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Studies on bacteria-like particles sampled from the stratosphere

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Abstract

Bacteria-like particles recovered from the stratosphere and deposited on cellulose acetate membranes have been analysed to confirm their bacterial nature. One particle appeared to be attached to an inorganic particle apparently by mucoid material typically produced by bacteria. A filamentous structure, morphologically similar to a fungal hypha, was also observed. EDS analysis showed that the particles were all non-mineral and therefore could be biological in nature. However, the composition of several clumps of nanobacteria-sized particles were found, by SIMS analysis, to be inconsistent with that of bacteria. The results show that it is dangerous to assume that bacteria-like particles seen under scanning electron microscopy are necessarily bacteria.

1. Introduction

Because of obvious sampling difficulties, there have been relatively few studies on the microbiology of the stratosphere. Russian workers, using rockets to sample the stratosphere at heights of 48–77 km claimed to have cultured a range of bacteria and fungi (Imshenetsky et al., 1978). More recently, using balloon-carried cryosamplers, Wainwright et al. (2003, 2004) have shown that bacteria are present in atmospheric samples collected at a height of 41 km. Here, we report scanning electron microscope (SEM) studies of stratospheric particles possessing biological morphologies. Energy dispersive X-ray spectroscopy (EDS) and SIMS analysis were used to investigate whether these bacteria-like particles are actually biological.

2. Materials and methods

Stratospheric air was sampled using a balloon at a height of 41 km and transferred to 0.2 μm cellulose acetate filters described by Wainwright et al. (2003). The membranes were then gold coated and examined by scanning electron microscopy (SEM)using a Phillips ESEM XL30 scanning electron microscope with a field emission gun; the elemental composition of the particles was determined using a Prism EDS X-ray detector.

Secondary ion mass spectrometry(SIMS) was performed using the Lawrence Livermore National Laboratory Cameca NanoSIMS 50. The $^{133}Cs^{+}$ primary beam had a 50 nm diameter. The mass spectrometer was tuned for simultaneous detection of $^{12}C^{-}$,$^{13}C^{-}$ and $^{12}C^{14}N^{-}$,$^{12}C^{15}N^{-}$,and $^{31}P^{-}$. N is detected as CN. Samples were located first by SEM and relocated in the NanoSIMS using real-time secondary electron imaging. Analyses were performed on control bacteria (a species of *Bacillus*) for sample comparison.

3. Results and discussion

A variety of bacteria-like particles having the dimension of normal bacteria (i.e. in excess of 0.5 μm) were observed on the membrane on which particles from the stratosphere had been deposited. Figure 1a, for example, shows an oval, bacterium-like particle. Except for its collapsed appearance (not usually associated with inorganic particles), this particle, like most others observed, possesses no morphological characteristics (e.g. flagella or fimbriae) that are typically associated with bacteria. The coccoid-shaped particle shown in Figure 1b is perhaps more convincingly bacterial because it is attached to an inorganic particle by what appears to be mucoid material, typically produced by bacteria (crystals of similar inorganic particles were subsequently shown by EDA analysis to consist of zirconium and chlorine). Such bacteria-like characteristics provide more suggestive, but not conclusive, evidence that this particle is bacteria in nature. A mass of bacterial-like nano-particles, some appearing as appendages is shown in Figure 1c. Such a clump could be regarded as being nanobacteria, but again, no typically bacterial structures are present to aid confirmation of this possibility. Very few filamentous organisms having morphologies similar to microorganisms are seen in stratospheric air samples. An exception is shown in Figure 2. Here, a filament with a rounded tip is seen emerging from a particle mass. Although spores are not present, this filament is morphologically similar to a fungus, or possibly a filamentous bacterium. The thickness of the filament might appear to indicate the latter. However, microorganisms surviving in the extreme environment of the stratosphere will be exposed to low nutrient conditions which would tend to induce coccoid cells to become smaller, and make filamentous organisms thinner than laboratory-grown bacteria and fungi.

Scanning electron microscope studies have been widely used in microbial ecology, often somewhat uncritically. For example bacteria- like structures found in soils and waters have been assumed to be bacteria in the absence of further defining analysis. Claims that bacteria exist in the stratosphere clearly require exacting standards of evidence. To this end, the particles seen in our very limited sample were examined using EDS analysis. Unfortunately it was not possible to confirm that the particles consist of carbon, so this had to be inferred by the fact they were not clearly inorganic. Electron dispersive X-ray analysis of non-bacteria-like particles from the atmosphere generally shows them to be rich in calcium, iron or silicon, or (like the particle shown in Figure 1b) more exotic metals such as zirconium. The bacteria-like parti-

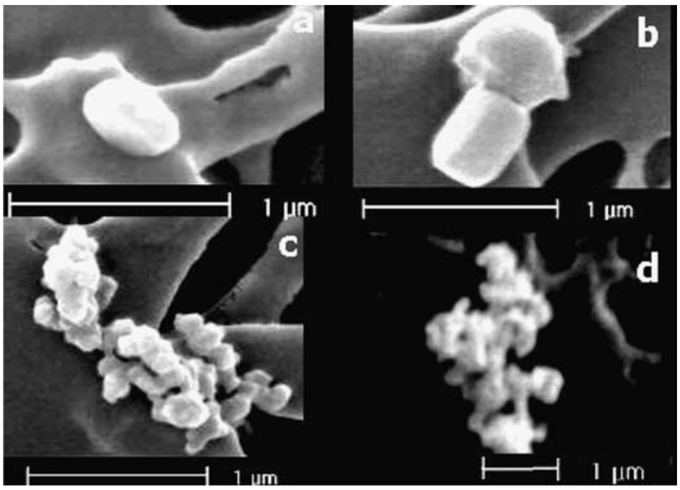

Figure 1. Bacteria-like particles from the stratosphere (at a height of 41 km).

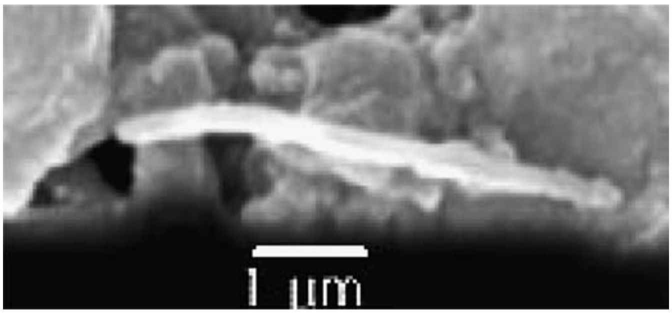

Figure 2. A stratospheric filament, morphologically similar to a fungus.

cles in contrast are not rich in these elements. From this it can be concluded that the bacteria-like particles are not mineral in nature. For further confirmation of this, we analysed clumps of bacteria-like particles by SIMS. Because of the relative difficulty of locating particles of interest, only three bacteria-like masses (e.g. the one shown in Figure 1d) were analysed. These three were of particular interest because they are morphologically similar to nanobacteria. Previously, Wainwright et al. (2004) provided presumptive evidence for the presence of nanobacteria in these stratospheric samples, a conclusion based on the apparent presence of fimbriae on one of the particles. The EDS spectrum of the particle mass shows that it was not mineral in nature (Figure 3). SIMS analysis however, showed that the C and CN count rates were significantly below count rates found for control bacteria, and that the CN/C ratio of the particle mass is not typical of an organic sample. Therefore, this particle is not a clump of nanobacteria. This of course does not mean that all of the bacteria-like particles, reported here, and elsewhere, are artefacts. It merely shows that in the absence of defining characteristics (such as fimbriae or flagella), it is dangerous to assume that particles with bacteria-like morphologies are always bacteria.

The use of the NanoSIMS is potentially of considerable benefit, since it can be used to analyse isotopic ratios, that may indicate whether a bacterium isolated from the stratosphere originates from Earth or space. It is worth noting that the choice of membranes onto which any stratospheric particles are deposited is best made with NanoSIMS analysis in mind during the initial design of the experiment. Here, cellulose acetate membranes, typically employed by microbiologists, were used. Unfortunately, these did not withstand the full period of NanoSIMS analysis. As a result, in future studies, stratospheric particles will be collected, or deposited, on more durable gold membranes.

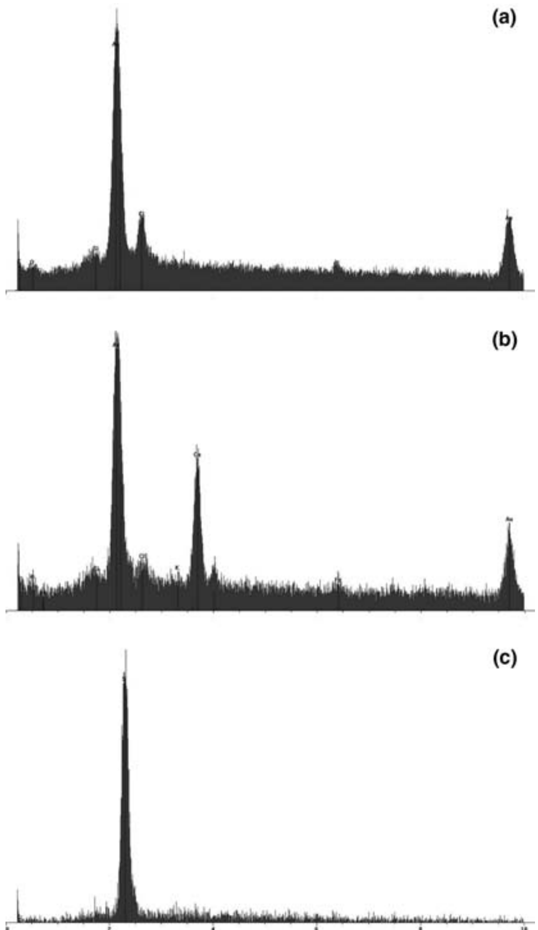

Figure 3. Typical EDA analysis of the particles obtained at 41 km. (a) Analysis of a typical bacteria-like particle, (b) Analysis of a typical inorganic-like particle; (c) Analysis of the control membrane.

In conclusion, we have demonstrated that several masses of stratospheric particles possessing bacteria-like morphology are not biological. This does not of course mean that all of the observed particles seen were of a similar nature; certainly particles possessing bacteria-like structures are probably biological and warrant further study. Finally, these results emphasize that SEM-based morphological characteristics alone cannot be used to confirm the presence of bacteria in stratospheric samples. This caveat applies to more routinely sampled environments, such as soils and water, where SEM-based morphology is often the sole criterion used for identifying bacteria.

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