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Science feature

Stratosphere microbes might hold clues to life on earth

Jayant Vishnu Narlikar

The balloon that carried the payload.

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 microorganisms[1]. 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' traveling across vast interstellar spaces was developed by Greek philosopher Anaxagorus. A scientific discussion of the idea in more recent times came from Lord Kelvin[2]. Svante Arrhenius[3] 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[4] objected to the idea on the grounds that microorganisms 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[5] 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[6] 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 microorganisms like bacteria and viruses originating in outer space.

Although previous studies[7,8] 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, one 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 was significant, while at greater heights the air density was too thin. Also, the theoretical abundance – height curve of dust particles coming from above – shows an exponential drop with height (because of Earth's gravity)[9], thus rendering a search at greater heights futile.

ISRO agreed to sponsor the first balloon flight[10,11]. 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 admitted.

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[12]. Sheffield University later detected two bacterial species, B. Simplex and Staphylococcus pasteurii as well as a fungus, Engyotontium album in the same sample. 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[11]. 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 latest experiment

 C. B. S. Dutt, U. R. Rao and J. V. Narlikar (L to R) studying the payload.

Considering the first balloon experimental results and some ambiguities over the issue on cryosampler contamination, ISRO convened a national level consultative meeting in microbiology led by its chairperson G. Madhavan Nair in June 2004. It resulted in modifying the cryosampler by incorporating pyro valve mechanism in the inlet valve, pre-testing of all the cryo cylinders to ensure high pressure, total environmental testing and strict adoption of mechanical and biological protocols while integrating the cryosampler payload. This ensured rigorous improvement in the payload fabrication as well as in the protocol for biological analysis.

During the launch on April 20, 2005, 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

A sterile environment was ensured while transferring the contents of the probes through two millipore filtration units, the first allowing anything smaller than 0.45 μm to go through, while the second limited the size to 0.22 μm. The filters were used for detection of bacteria by incubating one quarter of the filters on various agar solutions[1].

Of the 15 cryoprobes, the filter paper from Cryoprobe 11 corresponding to air sample from 40-41.4 km when placed on minimal salts agar showed a single cream coloured colony after 24 days of incubation at 300C. The NCCS scientists designated it as PVAS-1.

In the first balloon flight[10], it had been found that microorganisms not only remain in the air inside the probes, but preferentially tend to attach themselves to their walls. So the cryoprobes were injected with 200 ml of sterile phosphate buffer and agitated at 220C for 6 hours in a shaker. The liquid was then recovered through a filter. These filters, 47 mm in diameter, were cut into four quarters and cultured on four different media. The results were positive.

In all, 12 bacterial and six fungal colonies were detected. Based on 18S 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[14,15].


R. K. Manchanda, U. R. Rao and P. M. Bhargava (L to R) during a pre-experiment meet.

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.

Two 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 Aryabhata after the Indian astronomer of fifth century (and also the first satellite launched by ISRO), 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 microorganisms is not ruled out[17]. The ability of spores to survive interplanetary transfer[18],[19] has 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 can 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 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 microorganism collected in such a sample should help tell us whether it is extraterrestrial or not.

The author is an eminent astrophysicist at the Inter-University Centre for Astronomy and Astrophysics, Pune, India. He was the Principal Investigator in this balloon experiment. Significant contributors to the experiment were microbiologists S. Shivaji from the Centre for Cellular and Molecular Biology, Hyderabad and Yogesh Shouche from the National Centre for Cell Science, Pune, who identified the new isolates from the stratosphere. C. B. S. Dutt from ISRO, Bangalore, was the Coordinator for fabrication of the hardware. R. K. Manchanda from the Tata Institute of Fundamental Research, Mumbai, was in charge of the balloon flight. P. M. Bhargava of Anveshna Consultancy Services, Hyderabad was the biology expert and U. R. Rao from ISRO, Bangalore was the Chairman of the Project.

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