SEARCHES FOR MICROLIFE IN THE EARTH'S ATMOSPHERE

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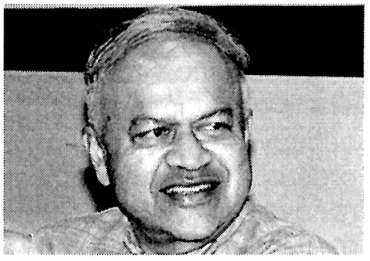
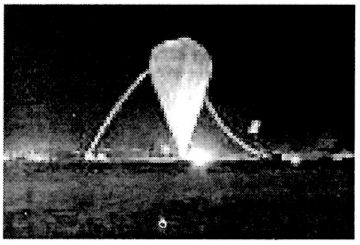
Life in the universe



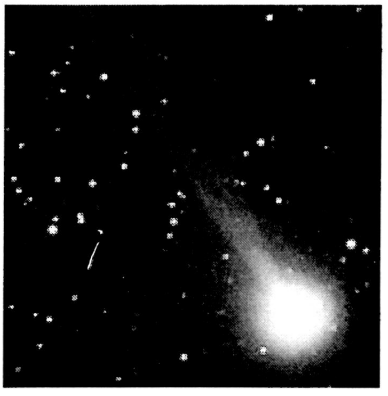
Is there life outside the Earth? This question has stimulated lot of thought, speculations, even active searches. For a long time this topic was considered limited to science fiction and not belonging to hard science. The situation changed in the 1970s when millimetre wavelength antennas began to reveal the existence of vast clouds of molecules, both organic and inorganic. The former set could very well be considered building blocks of life, as we know it on the Earth. Searches for this have begun and they range from sending messages to extrasolar planets in the expectation that they harbour advanced civilizations, to looking for micro-organisms right at our doorstep. Here we will concentrate on the latter approach.

A balloon experiment



On April 20, 2005, a 26.7 million cubic feet balloon carrying a 459 kg scientific payload with 38 kg of liquid neon was flown from the National Balloon Facility in Hyderabad operated by the Tata Institute of Fundamental Research (TIFR). The payload collected air samples from different heights ranging from 20 km to 41 km. After this operation, the payload was parachuted down and was safely retrieved. The collected samples were divided into two lots and independently analysed by the Centre for Cellular and Molecular Biology (CCMB), Hyderabad and the National Centre for Cell Science (NCCS), Pune. Both labs reported finding live micro-organisms. Such findings have enormous implications for the budding field of astrobiology besides providing important inputs into the question of how life started on our planet.

History of bugs in space



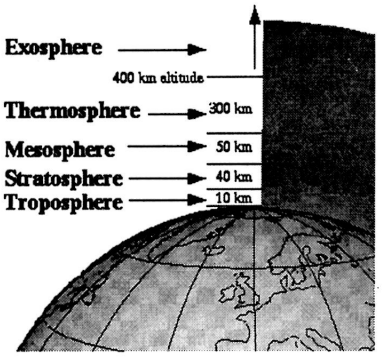
The history of speculations and searches for microbes in space dates back to the 5th century B. C. The concept of panspermia, or 'seeds of life' travelling across vast interstellar spaces was developed by Greek philosopher Anaxagorus. A scientific discussion of the idea in more recent times came from the distinguished physicist of the nineteenth century, Lord Kelvin.. Svante Arrhenius about a century ago advocated that panspermia, in the form of bacterial spores, could travel vast distances in the interstellar spaces. This concept was criticised by physicists and biologists. For example, Becquerel objected to the idea on the grounds that micro-organisms would not survive under the ultraviolet background existing beyond the Earth's atmosphere.

In the mid 1970s two British astronomers, Fred Hoyle and Chandra Wickramasinghe (H&W) went one step further in proposing a scenario that could bring bacteria to Earth from the outer reaches of the Solar System. According to H&W, 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. H&W conjectured that life on Earth might have been seeded by such bacterial showers.

Although originally attacked on similar grounds which Becquerel used against panspermia, the above hypothesis has received some support in recent times, through laboratory experiments demonstrating the survival of specific bacterial species like *Deinococcus radiodurans* under a dose of radiation. It is not clear, whether one can draw parallels between conditions in the International Space Station and the laboratory. Nevertheless some of us felt that an objective study needs to be carried out as to whether the Earth's atmosphere harbours living systems, especially extra-terrestrial 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.

The ISRO initiative

In 1998, once I initiated a brainstorming session sponsored by the Indian Space Research Organization (ISRO). Chandra Wickramasinghe from Cardiff University, UK also participated. We felt that the expertise developed by ISRO in recent years justified an attempt at sampling air from different heights using the balloon technology. Optimally the height range of 20-45 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, coming from above shows an exponential drop with height (because of Earth's gravity), thus rendering a search at greater heights futile.

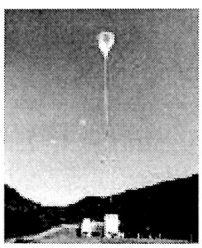


ISRO agreed to sponsor the first balloon flight which was launched in January, 2001. Briefly, the payload of that experiment consisted of a cryosampler containing sixteen evacuated and sterilised stainless steel probes. Throughout the flight the probes remained immersed in liquid neon to create a cryosampler 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 in CCMB and in Cardiff. The Cardiff group detected live cells and bacteria in the topmost sample. Milton wainwright at Sheffield University later detected two bacterial species, *B. Simplex* and *Staphylococcus pasteurii* as well as a fungus, *Engyotontium album* in the same sample. Shivaji et al.., at CCMB identified four new species of *Bacillus*, namely *B. aerius, B. aerophilus, B. strospericus* and *B. altitudinus* 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 neighbours. This may be linked to their survival in the stratosphere where the UV intensity is considerably more than on the surface of the Earth.

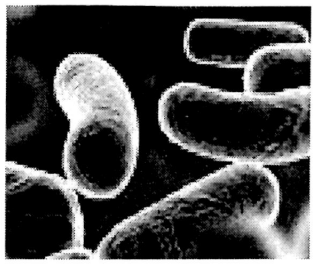
The recent experiment

During the launch on April 20, 2005, in a second experiment with several improvements over the first one, air samples were collected at six altitude ranges, 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 microorganisms, that would indicate contamination.



Of the remaining 15 tubes, eight were given to CCMB for examination while the rest were studied by the NCCS group. Care was taken that both laboratories followed similar protocols and there was frequent interactive discussion between the two groups to ensure homogeneity of procedure and interpretation.

What we found



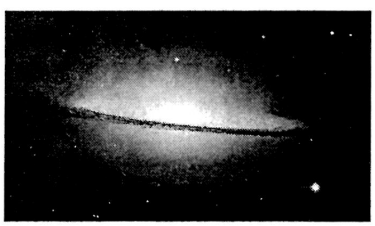
In all, 12 bacterial and six fungal colonies were detected. Based on 16S rRNA gene similarity, the fungal isolates were identified as *Penicillium decumbens* (*PVAS-7* and *PVAS-9*), *Cladosporium cladosporioides* (*B6W22-1* and *B6W22-2*), *Altermaria sp.* (*B8W22-1*) and *Tilletiopsis albescens* (*B8W22-2*). Out of the 12 bacterial colonies, nine based on 16S rRNA gene sequence showed greater than 98% similarity with reported known species. These include *Methylobacterium sp.* (*PVAS-2* and *PVAS-3*), *Acinetobacter radioresistens* (*PVAS-4*), *Stenotrophomonas sp.* (*PVAS-5*), *Acinetobacter calcoaceticus* (*PVAS-6*), *Stenotrophomonas rhizophila* (*PVAS-8*), *Bacillus pumilus* (*PVAS-10*), *Micrococcus flavus* (*B5W22-1*) and *Streptomyces maritimus* (*B5W22-2*). The remaining three strains *PVAS-1*, *B3W22* and *B8W22* based on 16S rRNA gene sequence similarity were identified as potential new species and were studied in greater detail. Morphological, growth and biochemical studies of the viable colonies were performed using standard methods.

PVAS-1 was identified as a member of the genus *Janibacter* and like members of the genus *Janibacter* PVAS-1 is a Gram-positive, coccoid, non-endospore forming, non-motile bacterium, which occurs singly or in clumps. It 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. These are Gram-positive, rod-shaped, endospore-forming bacteria. We named the first one *Bacillus isronensis* sp.nov. in honour of ISRO and the other after Aryabhata, the Indian astronomer of fifth century, as *Bacillus aryabhatai*. It is also significant that 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 microorganism 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 ability of spores to survive interplanetary transfer has also been seriously considered. 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. Thus in the 'survival of the fittest', only mutants which 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 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. Clearly there is need for more wide-ranging sampling of air at these heights. Likewise, while this study does not conclusively establish the extragalactic origin of microorganisms, it certainly requires us to take that alternative more seriously than has been done hitherto. Isotopic analysis of a micro-organism collected in such a sample should help tell us whether it is extraterrestrial or not. For example, 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 extragalactic.



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