Abstract

Direct evidence for living cells in the air samples collected aseptically over Hyderabad (India) at various altitudes is presented. In the preliminary experiment, samples collected from altitudes of 10 to 36 km yielded six identical bacterial colonies with phenotypic characteristics distinct from hitherto described *Pseudomonas stutzeri*, a terrestrial bacterium. Since in this earlier experiment the air-samples were collected over a long range of altitude (including the ones at low altitudes), it was not possible to completely rule out terrestrial contamination. On the other hand in the current experiment, the samples were collected at altitudes ranging from 20 to 41 km, well above the tropopause over Hyderabad. Voltage-sensitive dyes that could detect the presence of viable cells were used on these air-samples. Clumps of viable cells were present in samples collected at all the altitudes. The images obtained from electron microscopy are consistent with the above picture. Arguments are advanced against the detected cells (in the samples collected in the present experiment above the local tropopause at 16 km) being due to terrestrial contamination.

1 Introduction

Though the life was speculated to be of universal origin since very early times, scientific investigations indicating the life’s antecedents were carried out by Louis Pasteur, Alexander Fleming, Robert Hooke, and others. Over the years, the search for extraterrestrial life has been pursued by many scientists. The possibility of life in the stratospheric samples was first suggested by P. H. Allen et al. in 1984, followed by further studies by J. V. Narlikar et al. in 1989. The current study presents direct evidence of living cells in the air samples collected over Hyderabad, India, at various altitudes.
Pastur in the 1850s. Based on the experiments on the souring of milk and the fermentation of wine, Pasteur (1857a,b) concluded that microbial life had necessarily to be derived from pre-existing life-forms of a similar kind, this dealt a mortal blow to the idea of spontaneous generation (Pasteur, 1860).

However the goal of producing life from non-life starting from a constituent mixture of inorganic gases was advocated in the early part of the last century by Oparin(1935) and Haldane(1899). The Urey-Miller experiments of the mid-1950s showed how amino acids and nucleotides might form from a mixture of inorganic gases (Miller and Urey,1959). Since then several experiments have demonstrated the production of hexaglycine under conditions similar to those prevailing in terrestrial hot springs (Imai et al, 1999) and the production of biologically relevant molecules like alcohols, quinones and ether from UV irradiation of polyaromatic hydrocarbons in water-ice (Bernstein et al, 1999). But what is important to recognise is the fact that while the production of chemical building blocks is a necessary condition, it is not sufficient. What is relevant are the highly specific processes for the production of nucleic acids, enzymes and other proteins, membranes and organelles. Hoyle and Wickramasinghe (1980) have given theoretical arguments that life cannot originate in a small terrestrial pre-biotic pool; a cosmic origin is instead suggested.

If life is truly cosmic, then several questions arise: i) Is there evidence in favour of pandaspermia? ii) Can microorganisms survive in the harsh extraterrestrial environments? iii) What mechanisms are available for transport of such microorganisms to the earth? We consider these briefly.

Microorganisms expelled from any source into unshielded regions of interstellar space will firstly become deactivated and subsequently degraded by exposure to cosmic rays, UV and other intense electromagnetic radiation. This will lead to the production of free organic molecules and polymers. An impressive array of such molecules have been detected. The production of such a variety of interstellar organic molecules from non-biological sources is highly unlikely, if not totally impossible. In this connection the fitting of a highly detailed absorption profile extending over the 2.9 - 3.8 micron wavelength region, obtained for the IR Source near our Galactic Centre GC-IRS7, to the laboratory spectrum of the desiccated bacterium E.Coli (Wickramasinghe et al 2001) clearly stands out.

Over the last decade it has been demonstrated by several laboratory experiments that the microorganisms can survive extreme conditions of temperature, pressure and even radiation (Hoyle and Wickramasinghe 2000). Further a carbonaceous coating of even a few microns thick provides essentially total shielding against UV radiation (Secker et al 1994). So far as their transport to the Earth is concerned Hoyle and Wickramasinghe (2000) have been advocating the cometary transport of these microorganisms for more than two decades, adding several evidences in favour of this hypothesis. We will not go into details here but simply state that this hypothesis was one of the motivating factors for the present experiment.

2  ISRO/IUCAA/CCMB/TIFR/CARDIFF
Cryosampler Programme

Though some attempts to detect direct evidence for extraterrestrial life-forms entering our upper atmosphere were made in the 1960s and 1970s by mainly NASA supported Balloon Programmes (see Bruch,1967 for a summary) and a Soviet Rocket Experiment (Lysenkto,1979), no definite conclusions could be drawn due to the primitive nature of the sterilization procedures that were used. Actually some indications of extraterrestrially-derived microorganisms were claimed, but in view of the lack of sound techniques to conduct the experiments aseptically, it was quite impossible to rule out terrestrial contamination. In view of these difficulties, very stringent procedures to completely remove terrestrial contamination had to be evolved. Such techniques became available in the late 1990s (Shyamsundar et al 1996). Biochemical, chemical and molecular biological studies to identify the collected microorganisms were also being developed (Smibert and Kreig,1994). Furthermore extremely sensitive new dye-based detection methods for living organisms were developed in Cardiff (Lloyd and Hayes, 1995; Lopez-Amoros et al, 1995). So an experiment was proposed to collect direct evidence for extraterrestrial life in stratospheric balloon flights using the aseptic cryosampler and highly sensitive voltage-sensitive dyes (Narlikar et al, 1998).

2.1 Collection of Air samples using the Cryosampler

The cryogenic sampler instrumentation comprised a 16-probe assembly. Each probe had a volume of 0.35 L and was made of high vacuum grade stainless steel. It was capable of holding a vacuum of 10^-6 mb and pressure of 600 b. The temperature cycling ability of the probes were tested between -240°C and 140°C. To minimize contamination, the probes were machined from the above stainless steel stock, only the minimum required electron-beam welds were made, and the interior was electropolished. Just before the experiment, the probes and their manifold were cleaned with acetone and then four times with demineralised water. The assembly was then steam-baked and finally heated with infrared "lamps to temperatures of 140°C. To prevent collection of any outgassed substances from the gondola, an intake tube of 2 m formed a part of the payload ensemble and was sterilised as above. The probe mouth consisted of a metallic (Nupro) valve which was motor driven to open/close at a given altitude through ground command, using a telecommand-transmitter- receiver-decoder cryocontrol unit chain. During the flight, the probes remained immersed in liquid neon to create the cryopumping effect that allowed the ambient air samples to be collected on ground command.

2.2 First Experiment

In order to test the feasibility of collection of air samples aseptically and to test the rDNA sequencing and other procedures for identification of the microorganisms, a preliminary analysis was conducted by S. Shivaji and G.S.N. Reddy at CCMB and by P.M. Bhargava at Anveshana, Hyderabad, of one of the probes having air sample collected from the altitude range of 10-36 km. This sample was collected in
a balloon flight launched from Hyderabad on April 29, 1999. All procedures were carried out in a sterile equipment. The sample air was passed first through a 0.45 micron and then through a 0.22 micron pore filters. The exiting air was passed through a calibrated flowmeter. Each filter was placed in a nutrient agar plate; no growth occurred at 25°C in seven days. The filter was then transferred to a blood agar plate and incubated at 25°C for 11 days, when six distinct colonies had grown from the 0.45 micron filter; none were obtained from the 0.22 micron filter. These colonies were sub-cultured and maintained on nutrient agar. The cultures have been deposited in the MTCC Type Culture Collection of the Institute of Microbial Technology, Chandigarh, India.

Based on the morphological, physiological and biochemical characteristics and additionally on the basis of the 16S rDNA sequencing the isolates were identified as *Pseudomonas stutzeri*. But, these isolates were distinct from all the earlier described strains of *P. Stutzeri* with respect to i) ability to grow well on lysine, ii) high percentage of C15:0 and C18:0 fatty acids, iii) presence of carotenoid pigments, and iv) the yellow pigment which was produced only at room temperature (25°C), and then only in the stationary phase. In addition it is noteworthy that *P. Stutzeri* has so far not been described as an airborne organism, its natural habitat being soil and water. So it is tempting to speculate that this is evidence for an extraterrestrial bacterium. But the fact that the air samples were collected as low as 10 Km altitude, where even debris from jet planes can provide terrestrial contamination, puts a question mark over the extraterrestrial nature of the detected organism. At the same time, the unique properties of the detected colonies on comparison with terrestrially known strains of *P. Stutzeri* is remarkable. While the experiment demonstrated the capability of the techniques for collection of air samples and detection of microorganisms, it also showed the need for a more careful experiment, where samples are collected at heights well above the ones where normal terrestrial contamination is even remotely possible.

### 2.3 Results from Air samples collected by the Cryosampler balloon flight on 21 Jan. 2001

The balloon carrying the cryosampler payload was launched on 21 January 2001 from Hyderabad. Air samples were collected at different heights above the local tropopause at 16 Km. The lowest height was 19.8 Km and the highest at 41.06 Km. Probes in this flight were in duplicate sets at each height. One set is being analysed in Cardiff and the other one at CCMB, Hyderabad. In this meeting the preliminary results from the probes being analysed in Cardiff are given, whose details are given below.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Collection Height Range</th>
<th>Collected NTP Volume (litres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19.80-20.32 Km</td>
<td>81</td>
</tr>
<tr>
<td>B</td>
<td>24.36-27.97 Km</td>
<td>70.5</td>
</tr>
<tr>
<td>C</td>
<td>28.47-39.05 Km</td>
<td>38.4</td>
</tr>
<tr>
<td>D</td>
<td>39.75-41.06 Km</td>
<td>18.5</td>
</tr>
</tbody>
</table>
cells the number density of viable cells at 40 Km, corresponding to this would be about 7 per litre.

2.4 Terrestrial Contamination

Considering the extremely small number of about 7 living cells per litre of air at 41 Km, as determined above, the main concern will be one of terrestrial contamination. As noted earlier, the possibility of contamination due to the instrumental collection process is completely ruled out by the stringent sterilization methods adopted. Hence the only possibility is the presence of terrestrial cells being carried aloft to such great heights by some extraordinarily rare events or spacecraft debris.

Under normal circumstances atmospheric mixing takes place only up to the tropopause, that acts as a barrier against the transport of waves responsible for mixing the terrestrial material with the upper atmosphere. However during some extraordinary events such as a very powerful volcanic eruption, it is possible for the material to reach large vertical heights. This was demonstrated by the volcanic eruption of Mt Pinatubo in Philippines on June 15, 1991, which was estimated to have injected nearly 20 million tonnes of SO$_2$ up to a maximum vertical height of 32 Km (Grant et al., 1994 and references therein). The stratospheric mixing has been almost continuously monitored using several balloon flights (Deshler et al., 1992). It was shown that the gravitational settling to lower altitudes is rapid and after a few months, no significant mixing even in the lower stratosphere was taking place. Considering this direct evidence of no contamination of even as powerful a volcanic eruption as that of Mt Pinatubo, we can immediately rule out terrestrial contamination as an explanation of our results, as no volcanic eruption or any other extraordinary event took place even months prior to our experiment.

On the positive side, the observation of the depth profile of the organisms matches that predicted for extraterrestrial cells injected to the top of our atmosphere (Narlikar et al., 1998). Using the method of Kasten (1968) the infall of micron sized clumps in a standard atmosphere, the steady state number density of micron sized clumps at 40 Km is expected to be one tenth of that at 25 Km, which is consistent with what is observed, within the accuracy of the measurements completed so far. Furthermore, the same calculation yields the clearance of any transient injection (e.g. of space debris) through gravitational settling in a time of order of months. Hence it becomes even more feasible that the cells detected in our experiment are of extraterrestrial origin.

3 Concluding Remarks

Viable living cells have been detected using the cationic cyanine dyes at all heights ranging from 21 Km to 41 Km. Terrestrial contamination is ruled out because of the sample collection at altitudes well above the tropopause during a time when there were no extraordinary terrestrial events like volcanic eruption etc. Furthermore the use of stringent procedures for sterilization completely rules out the contamination due to the instruments or balloon. With an average falling speed for 3 micron sized clumps at 40 Km of about 0.3 Cm/s (Kasten, 1968), the infall rate of clumps with a number density of 0.068/litre over the entire Earth would be

\[(0.068 \times 10^{-3}) \times (0.3) \times (5 \times 10^{16})\] per second

Assuming an average of 100 individual bacterial cells each of mass $3 \times 10^{-14}$g in a clump, a daily mass input of about a third of a tonne of biomass is deduced. Although these estimates are very tentative, they serve the purpose of illustrating the amount of infall matter involved, if the results of this investigation are accepted, and a prima facie case for a space incidence of bacteria into the Earth is seen to be established.

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