteria have remarkably uniform morphologies, and their polar flagella indicate motility in an aquatic habitat. In light of the presumed oceanic origin of these bacteria, it is surprising that cells occur on the collection substrate without being aggregated with sea salt. Unless the bacteria leave the water surface by an unknown mechanism, they were ejected from the ocean in seawater droplets (Blanchard and Syzdek, 1982). In this case, however, they apparently separated from the sea-salt particles early in the process of drying of the droplets, since some fraction of the aerosol collected at Cape Grim and in low-altitude C-130 legs must have been newly formed. Crystallization of the sea-salt component of the internally mixed aerosol droplet could possibly separate the bacterium from the sea salt; however, the relative humidity ranged from 65% to 90% during the collection of our samples (Sievering et al., 1999), which makes it unlikely that sea-salt particles could have been crystalline before arriving onto the grids. At present, we do not have a satisfactory explanation for the lack of internal mixing of bacteria and sea salt.

Since the cells are neither attached to the strongly hygroscopic sea-salt particles, nor show signs of a former liquid coating, their surfaces are likely hydrophobic; thus, these bacteria are unlikely to play a role in cloud nucleation. Nevertheless, we note that the degree to which bacteria attach to the air-water interface is influenced by several factors including the ionic strength of the medium and hydrophobicity of the cell surfaces, and much detail remains unknown about the interactions between bacteria and the air–water interface (Schäfer et al., 1998). Also, we observed these bacteria in the same part of the Southern Ocean where high atmospheric ice nucleus concentrations (Bigg, 1973) coincide with high biological productivity in the ocean, as noted by Schnell and Vali (1976). To obtain more certain knowledge about the nucleating abilities of airborne bacteria over the Southern Ocean, it would be necessary to collect aerosol particles by using a method that is specifically designed for sampling biological particles.

Since the dominant particle types in the Southern Ocean atmosphere are sea salt and sulfate (Murphy et al., 1998), and these particles do not contain P, the bacteria could represent the most significant reservoir of P in this region during clean conditions. The bacteria also contain K within the phosphatic bodies and are certainly contributors to the organic compounds in the atmosphere of the Southern Ocean. However, the aerosol organic material at Cape Grim was found to be internally mixed with sea salt (Middlebrook et al., 1998).

The number concentration of microorganisms relative to other particle types in the atmosphere seems to vary widely depending on the sampling location, meteorological conditions (Bovallius et al., 1978), and collection methods (Nevalainen et al., 1992). For example, in the Amazon Basin biogenic particles represent as much as 55% to 95% of the total particle mass concentration during the wet season (Artaxo et al., 1990). In continental air over Germany, Matthias-Maser and Jaenicke (1994) found that 37% of the total particle number are bacteria, fungi, and pollen. On the other hand, many studies, including those performed within large international aerosol characterization experiments that were...
designed to obtain comprehensive data on various types of aerosols (such as ACE-1 (Bates et al., 1998b), INDOEX, ACE 2, TARFOX, etc.), did not report a significant biogenic aerosol component. We believe that this large variation in the observed concentrations of microorganisms is partly a result of their highly variable numbers in the atmosphere, and partly a consequence of the different collection and analytical methods that were used in the above studies.

Potential problems with sampling bacteria are also highlighted by our present study. As mentioned above, the rod-shaped cells occur aligned on the grids, indicating their special aerodynamic behavior. In addition, the ratios of the numbers of bacteria and sea-salt particles were affected by different collection efficiencies for the two particle types, as indicated by the high relative concentration of cells in the 4th-stage sample. In this study we found that bacteria represent about 1% of the total number of particles with diameters >0.2 μm. This number seems small since the study area is characterized by upwelling and convergence of oceanic waters, resulting in high biological productivity (Schnell and Vali, 1976). It is even more surprising that our previous single-particle TEM studies of marine and continental, clean and polluted samples (Pósfai et al., 1994, 1995, 2003; Buseck and Pósfai, 1999; Pósfai and Molnár, 2000; Li et al., 2003a,b; Buseck et al., 2002) did not indicate the presence of bacteria.

In addition to the relative number concentrations of aerosol microorganisms, it would be important to know whether they occur internally or externally mixed with other aerosol species. Such information is almost completely lacking, despite the great significance of the mixing state in determining the efficiency of microorganisms in nucleating cloud droplets and ice crystals. Since microbes are assumed to be attached to dust particles transported over the oceans (Griffin et al., 2001), their mixing state affects the global spreading of allergens and pathogens. Our report of the bacteria from the Southern Ocean attempts to draw attention to this problem; the uncertainties of the impactor’s collection efficiency for bacteria notwithstanding, we believe that the confirmation of the very existence of bacteria, their apparent relative abundance, and external mixing states with sea salt are important results of this study. Future studies should address the problem of the mixing states of aerosol microorganisms by using sampling procedures that allow efficient collection of both microbes and other aerosol constituents such as sea salt, sulfates, mineral, and organic particles. Regarding analytical methods, a combination of epifluorescent microscopy with analytical transmission electron microscopy could provide useful data on both the relative number concentrations and mixing states of aerosol microorganisms.

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References


