

REPORT

Isolation Of Biological Entities From The Stratosphere (22–27km)

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Putative biological entities were isolated at a height of between 22–27 Km in the stratosphere, varying from a presumptive colony of ultrasmall bacteria to two unusual individual organisms. Biological structures of this nature have not previously been reported as occurring in the stratosphere. The likely origin of these structures is discussed, with the conclusion that some at least may be plausibly attributed to an ingress from space.

Introduction

We previously reported the discovery of a fragment of a diatom frustule in the stratosphere obtained by a recent balloon-sampling of the stratosphere at a height of between 22–27km (Wainwright, et al 2013). We also speculated on the origin of this unequivocally biological particle and concluded that, since there was no mechanism by which it could have reached the stratosphere from Earth, this diatom fragment must have been falling to our planet from space. A number of other putative biological entities were obtained during the same sampling exercise, details of which are presented in the present paper. We also discuss here the problems and pitfalls in deciding whether particulate material found in stratospheric samples are of biological origin or are made up of inorganic, cosmic dust.

Materials and Methods

A balloon-borne stratospheric sampling device was launched from Chester, NW England on 31 July 2013. The sampler included a drawer mechanism that could be automatically opened and closed at any desired height. The stratosphere sampler also carried a video camera by which the opening and closing of the sampling drawer could be viewed, confirmed and recorded. The sampling apparatus was protected from downfall of particulate matter from the balloon by the use of a cover. Prior to launch, the interior of the drawer device was scrupulously cleaned, air blasted and finally swabbed with alcohol. New scanning electron microscope stubs were

placed in rows inside the drawer with their top surfaces facing outwards so that when the draw was opened any particulate matter in the stratosphere would attach to them and they could later be removed for examination under the scanning electron microscope. The protective layer on the surface of the stub was peeled off just before launching under a cover to prevent any particulate contamination. After sampling, the apparatus was transported to the laboratory and opened under conditions which avoided exposure of the stubs to contaminating dust, and the stubs were similarly transferred under cover to the scanning E/M. The stubs were then sputter-coated with gold for 30secs at 30mA and examined using a SEM (JEOL 6500F).

Balloon launch

The balloon was launched from an open field near Dunham on the Hill (near Ellesmere Port, Cheshire, England) during daylight hours and traversed to just south of Wakefield in West Yorkshire (England). The sampling drawer was opened for 17 minutes as the balloon rose from 22026m to 27008m. The sampling apparatus was returned to Earth (by parachute) undamaged and completely intact.

Control flight

A separate control flight was made to the stratosphere prior to the sampling flight, when the draw was not opened, but all other sampling procedures were observed. No particulate matter was found (using the SEM) on any of the unexposed microscope studs, showing that the draw remained airtight and that none of the stubs was exposed to particles at, or near, ground-level or at any height up to the stratosphere. These results also show that no particles contaminated the stubs during any of the sample processing procedures, thereby demonstrating that the scrupulous procedures used to prevent ground-level contamination proved effective.

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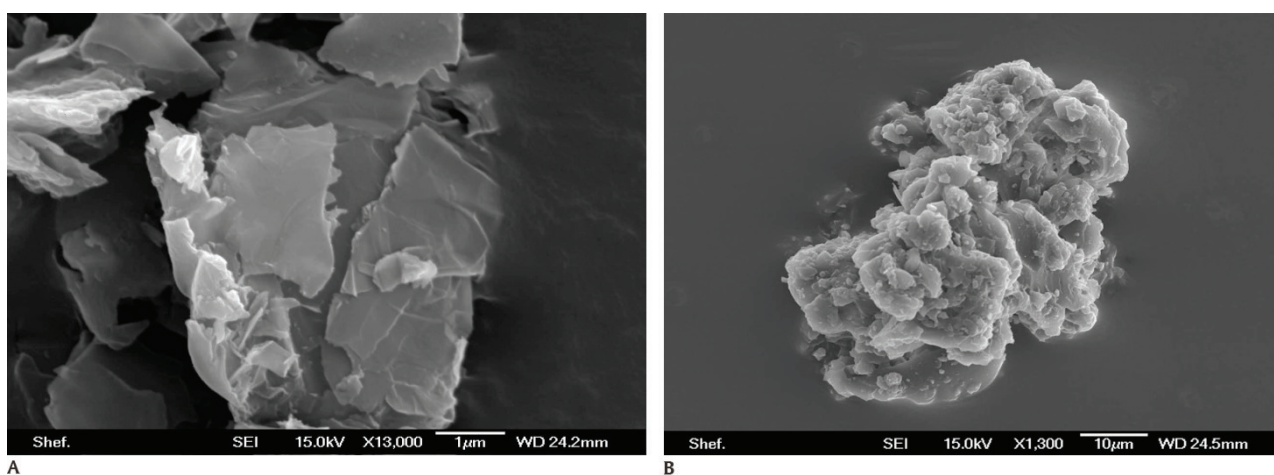


Fig. 1: A) Sheet-like inorganic material recovered from the stratosphere which is clearly not biological; and **B)** a clump of stratospheric cosmic dust which includes coccoid and rod shaped particles which may, or may not, be bacteria.

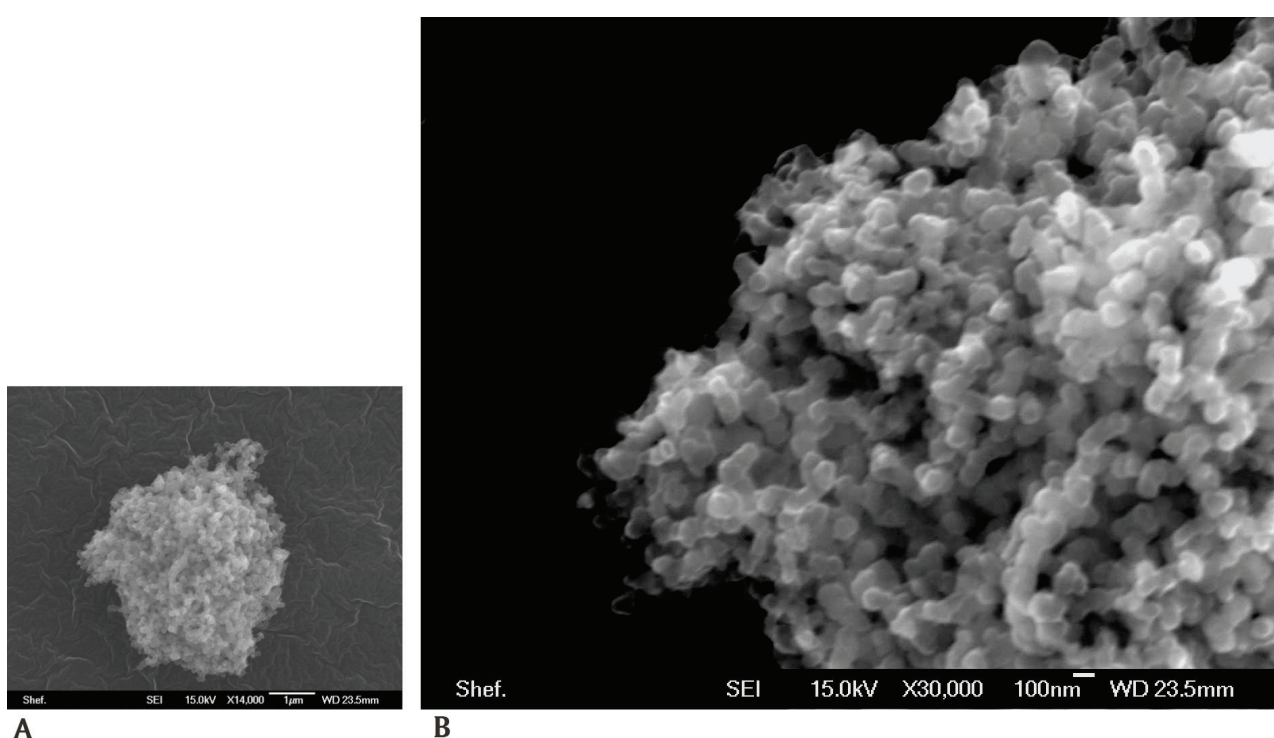


Fig. 2: A) A colony of putative bacteria sampled from the stratosphere. **B)** a detailed image of the colony, note the “budding” and complex interconnection of the particles suggesting bacteria.

Results and Discussion

A major problem which faces anyone researching the microbiology of the stratosphere is the difficulty of distinguishing between biological entities, particularly bacteria and fungi from particles of cosmic dust which in some cases can mimic the morphology of these entities, notably bacteria (Wainwright et al, 2004a, b, Wainwright et al, 2006, Wainwright, 2008). While structures such as diatom frustules can be readily and unequivocally ascribed to being biological, in the case of bacteria such certainty is rarely forthcoming. This is because structures seen on electron micrographs as naked cocci, or rods are often difficult to distinguish from some types of cosmic dust particles. The use of EDX analysis is of limited value since it cannot identify biological compounds. Moreover strato-

spheric microbes are often associated with or attached to inorganic particles and the resolution of spot EDX is not always precise. Previous studies have shown that some particles which morphologically appear to be bacteria turn out under NanoSims analysis to be wholly inorganic. (Wainwright et al, 2004c).

Fig. 1A shows inorganic material which was isolated during the current study and which has also frequently been seen in samples obtained from 41km (Wainwright, 2008). Such material is clearly inorganic and could not be confused with biological entities. The situation in regard to clumps of cosmic dust is, however, not so straightforward as is shown in **Fig. 1B** where coccoid and rod-shaped particles of the general size of bacteria (around 1micron) can be seen amongst the dust particles. In

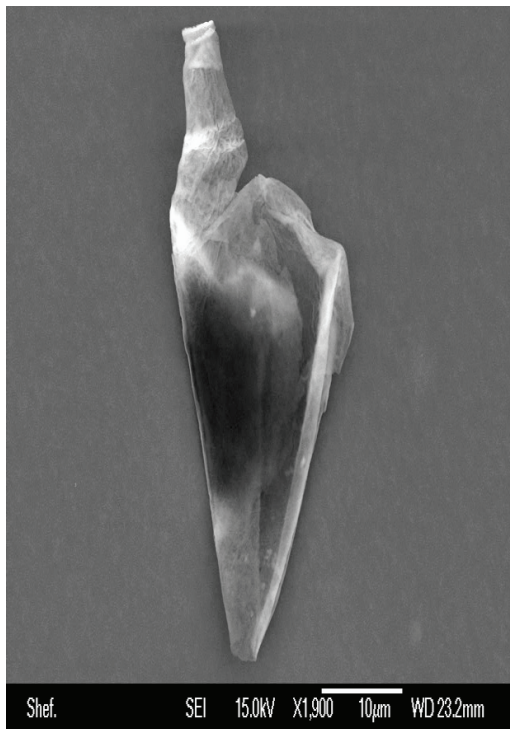


Fig. 3: An unknown biological entity isolated from the stratosphere.

this case, there are no distinguishing biological features present, such as flagella or pilli which could be used to distinguish such bacteria-like particles from inorganic dust and claims that such particles are bacteria therefore remain open to dispute.

The situation is more clear-cut in the case of what appears to be a group of particles which have the appearance of a bacterial colony as shown in **Fig. 2** (Upper frame). A detail here is that the particles in the “colony” are exceedingly uniform in appearance (**Fig. 2** Lower frame). They show signs of “budding” as well as the formation of interconnections by way of bridges. The individual particles here are much smaller than “normal” sized bacteria, being around 100nm; bacteria of this size (so-called nanobacteria) do however, exist, so the small size of these particles does not automatically exclude them from being bacteria (Wainwright, 2004a).

While the colony shown in **Fig. 2** appears to be biological it could still be argued by the sceptic that it is instead made up of minute inorganic particles. The structure shown in **Fig. 3** however is unequivocally biological. Here we see a complex organism which has a segmented neck attached to a flask-shaped body which is ridged and has collapsed under the vacuum of the stratosphere or produced during E/M analysis. The top of the neck is fringed with what could be cilia or a fringe which formed the point of attachment of the neck to another biological entity. The complexity of this particle excludes the possibility that is of non-biological in origin. It is clearly too large to be bacterium, but could be an algae or a protozoan; it appears however, too small to be an angiosperm seed, and the fact that the main body has a thin wall appears to exclude the possibility of it being a pollen grain, which generally

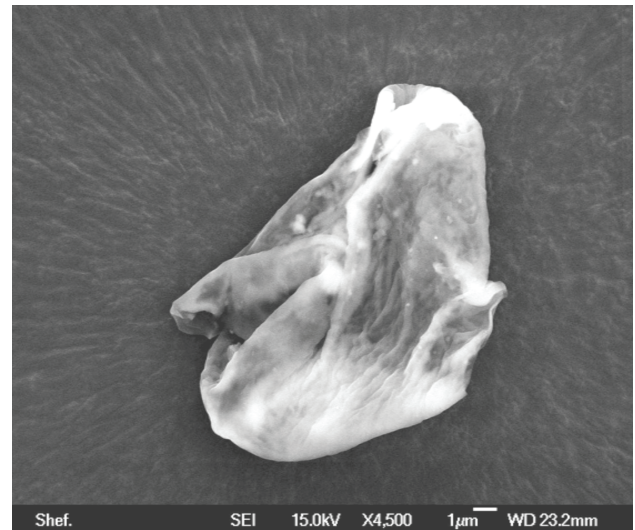


Fig. 4: A collapsed balloon-like biological entity sampled from the stratosphere. Note the “proboscis” to the left, with nose-like openings and the “sphincter” present at the top of the organism.

have rigid outer coats that do not generally collapse when exposed to low pressure.

The structure shown in **Fig. 4** is also clearly biological in nature; here we see a somewhat phallic balloon-like structure which has presumably collapsed under low pressure. A “proboscis” is seen emerging from the left of the main cell which has two, nostril-like openings. At the top of the collapsed “balloon” is a sphincter-like opening. Again, this entity is clearly biological in nature, and is not an inorganic artefact. Although it is clearly not a bacterium it could well be an alga or a protozoan of some kind. The organisms shown in **Figs. 3** and **4** are presumably clear enough for experts in the relevant branches of taxonomy to provide some kind of identification.

From where do these particles originate: space of Earth?

While parsimony and prudence might suggest that the biological entities shown here originated somehow from Earth, the situation is not as clear-cut as it first appears, This is because the tropopause is considered to act as a barrier to the upward movement of particles (as opposed to gases like ozone and aerosols such as a nitric acid), and as a result it is difficult to explain how a structures well over 5 microns in size could make the journey from Earth to the lower, and indeed upper stratosphere.

These conclusions are strengthened by virtue of our earlier unambiguous detections of 10 micron clumps of bacteria, some in viable form, in the stratosphere at an altitude of 41km (Harris et al, 2002; Wainwright et al, 2003, 2004b). In a model calculation of infalling bacteria of various sizes it can be seen that the steady-state distribution of particles in the size-range 0.03–1 micron yields a number density 10 times higher at 27km altitude than at 41km (Kasten, 1968). This is shown in **Fig. 5**. The higher altitude collections of 2001 using a totally different cryosampler technique (Harris et al, 2002) led to the discovery of struc-

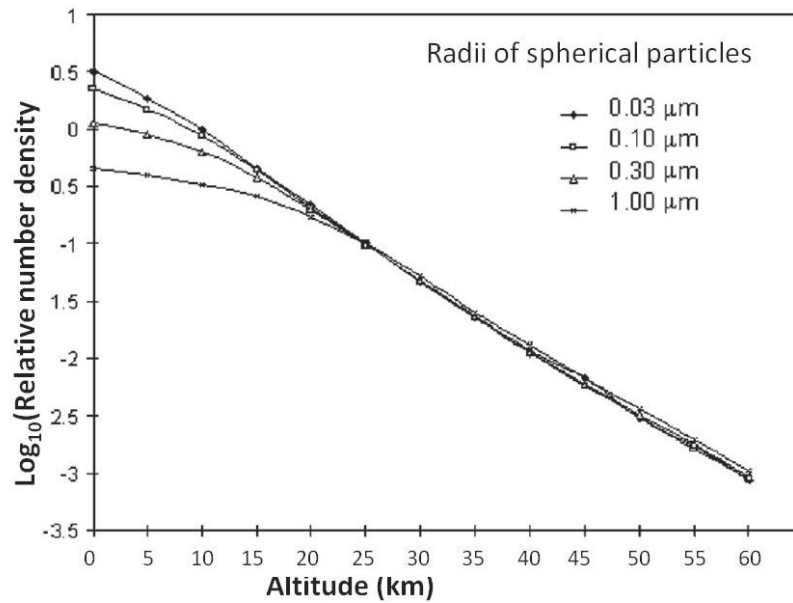


Fig. 5: The relative number densities of bacteria of various radii falling in at a steady rate from space, at various altitudes (Kasten 1968).

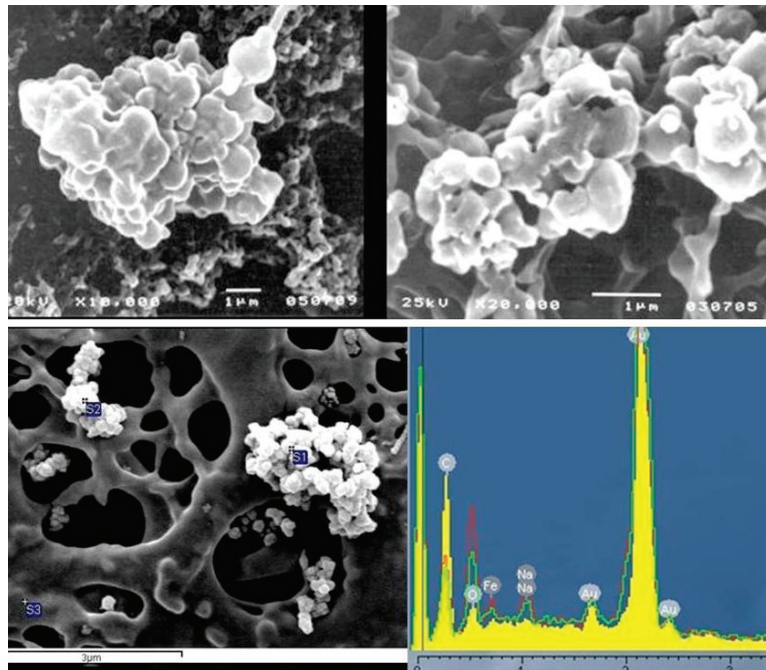


Fig. 6: Carbon-rich structures with putative identification as bacteria from 41km using cryosampler collections in 2001 (Harris et al, 2002).

tures shown in **Fig. 6** which can be seen to resemble some of the structures obtained in the present sampling. The biological nature of some of the 2001 structures that was confirmed by EDX, staining tests to detect DNA, as well as by culturing. This gives us added confidence to argue that the structures resembling bacteria in **Figs. 1–4** have a high probability of being biological.

The obvious means by which such relatively large biological entities (e.g. **Figs. 1–4, 6**) could make the journey to 22–41km in the stratosphere is by being lofted by a violent volcano, although other possible, but unproven, mechanisms have been suggested. It is crucial to note here that no such eruption occurred close to the date when this stratosphere sampling described here was performed and

that structures such as the ones shown here will have a short residence time (< 1 day) in the stratosphere. We are therefore confronted with an obvious dilemma in that while Occam's razor informs us that these biological entities must somehow be carried to the stratosphere from Earth, there seems to be no known mechanisms by which such transfer could be achieved. The corollary is that these biological entities arrived in the stratosphere from above. Commercial aircraft flying at around 30,000 feet could not be the source of this biology, although it is possible, although statistically highly unlikely, that they originated from satellites such as the orbiting space station. A cosmic origin of these particles would of course be weakened if taxonomists could definitely prove that the

biological entities shown here are angiosperm seeds or pollen grains, since there would be obvious (although not necessarily rational) resistance to idea that such structures arrive from space.

It is noteworthy that the biological entities shown in **Figs. 1–4** are manifestly clean and free of cosmic dust or soil particles, thereby suggesting they have an aquatic origin. It is unlikely that a water spout from the Earth's oceans could ever reach the stratosphere to act as a carrier of marine organism to this region. Comets provide a vast cosmic source of water from where such biological entities could have arisen (Hoyle and Wickramasinghe, 2000). It is likely that, because of the very low temperatures and high exposure to UV radiation, such biological entities would not be alive in the stratosphere, although bacteria existing within clumps of cosmic dust would be protected from such radiation and could arrive to Earth in a viable state. Even if these entities were not alive when they arrived in the stratosphere and later fell to Earth, they might still carry DNA and RNA, which could possibly integrate with the genomes of Earth's life forms, thus contributing to the evolution of life on the Earth (Wickramasinghe, 2011, 2012; Wesson, 2010).

The use of isotope fractionation provides a possible means of determining whether the biological entities described here and the stratosphere-isolated diatom fragment previously described are of terrestrial or comic origin. We eagerly look forward to performing this test on our stratosphere-samples.

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