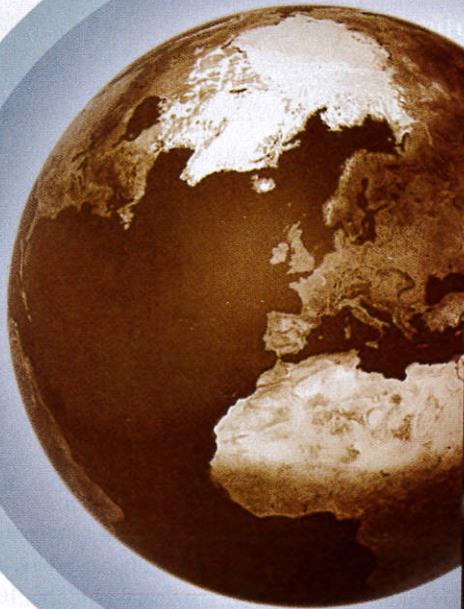


MICROBES

IN THE

STRATOSPHERE



KEY CONCEPTS

- Panspermia is the idea that microbes can travel inter-planetary and inter-stellar distances to "seed" planets, including Earth.
- The idea has been criticized on the grounds that space is too inhospitable for even microlife to survive.
- But an experiment found UV-resistant microbes very high in the stratosphere, suggesting extremophiles may survive.
- There is no obvious mechanism that can lift microbes to 40 km above the Earth's surface, implying that they may not originate on Earth.

—The Editors





Resistant to ultraviolet rays, new species of bacteria were found high in the stratosphere. Is there a sliver of possibility they are extraterrestrial in origin?

By Jayant Vishnu Narlikar

In April 1960, astronomer Frank Drake turned a 26-meter-diameter radio telescope heavenwards to study the stars Tau Ceti and Epsilon Eridani and begin the first systematic search for extraterrestrial life forms. Half a century later, SETI, or “Search for Extraterrestrial Intelligence” has grown into a major international enterprise. For the moment, though, it listens in to what scientist Paul Davies of Arizona calls “the eerie silence.”

But there is another, more modest way of approaching the issue of extraterrestrial life. Instead of setting our sights on advanced forms of life located several light years away, we look for them in microbial form right on our doorstep: that is, in the atmosphere of the Earth.

Panspermia

The history of searches for microbes in space dates back to the 5th century B.C. The concept of panspermia, that is, “seeds of life” travelling across vast interstellar spaces was mooted by Greek philosopher Anaxagoras. A scientific discussion of the idea in more recent times came from no less a person than the distinguished physicist of the nineteenth century, Lord Kelvin. Later, about a century ago, Swedish physicist and chemist Svante Arrhenius advocated that panspermia, in the form of bacterial spores, could travel vast distances in the interstellar spaces. This concept

ALANKAR VISHAL

EVERY DAY, 100 TONNES of cometary material reaches the earth. A theory dating back to the mid-1970s suggests frozen microbes may piggyback on comets and “shower” on the Earth as they approach the Sun and heat up.

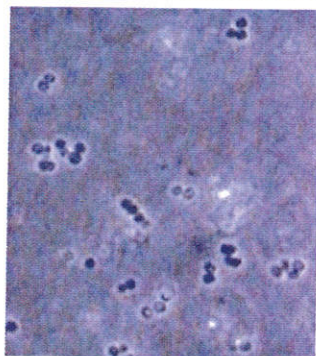
was roundly criticised by physicists and biologists. For example, Antoine Henry Becquerel, the discoverer of radioactivity, objected to the idea on the grounds that micro-organisms would not survive under the ultraviolet background existing beyond the Earth's atmosphere.

Nevertheless, in the mid-1970s two British astronomers, Fred Hoyle and Chandra Wickramasinghe went one step further in proposing a scenario that could bring bacteria to Earth from the outer reaches of the solar system. According to Hoyle and Wickramasinghe, the bacteria in frozen form encased in comets travel to the vicinity of the Sun. As they approach the Sun, the cometary tail develops out of material evaporated by the Sun's heat. Some of the bacteria spread out onto the cometary tail. In the event of a tail brushing the Earth's atmosphere, as happens not infrequently, the bacteria get transferred to it from where they descend to the Earth attracted by its gravity. Hoyle and Wickramasinghe conjectured that life on Earth might well have been seeded by such bacterial showers.

Initially attacked on the same grounds that Becquerel had used against panspermia, the above hypothesis has received some support in recent times, through laboratory experiments demonstrating the survival property of specific bacterial species like *Deinococcus radiodurans* under a dose of radiation. Hence a definitive study needs to be carried out as to whether the Earth's atmosphere harbours living systems, especially extraterrestrial micro-organisms like bacteria and viruses originating in outer space. Although previous studies had been carried out in the sixties and seventies, the biological controls used had not been rigorous enough to guarantee the absence of contamination. So, how can one sample the atmospheric air at great heights under aseptic conditions and confirm the existence of bacteria and viruses over there?

The ISRO Initiative

In 1998, this author initiated a brainstorming session sponsored by the Indian Space Research Organization (ISRO). Experts in balloon experiments, biologists like Pushpa Bhargava and S. Shivaji from the Centre for Cellular and Molecular Biology (CCMB) took part in the session. Chandra Wickramasinghe from Cardiff University, UK also participated and some useful inputs were provided by Hoyle. It was felt that the expertise developed by ISRO in recent years justified an attempt at sampling air from different



[ISOTOPIC ANALYSIS]

ET Test

Is there a hard test to establish the extraterrestrial origin of microorganisms, such as those found by Wainwright (above)? It could be isotopic analysis. If the relative proportions of different carbon isotopes in the bacterium turn out to be different from the terrestrial standard, we have a strong reason to consider its origin as extraterrestrial.

But such an analysis is not easy to carry out since it must be applied to the tiny target of an uncontaminated, pristine bacterium collected at a balloon height of around 40 km. Such a bacterium will be caught on the filter through which the air in the payload cylinder is passed. If we manage to locate it, the filter can then be passed on for nuclear examination. We are hopeful that the technological challenges facing this approach will be met in future.

heights using the balloon technology. Optimally the height range of 20-45 km (a commercial aircraft flies at a height of around 10 km) was considered viable. At lower heights the possibility of biological contamination from the Earth's surface would be significant, while at greater heights the air density is too thin. Also, the theoretical abundance-height curve of particles of any species (See *Cometary Trail* on next page), coming from above shows an exponential drop with height (because of Earth's gravity), thus rendering a search at greater heights futile.

The discussion led to the proposal to send the payload for collection of air samples attached to a balloon, the kind that cosmic ray scientists use for their studies.

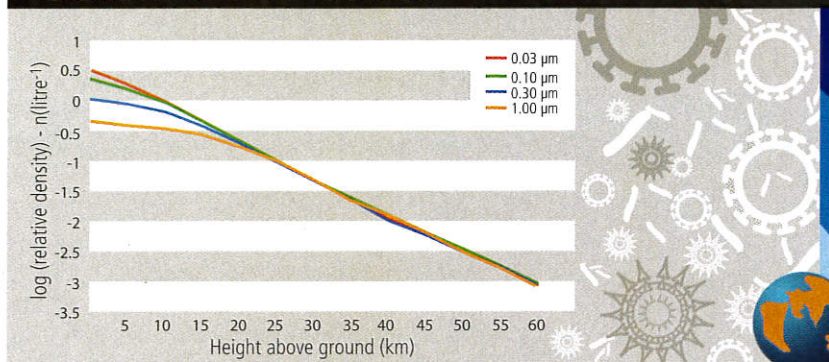
ISRO agreed to sponsor the first balloon flight which was launched from the National Balloon Facility run by the Tata Institute of Fundamental Research in January 2001. Briefly, the payload of that experiment, prepared under the supervision of Dr Rajarathnam consisted of a cryosampler containing 16 evacuated and sterilised stainless steel probes. Throughout the flight the probes remained immersed in liquid neon to create the cryosampler effect. Thus, whenever the valves attached to the cylindrical probes were opened by a remote command from ground headquarters, air could be pumped in.

Samples were collected in four height ranges: 19-20, 24-28, 29-39 and 39-41 km. After the payload was parachuted down, the contents were sent for analysis to CCMB in Hyderabad and to the Centre for Astrobiology in Cardiff. In Cardiff, David Lloyd and Melanie Harris detected live cells and bacteria in the topmost sample. Milton Wainwright at Sheffield University later used one of the Cardiff samples and detected two bacterial species, *B. Simplex* and *Staphylococcus pasteurii* as well as a fungus, *Engyodontium album* in the same sample. Shivaji et al, at CCMB identified four new species of *Bacillus*, namely *B. aeri*, *B. aerophilus*, *B. stratosphericus* and *B. altitudinis* from air samples at the upper three strata. In the CCMB samples, the four isolates were found to be more ultra-violet resistant compared to their nearest phylogenetic neighbors. This may be linked to their survival in the stratosphere where the UV intensity is considerably more than on the surface of the Earth.

The success of the first flight led to the launching of a second one with a few structural changes in the payload to ensure improvements in the collection technique. Senior space scientist and

[ABUNDANCE-HEIGHT CURVE]

COMETARY TRAIL



former head of ISRO, U.R. Rao, and senior biologist and Founder-Director of CCMB, Pushpa Bhargava, acted as advisors to the team. In place of Rajaratnam (now retired) C.B.S. Dutt undertook the responsibility of preparing the payload. Ravi Manchanda, who had taken over from Prahlad Agrawal, streamlined the procedures at the TIFR Balloon Facility.

During the launch on April 20, 2005, air samples were collected at six altitude bands, 20-24 km, 24-27 km, 27-30 km, 30-35 km, 35-40 km and 40 km (and above). Out of the 16 tubes, one was kept unopened throughout. The contents (-or lack of them) of this tube would serve as control for the rest of the tubes. For example, if the control tube showed micro-organisms, that would indicate contamination.

Also, this time the samples collected were shared by two labs in India, namely CCMB and the National Centre for Cell Sciences (NCCS) in Pune. Biologists S. Shivaji at CCMB and Yogesh Shouche at NCCS led the task of examining what was found. Of the remaining 15 tubes, eight were examined at CCMB while the rest were studied by the NCCS group.

What Was Found?

In all, 12 bacterial and six fungal colonies were detected. Based on 16S rRNA gene sequence similarity (See *How to Map a New Species*), the fungal isolates were identified as *Penicillium decumbens* (labelled as PVAS-7 and PVAS-9), *Cladosporium cladosporioides* (B6W22-1 and B6W22-2), *Alternaria* sp. (B8W22-1) and *Tilletiopsis albescens* (B8W22-2). Out of the 12 bacterial colonies, nine showed greater than 98 per cent similarity with previously known species based on 16S rRNA gene sequence. The remaining three strains, PVAS-1, B3W22 and B8W22, were identified as potential new species. Mor-

THE EARTH IS CONTINUOUSLY bombarded by tiny pieces of dust, micrometeorites, etc. The typical piece is subjected to air friction, leading to a finite terminal velocity for downfall of matter. Because of the tendency to "fall" and seek lower and lower levels of the atmosphere, the steady state distribution of such matter shows higher and higher densities at lower and lower heights. As the density drops exponentially with height, to look for microbes beyond a 60 km height may prove fruitless.



[COMPLEX MOLECULES]

FRED HOYLE AND THE BLACK CLOUD

Maverick British astrophysicist Fred Hoyle had argued, back in the mid-1950s, that interstellar space contains large clouds of molecules. In those days the astronomical community believed that only the simplest chemical structure, namely atomic hydrogen, can exist in space. So Hoyle encountered considerable resistance to his idea and his paper proposing it was rejected by the leading journals in astronomy and physics. Undaunted, he took a remarkable way out: He wrote a science fiction novel containing this idea. The novel, *The Black Cloud*, became a great success. Hoyle was, however, vindicated a few years later when the discovery of molecular clouds, as reported in the text, became a reality.

Today, we know that clouds extending several light years in size exist in the interstellar space. Not only that, the organic molecules amongst them include those which make up the DNA molecule. Thus, here are the building blocks of life present in space and one could argue that life is very likely present around some other stars in such clouds, provided suitable planetary habitats exist. Indeed, the case has become stronger in recent years with the discovery of extra-solar planets. At the time of writing this account, more than 400 planets have been discovered around other stars than the Sun.

phological, growth and biochemical studies of the viable colonies were performed using standard methods.

Detailed analysis shows that PVAS-1 represents a novel species of the genus *Janibacter*, which we named *Janibacter hoylei*. sp. nov. after Fred Hoyle. The other new species were B3W22 and B8W22. We named the first one *Bacillus isronensis* sp.nov. in honor of ISRO and the other after Aryabhata, the 5th century Indian astronomer, as *Bacillus aryabhatai*. All the three new species found in this experiment are more UV-resistant than their nearest phylogenetic neighbours.

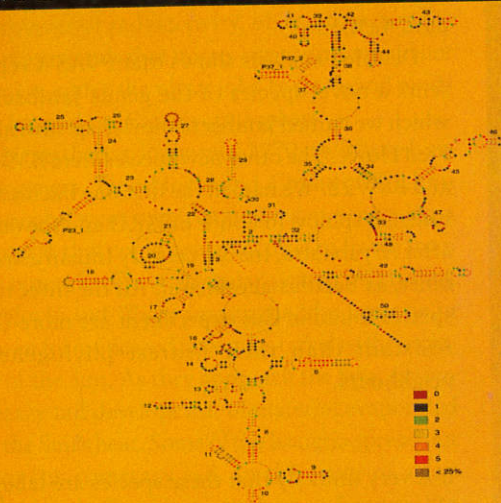
What This Means?

It is very unlikely that these species are laboratory contaminants, as no such cultures were handled in the laboratory. The control cylinder did not yield any micro-organism nor did the instrumentation involved in the filtration. Thus, we can say with some measure of confidence that these species were picked up in the stratosphere. The possibility of routine meteorite exchanges between the Earth and Mars carrying micro-organisms, is not ruled out. The process giving rise to this ejection is generally believed to be spallation, in which a heavy projectile hits one planet ejecting smaller pieces outwards, some of which will eventually fall onto another planet.

The ability of spores to survive interplanetary transfer has also been seriously considered. We, the inhabitants of this planet are able to survive

HOW TO MAP A NEW SPECIES

THE PICTURE depicts secondary structure of 16S rRNA gene of bacterium *Escherichia coli*. This is representative of all bacteria. A single point represents an individual base in the sequence of the gene and the color code indicates degree of conservancy amongst various bacterial groups.



Given a bacterial species, it is of interest to find if it resembles something we already have on Earth, or if it is very different. One way of deciding this is through a detailed examination of the 16S rRNA gene. Why choose this particular gene? Because, this gene is regarded as “Molecular Chronometer”, where the extent of similarity of the sequence of this gene between two organisms tells us about their relatedness. Several characteristics of this gene, such as its essential function, ubiquity and evolutionary properties, have allowed it to become the most commonly-used molecular marker in microbial ecology. With more than 1,20,000 DNA-sequences available in public databases like the ones at the National Center for Biotechnology Information (NCBI), Ribosomal Database Project at Michigan State University, 16S rRNA sequencing is considered by taxonomists to be the gold standard in bacterial identification and classification.

A comparison of these sequences allows the assembly of a Tree of Life, the evolutionary order and relational structure of all species we have. It is this Tree of Life that forms the baseline on which any new isolate can be placed by studying its own 16S rRNA. In this same way, the novelty of a completely new isolate can also be ascertained.

the harmful ultraviolet (UV) radiation from the Sun because it is absorbed by the protective layers of the atmosphere before it can reach us. Having got used to this protection bacteria on the ground level will have very little immunity against such radiation. In contrast, the greater UV resistance found in the three new species suggests that they may have passed some time at least in the upper atmosphere (above 24 km) where the UV flux is much more intense than on the ground level. Thus in the “survival of the fittest”, only those mutants that could withstand the UV flux at high altitudes would remain.

The two balloon flights conducted so far have led to four biological examinations of samples from 41 km heights. These are small samples when one considers the variety of bacteria found on the surface of the earth. Even if one argues that only a fraction of those may survive at such

[THE AUTHOR]



Educated in Banaras Hindu University and the University of Cambridge with an ScD degree, **Jayant Narlikar** has worked in cosmology and theoretical astrophysics. Amongst the minority critical of the Big Bang, Narlikar, with his mentor Fred Hoyle and senior colleague Geoffrey Burbidge has worked on an alternative cosmology, the Quasi-Steady State Cosmology.

heights, the probability of these four studies picking up the same species is small. This may explain why there has not been any repetition in the bacterial species found so far.

Can the bacteria be lifted up from the surface of the Earth to 41 km? There are mechanisms for lifting dust particles, e.g., in storms; but reaching this height through such processes is hard if not impossible. Even the volcanic ash does not normally rise beyond 25 km. There had been no major volcanic eruption prior to the two balloon flights. So we consider the “riding bacteria” hypothesis somewhat unlikely although not ruled out. Clearly, there is need for more wide-ranging sampling of air at these heights.

Back to Panspermia

The above experiments do not conclusively establish the panspermia hypothesis, although their findings do make it more credible than before. There are other possible observations that could test this hypothesis. Now that it is possible to launch spaceships that rendezvous comets, as was done in 2006 for Comet Wild 2 in NASA’s Stardust mission, future such missions could specifically look at the cometary material for micro-organisms. The Stardust spacecraft was able to collect material from the comet and parachute it down to Earth. The collected material included glycine, an amino acid which is a basic building block of life. More such missions are indeed planned. The mission called Stardust NExT, for example, is going to fly by comet Tempel 1 in February 2011.

Another possibility advocated by Paul Davies is that of exchanges of material between planets of our solar system, with some of it carrying live micro-organisms. In 1996, the examination of a meteorite, ALH 84001, found in the Allen Hills Region of the Antarctica, led to the controversial finding of what looked like fossilized micro-life within it. The chemical composition of this meteorite suggests that it is of Martian origin. In a preprint posted online as recently as February 22, a group of Italian scientists found that terrestrial microbes could survive a simulated Martian environment.

It is too early to pass a final judgement on such findings: But one thing that these findings suggest is not to rely on preconceived notions of what life outside the Earth may be like. Fred Hoyle had the last laugh when giant molecular clouds were discovered. Now, along with his former student Wickramasinghe, his suggestions that comets carry panspermia may still be proved correct. ■