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How do microorganisms reach the stratosphere?

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Abstract: A number of studies have demonstrated that bacteria and fungi are present in the stratosphere. Since the tropopause is generally regarded as a barrier to the upward movement of particles it is difficult to see how such microorganisms can reach heights above 17 km. Volcanoes provide an obvious means by which this could be achieved, but these occur infrequently and any microorganisms entering the stratosphere from this source will rapidly fall out of the stratosphere. Here, we suggest mechanisms by which microorganisms might reach the stratosphere on a more regular basis; such mechanisms are, however, likely only to explain how micrometre to submicrometre particles could be elevated into the stratosphere. Intriguingly, clumps of bacteria of size in excess of 10 µm have been found in stratospheric samples. It is difficult to understand how such clumps could be ejected from the Earth to this height, suggesting that such bacterial masses may be incoming to Earth. We suggest that the stratospheric microflora is made up of two components: (a) a mixed population of bacteria and fungi derived from Earth, which can occasionally be cultured; and (b) a population made up of clumps of, viable but non-culturable, bacteria which are too large to have originated from Earth; these, we suggest, have arrived in the stratosphere from space. Finally, we speculate on the possibility that the transfer of bacteria from the Earth to the highly mutagenic stratosphere may have played a role in bacterial evolution.

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Introduction

Because of difficulties with sampling, little is known about the upper limit of the biosphere. It is generally accepted however, that most microorganisms are restricted to the region below the tropopause, at an average height of 17 km. However, Russian workers, using rocket probes, isolated a widerange of common bacteria and fungi from the stratosphere, at heights up to 60 km (Imshenetsky *et al.* 1978). Our knowledge of the microbiology of the stratosphere has been recently extended by the use of balloon-lifted cryosamplers, by which large volumes of stratospheric air can be sampled and then returned to Earth for analysis. By using scanning electron microscopes, clumps of bacteria-like particles, have been found in the samples, which have been shown, with fluorescent stains, to be viable cells (Harris *et al.* 2002). In addition, very small, bacteria-like particles possessing what appear to be fimbriae have been obtained from a height of 41 km (Wainwright *et al.* 2004).

The majority of attempts to isolate microorganisms from such samples have been unsuccessful, showing that most of the fluorescent-stained cells are viable, but non-culturable bacteria. However, Wainwright *et al.* (2003) reported the

isolation of two bacteria (*Bacillus simplex* and *Staphylococcus pasteurii*) and the fungus (*Engyodontium album*) from a single sample. In a separate study, two further bacteria (*B. licheniformis* and *B. pumilus*) were isolated (in India) from a different subset of the same stratospheric samples (Shivaji, *et al.*, unpublished observations).

The fact that microorganisms are present in the stratosphere has also recently been confirmed by Griffin (2004), who isolated a species of *Penicillium* and two bacteria (*B. luciferensis* and *B. sphaericus*) from a height of 20 km; Griffin (2004) concluded that 'the presence of viable microorganisms in Earth's upper atmosphere may not be uncommon'.

The stratosphere is an extreme environment and it could be argued that bacteria are unlikely to survive the prevailing low temperatures, and high exposure to ultraviolet radiation. However, a number of mechanisms have been suggested to explain how such stratospheric bacteria could survive such extremes, as well as those found in deep space (Wickramasinghe 2004). Protection from ultraviolet radiation could, for example, be afforded by the clump formation and the carbonization of outer layers (in both cases, thereby affording protection to the innermost cells) or by association with porous, stratospheric dust.

Mechanisms by which microorganisms could reach the stratosphere from Earth

The possibility that the above findings result from contamination is obviously reduced by the fact that independent studies have demonstrated the presence of a stratospheric microflora. Since, however, a negative cannot be proven, it is impossible to rule out sampling or laboratory-based contamination. However, if we accept that microorganisms are indeed present in the stratosphere we are left to answer the following question: how did they get there? The obvious answer – that they were carried up from the Earth – unfortunately presents a number of problems. Firstly, the tropopause (at an average height of 17 km) is generally thought to act as a barrier to the upward movement of particles (although not necessarily gases or volatiles). As a result, particles must be ejected with force before they can pass through this barrier. Volcanic eruptions provide an obvious means by which particles could be ejected through the tropopause into the stratosphere. However, such eruptions occur infrequently and, since any particles reaching the stratosphere are subject to sedimentation under gravity, such eruptions cannot provide a constant supply of stratospheric microorganisms.

Although there seems to be no obvious means by which microorganisms could regularly reach 41 km, a number of possibilities can be suggested. For example, it is possible that particles, including microorganisms, are transported to the stratosphere in the updraft resulting from so-called blue lightning strikes. These are conical blue jets that travel upwards from cloud tops and travel at a hundred thousand metres per second to a height of 70 km, charging the atmosphere as they go (Pasko *et al.* 2002); whether microorganisms could survive such charging and the high rate of upward movement is of course open to question.

Microorganisms might also be carried above the tropopause when thunderstorms and forest fires occur together. Apparently, forest fires strengthen thunderstorms, which then pump immense plumes of smoke and soot (as well as presumably microorganisms) into the stratosphere (Fromm *et al.* 2004).

Finally, so-called gravito-photophoresis (GP) might provide yet another mechanism by which microorganisms could reach the stratosphere (Rohatshek 1996). As a result of GP, soot can be carried to a height of 10–20 km, and beyond. Calculated vertical velocities exceed settling velocities by a factor of 30, and it takes about 30 years to transport soot from 10–20 km, and a further 20 years to transport it from 20–80 km. The effect of GP is strongly altitude dependent with the lofting force most effective at an altitude of between 10 and 85 km. It is therefore possible that small microorganisms could be carried by this mechanism in soot particles. Such transfer would be particularly advantageous, since a coating of soot would protect the organisms from the damaging effects of ultraviolet radiation. However, a major problem with this suggestion is that GP only works on soot particles of diameter around 1 µm; submicrometre-sized

bacteria might however, reach the stratosphere by this mechanism, either independently or when travelling on soot particles. This caveat immediately excludes the possibility that fungal spores, which are relatively large, are carried up from the Earth to the stratosphere in this way. It is possible, however, that microscopic fragments of fungal hyphae are carried from Earth to this region and then, infrequently, cultured from stratospheric air samples. It is particularly noteworthy that bacteria and fungi growing under low nutrient (i.e. oligotrophic) conditions are usually much smaller than equivalent cells grown in nutrient rich environments (Wainwright *et al.* 1991); oligotrophically growing microorganisms are therefore likely to be the requisite size to enable them to be transported through the stratosphere.

Is the stratospheric microflora made up of two bacterial populations of distinct origin?

We suggest that the view that all of the microorganisms found in the stratosphere originate from Earth is questionable for the following reasons. Firstly, many of the clumps seen by scanning electron microscopy are relatively large. Harris *et al.* (2002), for example, reported clumps (exceeding 10 µm across) of viable, but unculturable, bacteria. It is difficult to see how any mechanism could carry such large masses into the stratosphere. It also seems to us unlikely that a single bacterium, carried up to 41 km, for example, would replicate to form such a clump under the extreme conditions prevailing at this altitude. Such clump formation might possibly have occurred as the samples were being processed in the laboratory, as they were being exposed from freezing to room temperature and prepared for scanning electron microscopy. However, since no nutrients were added at this stage, such replication and growth to form clumps would have had to have occurred under oligotrophic conditions; presumably such actively dividing bacteria would have been readily cultured, which was not the case (alternatively, bacteria might have physically clumped together).

If such relatively large clumps of bacteria do in fact occur in the stratosphere then we are left with the possibility that they originate from space. Provisional evidence in support of this view was recently provided by Narlikar *et al.* (2003). Based on the determination of numbers of presumed bacteria at two heights in the stratosphere, they concluded that the viable, but non-culturable, bacteria that occur as clumps must be incoming to Earth, rather than exiting.

A mechanism by which evolution may have been speeded up by the transfer of bacteria from Earth to the stratosphere

It has occurred to us that the possibility that transfer of bacteria (and other microorganisms) from Earth to the stratosphere may have impacted (and continue to impact) on bacterial evolution. The continued development of life on Earth has depended upon the presence of an atmosphere that shields the biota from the lethal effects of ultraviolet

radiation. Such protection will necessarily have reduced the exposure of bacteria to the potentially mutagenic ultraviolet and other radiation. This problem can obviously be overcome if a mechanism exists whereby bacteria can be transferred from the relatively benign environment of Earth to the highly mutagenic conditions found in the stratosphere. The above-described mechanisms provide a means by which such transfer of bacteria between Earth and the stratosphere can occur. Those bacteria that are mutated, but not killed, by exposure to stratospheric ultraviolet radiation (a mixture of UV-A, B and C), will be returned to Earth as the result of sedimentation. The novel, multiple or increased number of mutations they carry with them will increase the mutation pool that is available for natural selection to act upon and, as a result, the rate of bacterial evolution will have been greater than if such evolution was solely dependent on mutations occurring below the stratosphere. It is likely that such mutations will occur most often in the lower regions of the stratosphere where numbers of bacteria are greatest and where residence times allow for rapid mutation, but not sterilization. Of course, such a view is dependent on a large number of bacteria being exchanged between the Earth and the stratosphere. However, even if we consider volcanic transfer alone, the number of bacteria transferred is likely to be extremely large over the aeons that bacteria have existed on the Earth.

Conclusion

Recent findings relating to the microbiology of the stratosphere suggest that there exist at least two separate populations of stratospheric microorganisms. One population consists of common Earth bacteria and fungi that are carried on a relatively regular basis (by phenomena such as blue lightning, fire-associated storms and GP) to heights above 17 km; these organisms can be cultured, albeit rarely, from stratospheric air samples. The fact that such bacteria are, essentially, genetically identical to the same species derived from Earth suggests to us that they are from Earth, as it would seem highly unlikely that space-derived bacteria would have evolved at exactly the same rates as their Earth-derived counterparts. Occam's Razor can thereby be invoked: Earth-like bacteria come from Earth (Wainwright *et al.* 2003).

The second component of the stratospheric bacterial population consists of bacteria that exist as relatively large clumps; this population is viable, but non-culturable. It is assumed here that such clumps (around 10 μm across) are too large to have originated from Earth and their distribution in the atmosphere indicates that they have a non-terrestrial origin. Since the majority of bacteria found on Earth have yet to be cultured, the possibility exists that an unknown fraction of these bacteria may have originated from space. The possibility still remains however, that all components of the stratospheric microflora are incoming to Earth. We recognize of course that our views are highly controversial and will need to be verified by further studies of the microbiology of the stratosphere.

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