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An Overview of Astrobiology and Microbial Survival in Space

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Abstract:- With this review article, we aim to give a general overview on the environmental conditions in the different layers of space extending to above the Kármán line and also assessing the various microorganisms sent to space for experimentation and how they are able to survive there even under extreme environmental conditions. The vast outer space is a boundless field of opportunities and new discoveries and has always been appealing to mankind. The harsh environmental conditions of space are a challenge for any living form ranging from microorganisms to humans. However, using microorganisms and testing their survival paves way to prove theories of panspermia and extraterrestrial life. Astrobiology is a rapidly growing field and this review article hopes to ignite interest on the same by giving a simplified yet coherent information about the history, the present and ultimately, the future and scope of astrobiological studies.

Keywords:- Astrobiology, Microbiology, Kármán line, Space, International Space Station, Survival, Extraterrestrial life, Microgravity, UV-irradiation.

I. INTRODUCTION

Astrobiology is the study of microorganisms in space through *in situ* exploration. The main goal of astrobiology is to find signs of life on planets other than earth. Most of the microorganisms present in the outer space are found to be bacteria which are also capable of colonizing any environment, as they would have already been used to it. We all know that outer space is a very complex environment, as microbes undergo changes like cosmic radiation, weightlessness and pressure.

Outer space also provides a challenging environment with regard to temperature in absence of appropriate survival condition, which are thermal extremes that usually exceeds earth's hottest and coldest locations are expected.[1] The solution on how bacteria survives through extreme conditions is determined by the anatomy of the particular bacteria, which makes it possible for them to stay in outer space vacuum without being deteriorated. Experiments in space is complemented by terrestrial laboratory experiments formulated to simulate the required parameters of outer space, such as, space vacuum, thermal extremes, and hypobaric chambers etc. Most of the microbes in space are believed to be there because of humans, spaceships and cargo. Some microbes like *Staphylococcus aureus* come from nasal passages of human. The scientists have also noted that the similar microbes are also found in offices and gyms etc. so the space station is also considered as another built environment by humans.

II. ENVIRONMENTAL CONDITIONS IN SPACE FOR MICROBIAL GROWTH

Environmental conditions play a crucial role in the growth of microbes. Factors like microgravity, solar UV radiations, vacuum of space and the galactic cosmic radiations present new boundaries to be explored especially in the growth, survivability and culturing of microbes in space. The microbial presence beyond a certain altitude and atmospheric temperature was still not fully analyzed and studied. In order to examine the vertical distribution of the microorganisms in the air, a helium balloon payload system was developed to sample bioaerosols at stratospheric altitudes of up to 38 km, where the temperature, air pressure, relative humidity, and ultraviolet- C (UV-C) radiation conditions were made to be similar to the surface of Mars.[2] Different layers in the earth's atmosphere present different living conditions for microbiome. Earth's atmosphere consists of four layers which are called Troposphere, Stratosphere, Mesosphere, Thermosphere and Exosphere.

Troposphere being the first layer of earth's atmosphere expands from 0-10 miles of altitude in the equator and it contains 75% of the atmosphere's mass and 99% of the total quantity of water vapour and biological aerosols. This part of atmosphere presents the best temperature variant for most microbes to thrive. Viable microorganisms have been isolated from the upper layer of troposphere. The tropopause is the boundary between the troposphere and the stratosphere, where the heated air masses containing the microbes, stops cooling with height, remains at approximately -56 degrees Celsius and is completely dry.[3]

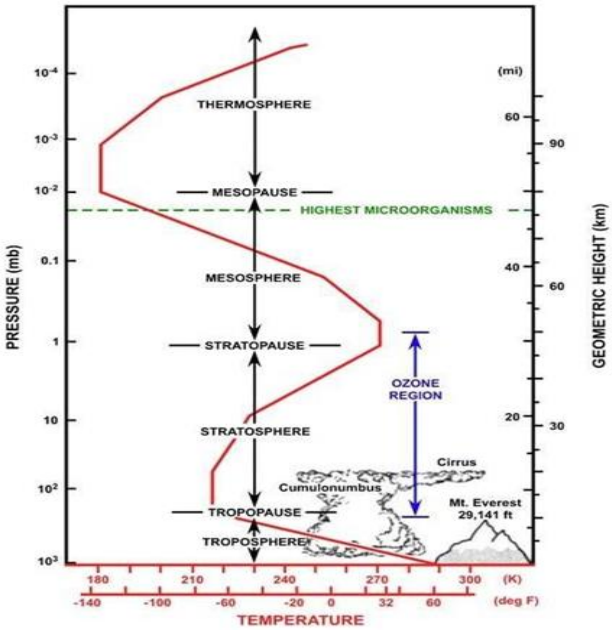

Fig 01: Layers of the Earth's Atmosphere7

Since the advent of space travel, the hypothesized presence of microbes in the stratosphere has been confirmed and steps to study it has been taken continuously ever since. Pressure in this region can reach values below a hundredth of that at sea level. Temperatures can drop up to as low as -70 degrees Celsius. Microorganisms are not restricted to the troposphere, aerosol particles can be carried further above into the stratosphere even by natural meteorological phenomena like thunderstorms.[4] Spores of *Bacillus subtilis* were exposed to the extreme habitable conditions of the stratosphere in lab simulations, After six hours of exposure to stratospheric environment 99.9% of *B. subtilis* spores were killed due to UV radiations.[5] But when the spores were exposed to other stratospheric conditions like temperature, desiccation, and pressure, their viability lasted up to 96 hours.[5]

Mesosphere is the third layer of atmosphere located at the altitude of around 31-40 miles above earth's surface. This layer is humorously referred to as "Ignorosphere", as it is much less explored for the presence of microorganisms, compared to the various studies conducted in the stratosphere. For the first time microbes have been detected in the mesosphere at an altitude of around 48-77Km.[6] The microbes found are fungi having black conidia or spores (*Circinella muscae, Aspergillus niger, Papulaspora anomala)*and one species forming green conidia (*Penicillium notatum)*, colonies of *Mycobacterium luteum and Micrococcus albus* have also grown.[5] Five of these six species are shown to have synthesized pigments.[6] The discovery of these pigmented microbial forms suggests that natural selection is occurring in the mesosphere, since cells having chromogenous pigments(carotenoids, melanin) are more resistant to UV radiation.[6] Microorganisms have been registered in a large number in the mesosphere during sand storms than in the absence of heavy winds.[6] It is observed that certain microbes has adapted to live in the extreme radiations, pressure and temperature of the mesosphere.

The layer of atmosphere which has the highest temperature is the Thermosphere. The temperature in this layer increases with altitude. The thermosphere begins at about 50 miles and reaches up to roughly 370 miles. Temperature here depend upon solar activity and can rise to 1700 degrees or more. Microbial presence in this layer is not detected. Because of this fact only the mesosphere, stratosphere and troposphere are considered to be the part of biosphere.[7] The Kármán line which is commonly referred to as the boundary between atmosphere and space, lies in the thermosphere at an altitude of 100km above sea level.

No viable microorganisms naturally exist in the last two layers of atmosphere (thermosphere and mesosphere) mainly because the meteorological disability for the microbes to reach such altitudes, but the extreme conditions of outer space can be simulated in a laboratory. One of the spaces like conditions widely simulated on ground-based laboratories to monitor the growth and viability of microbes is microgravity. Many of the experiments were performed under simulated microgravity conditions by using ground based microgravity simulators owing to the scarcity and expensiveness of spaceflight experiments. The term "microgravity" which refers to "weightlessness" or "zero-g" that only exists only in space, is labelled "µg", referring to the fact that gravity in this scenario isn't entirely equal to zero and ranges from approximately 10^{-3} to 10^{-6}g and is dependent on location and frequency of vibrations of the simulator.[8] Bacteria grown under microgravity conditions express pronounced enhancements in their resistance to extreme environmental conditions (acid, thermal and osmotic) and metabolism. When mice were inoculated with *Salmonella enterica serovar Typhimurium* grown in microgravity conditions, only 20% of the mice survived after 10 days while 60% of the mice remained, which were infected with the bacteria grown in normal conditions.[9] Microgravity presents an environment in which the shear and buoyant forces does not interfere with the molecular activities of the cell physiology[10], which results in enhancements such as in the case of *Salmonella enterica serovar Typhimurium.*

The other factor which poses a challenge to microbial growth is the vacuum of space. Experiments of this kind are performed either directly in space or in vacuum chambers in laboratories. This factor is comparatively easier to simulate in ground- based laboratories. Although the experiments conducted with *Deinococcus radiodurans* in both simulated vacuum and space vacuum were at the same pressure of 10^{-6} PA, the bacterial cells exposed to space vacuum displayed a higher loss of viability, which can be hypothesized to be caused by a composite of stress factors including zero gravity and ionizing radiation. The results of this experiment emphasize the necessity for space experiments, in addition to the experiments performed on the ground under simulated vacuum conditions.[11]

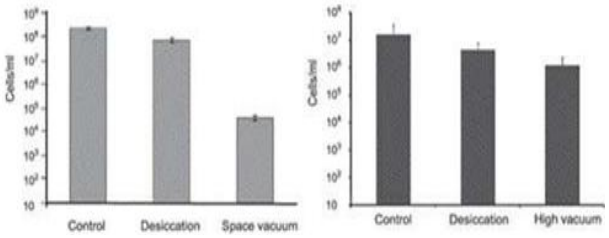

Fig:02: The differences in the results of the similar experiments conducted on space vacuum and simulated vacuum.11

The lowest pressure barrier for the growth of earth bacteria is found to be 25hPa.[12] At the near inhibitory low pressure of 50hPa, the bacteria *Bacillus subtilis* showed and enhanced growth kinetics.[12] A pressure downshift inhibited by up-regulation of some genes, and the regulation was different in response to the temperature vs the pressure downshift.[13]

Radiations in space also play a crucial role in reducing the viability of microorganisms. These radiations include solar UV radiations, which is present at very lethal levels that could kill most of the microbes when they are fully exposed, and galactic cosmic rays which are lethal to a greater extent than that of solar UV rays. Some strains of microorganisms are more resistant to these radiations than the other.[14] For example spores of various species of *Bacillus* are 10-100 times more resistant to UV radiations, the reason for which is still unknown. The major lethal target in the spores for UV radiation is almost certainly the DNA and this appears to be true for the whole UV spectrum.[15] UV radiations create reactive oxygen species in addition to lesions directly in DNA and appear likely to terminate the spores by generating single and/or double strand breaks in DNA.[16] The most effective UV wavelength for killing the spores of most strains of microorganisms is UVC, which is more than 300 times effective compared to UVB or UVA.[14]

One of the physical factors that is necessary for the microbial growth kinetics is temperature. In space the average temperature is just about the absolute zero, standing at 2.7 kelvin (-235 degrees Celsius). Like the other factors, the effect of temperature varies along different strains of microorganisms. Psychrophiles are microorganisms that can grow at low temperature environments on earth. For example, *Psychrobacter cryopagella* is a bacterium that can grow at -10 degrees Celsius and still stay alive, even keep metabolizing at -20 degrees Celsius.[17] As we discussed the temperature in space can drop to even much lower values, and in such low temperatures the enzyme activity in the cells comes to a halt. For example, after 3 months of storage of *Haloarcula hispanica* at -195 degrees Celsius, it showed a lower resistance to this temperature, indicated by the low capability of the microbe to grow again after restoration to normal temperatures, while with the other bacteria *Thermotoga neopolitana*, the growth after the treatment with a temperature of -196 degrees Celsius was almost similar to the growth kinetics when it was subjected to temperatures such as -20,10,50,85 degrees Celsius.[18] Thus long exposure to extreme low temperatures as in space can affect the growth of the microbes after they are restored to normal conditions.

III. EXPERIMENTING IN SPACE: TECHNOLOGIES AND FACILITIES

Beyond the Kármán line, which is a feeble attempt to mark a definite boundary between atmospheres a space, environmental conditions reach extremes. This line, at 100kms above the Earth’s surface marks the end of our atmosphere and this definition is accepted by the Fédération Aéronautique Internationale (FAI).[19] However, it is not like after the 100km mark, the environmental conditions change drastically and we reach a new territory. The changes are gradual and with every kilometer from the surface, the air becomes thinner, gases start to dissipate decreasing the density, accompanied by an exponential decrease in pressure.[20]

The lingering question of whether microorganisms are able to survive in space environmental questions depends to major factors: The magnitude of damage that an airborne microbe has to endure during flight and whether or not that microbe is able to repair the damage endured by it, on its own and begin proliferation, i.e. its natural growth cycle. Scientists already did realize the importance of such studies and began experimentation from as early as 1800s, often using balloons to reach heights needed to collect the microorganism.[21] However these experiments were futile due to contaminations and other errors and thereby didn’t give any accurate results. With advancement of technology, more studies were made and microorganisms, mainly spore forming fungi, were isolated from even heights of 77km above the surface. [22] Beyond this, earth’s gravity decreased significant and is now termed as microgravity and/or a feeling of weightlessness and the immense magnitude of radiation that are not yet filtered by the atmospheric layers.[23] It is here where astrobiological experiments are conducted.

The first experiments to study the survival of microorganisms under exposure of space radiation were conducted in 1965 with Gemini 9 and Apollo 16, ranging from 150 to 300 kms. [24] This experiment revealed the potential ability for the survival of microorganisms even in the harsh conditions of space. Much progress has been made after this experiment and the following list includes the various technologies and facilities used for such experiments:

LDEF (Long Duration Exposure Facility)

LDEF orbited the earth for 2107 days, making it the longest duration space exposure experiment till date. This was launched by NASA Space Shuttle Challenger in April 1984 and was expected to be retrieved by 2985 but due to unforeseeable circumstances it was retrieved only in January 1990 by the Space shuttle Columbia.[25] The main objective of NASA’s LDEF was to build a low-cost retrievable platform, which can be reused, efficiently. Some of the experiments included survival of spores in the presence of radiations and their resistance was proved.[26]

BIOPAN

BIOPAN, as its name suggests, is a pan shaped exposure facility that can be retrieved. They are known to help conduct experiments on astrobiology, radiobiology and addition dosimetry.[27] BIOPAN works by exposing samples to harsh space environment and are carried by unmanned recoverable satellites.

EURECA

EURECA stands for European Retrievable Carrier was actually built to survive 5 flights over 10 years but due to the lack and shortage of funds, it was used only one time. EURECA is one of the very few unmanned space vehicles that have been returned to the Earth unharmed. During the flight, a total of 15 experiments were conducted which included the study of survival and evolution of organic molecules in space.[28]

Fig 03: Eureca retrieval [44]

ISS

The International Space Station also harbors microorganism inside them, estimating to have around 55 genera of core microbiome thriving inside.[29] These microorganisms have entered the space station solely through the cargo and passengers and have efficiently adjusted to the environmental conditions of the ISS. In flight sampling and further cultivation was done on R2A and TSA agar to identify the radiation resistant microorganisms.

These are some of the facilities used to conduct experiments in space. However, the future of astrobiology is not in space but on earth itself. Research should be done on how to stimulate and mimic the icy, harsh environments of space in laboratories so as to reduce cost and conduct experiments much more efficiently. Synchrotron facilities that provide high spectral purity and resolution can be used to conduct photochemical experiments on biological samples.[30]

IV. MICROORGANISMS IN SPACE

In the previous section, we discussed about the various facilities available to help conduct experiments. The results obtained from these experiments were almost always unpredictable and moreover some microorganism demonstrated an increased disease causing potential even after being weakened in space. [31] Let us first look into the two broad categories of microorganisms that are tested.

- *HUMAN-BORNE MICROORGANSIMS:*

These are the microorganisms that can survive inside the human body and will be able to infect and cause diseases in the body. It is very important to study these types of microorganisms as they can directly impact the health of the crew members and thereby future space missions.

- *EXTREMOPHILES:*

Extremophiles are microorganisms that have the ability to survive in the most extreme environmental conditions, like their name suggests. These organisms are especially vital for studying the physiological requirements for survival in space.[32] They can also be used to prove theories about life in other plants and also the highly controversial theory of panspermia.

Following are the some of the many microorganisms that have been tested and their respective experimental results:

Bacillus subtilis

Bacillus subtilis a Gram-positive, catalase- positive bacterium which can be found in soil and the gastrointestinal tract of ruminants and humans. UV-resistant strains were exposed to stimulated Martian surface conditions and low-earth orbit (LEO) using European Space Agency's exposure facility (EXPOSE-E). Results did prove the survivability of spores with the biggest challenge for survival being the high inactivation potential of extra-terrestrial solar UV radiation. [33] Moreover, survival of spores after hypervelocity atmospheric entry does pave way for lithopanspermia theory. [34]

Chroococcidiopsis

Chroococcidiopsis is a primitive, unicellular cyanobacteria implying that it is able to conduct photosynthesis. They are known to survive in extreme conditions, i.e., are extremophiles. As it is able to resist low temperatures, tolerate radiations and survive in low moisture, they were considered to be an ideal organism that would be capable of surviving on Mars. Many experiments were conducted to determine the extent of tolerance of Chroococcidiopsis against UVC radiation and the results do support the endurance of this cyanobacterium under extra-terrestrial conditions and is now very relevant in the development of life detection strategies. [35]

Deinococcus radiodurans

Deinococcus radiodurans is an extremophilic bacterium and is by far the most radiation resistant organism known to man and is even in the Guinness Book Of World Records as the world's toughest bacterium. This ability arises from its unique ability to repair its own DNA from damage. Recently, a study of dried *Deinococcus radiodurans* pellets were found to survive 3 years of space exposure by being able to efficiently repair DNAwhich were damaged by UV. These results thereby provide evidence for the possibility of interplanetary transfer of microbes.[36]

Escherichia coli

Escherichia coli is one of the most experimented bacteria and is a gram negative, rod shaped bacteria that are commonly found in the intestine and gut of humans and other animals. It was concluded that during space flight, the exponential growth phase increased and the cell density was almost doubled, indicating that direct influence of gravity might not cause any significant changes in their metabolism and can be deemed negligible.[37]

Methanobacterium

Methanobacterium are methane reducers of the archaeal domain. They non-motile, anaerobic and are found in hot springs, anaerobic digestors etc. They are involved in biogas production from organic wastes. Permafrost archaea were exposed to the thermo-physical conditions of the low and high altitudes of Martian environment and scientists observed that around 90% of the sample survived suggesting subsurface lithoautotrophic life on mars can be done with the same. [38]

Euglena gracilis

Euglena gracilis is a single celled freshwater alga capable of photosynthesis. It has a tail like structure called flagellum which helps in movement. Experiments were done to test the pattern of flagellum under microgravity and even whether dormant cells of *Euglena gracilis* had the capability to restart and recover after a period of 9 months in space. Test results indicates that even after such a long period in space dormant, inactive cells were able to efficiently metabolise and move when transferred to a fresh medium. So dormant states of organisms can be preferred over live states as they can more easily tolerate the large environmental stress.[39]

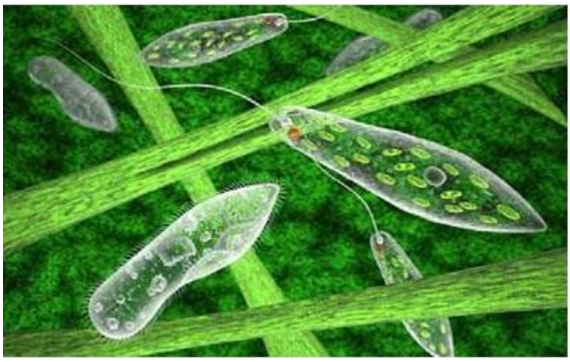

Fig 04: Microscopic view of Euglena gracilis (coloured).[45]

Xanthoria elegans

Xanthoria elegans is a bright red or orange fungus that can be seen in lichenised symbiotic organisations. They are usually found on rocks in Antarctic regions. They are model organisms for astrobiological studies as they have the potential to survive extreme conditions. In an experiment conducted on the International Space Station (ISS), *Xanthoria elegans* was exposed to stimulated Mars like conditions along with space exposure, for 18 months. Nevertheless, they showed a good percentage of viability and survival rate even after being exposed to cosmic radiation, vacuum, UV radiations and the very cold temperatures of space. [40] Moreover, among various lichens *Xanthoria elegans* displayed the highest viability survival rates under space exposure. [40]

Apart from the above few *Zygosaccharomyces bailii, Saccharomyces cerevisiae Canine hepatitis, Nostoc microscopicum, Xanthoria elegans, Trichoderma longibrachiatum, Aspergillus niger, Streptococcus mutans, Staphylococcus aureus, Klebsiella pneumoniae* etc. have been tested for survival conditions.

V. HOW DO MICROBES SURVIVE IN SPACE?

The Space technology over the past years has developed for transporting life beyond the stratosphere to study their survival characteristics and responses.

Some microbes have the ability to survive in space can also be known as Extremophile. An extremophile is an organism is an extremely tolerant organism that can survive even in extreme environmental conditions.[41] Among the many extremophiles on Earth, the halophilic archaea (Haloarchaea) are especially attractive models for Astrobiology Halophilic archaea (family Halobacteriaceae) are the microorganisms best adapted to life at extremes of salinity on Earth. This paper reviews the properties of the Halobacteriaceae that may make the group good candidates for life also on Mars.

Fig 05: Tardigrade, an Extremophile.[43]

Our Earth was likely very inhospitable more than 4 billion years ago, but microbial life started forming and evolved over time. Solar radiation is also a primary energy source for surface planetary life, so that the pigments produced are important components for any organism that lives on the surface. This is believed to be the reason why there is evolution in some way on Mars too.[42]

VI. CONCLUSION

Astrobiology is yet a new field which is being explored since recently and not many breakthroughs have been made. This presents new horizons to be researched and even proposes the existence and the search for extra-terrestrial life in our own star system and beyond. It is evident that life on earth ultimately finds it difficult to survive in space for elongated periods of time. Though some microbes grow indistinctly in space conditions such as microgravity, as they do under normal conditions, factoring in all the variables that come with the outer space environment, we can see that microbes cannot thrive and sustain in such environments. This field of study opts to find other habitable environments on planets both nearby and of exoplanets. It explores the idea that life could exist out of our biosphere and could even be non- carbon-based organisms. Till now microbes are being taken to space only by manned or unmanned spacecrafts and detailed studies and observations are taken on their resistance and growth under such conditions. The environment in the space proves to be fatal for most microbes when they are exposed for longer periods of time, still some have shown natural ability to retain their viability. Hence astrobiology provides a wide range of research interest that can be explored to many biologists aspiring to make their mark in the development of our knowledge on the evolution and migration of life through space. As steps are being taken in an accelerated manner, the field of astrobiology is only expected to grow exponentially in the coming decades. Interplanetary space missions are being planned which only widens the necessity for the study of astrobiology.

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