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Space Microbiology: Modern Research and Advantages for Human Colonization on Mars

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ABSTRACT

Astromicrobiology or exomicrobiology, is the study of microorganisms in outer space. Microorganisms in outer space are most wide spread form of life on Earth, and are capable of colonising any environment, this article usually focus on microbial life in the field of astrobiology. Microorganisms exhibit high adaptability to extreme environments of outer space via phenotypic and genetic changes. These changes may affect astronauts in the space environment as well as on earth because mutant microbes will inevitably return with the spacecraft. In this article, the advantages and disadvantages of microbes in outer space are discussed. We all know that outer space is extreme and very complex environment, microorganisms readily adapt to changes in environmental variables, such as weightlessness, cosmic radiation, temperature, pressure and nutrient levels, and these microorganisms exhibit a variety of morphological and physiological changes. Space conditions may significantly increase the mutation frequency of certain genes in microorganisms, which could allow the cultivation of the bacterial mutants, followed by screening of the bacteria for large scale production. Also we can extract microbial secondary metabolites as medicine, flavouring and nutritional drugs. This article provides the planetary exploration and also provides the microbial observatory program on ISS. The aim of this article will also help us to determine the benefits of bacteria and other microorganisms in case of "Human colonization on Mars".

Keywords-- The ISS as a Microbial Observatory, Planetary Exploration, Discoveries, Experiments, Microbes Tested in Outer Space, Extremophiles, Antibiotic and Food Production in Outer Space, Alcohol Production by Bacteria in Outer Space, Changes in Bacterial Invasion, Changes in Bacterial Adaptability in Environment, Changes in Antibiotic Resistance, Advantages of Space Microbiology for Human Colonization on Mars, Benefits on Microbial Observatory on ISS, and Multipurpose Facilities Available on ISS.

I. INTRODUCTION

The microbial ecosystems on the International Space Station are no different. Some microbes were inhabitants from the time station was assembled. Some join each time a new crew member or payload arrives. People, their habits, their physiological reactions to their emotional states, and their physical environment are all variable that have the potential to continue to alter microbe systems on the space station. With the potential to affect future space exploration missions, researchers founded by Human research program plan to gather and analyze biological samples to study better the micro-biome of the space station, the ever changing microbe environment that can be found on the space station. The vast cold and radiation filled conditions of outer space present an environmental challenge for any form of life. The biosphere of Earth has evolved for more than 3 billion years, shielded by protective blanket of the atmosphere protecting terrestrial life from the hostile environment of the outer space (Clement et al). Recently space technology provide the opportunity to expose microorganisms intentionally to the harsh external environment or selected parameter of it. Owing to the limitations of space flight time, specific environmental conditions and other uncontrollable factors, implementation of space ship based experimental research is more difficult. To overcome this weakness, terrestrial laboratory facilities are designed to stimulate parameters of outer space, such as rotator cell culture systems, parabolic flight stimulation and diamagnetic levitation. Researchers have recognized that the ground based stimulation of conditions such as microgravity and ionizing radiation only imitates the real space environment in a limited manner for the microbes being analyzed (Horneck et al). the short generation time of microorganisms makes them uniquely suited for studies assessing responsible to "altered environmental conditions". Microbial cells were among the first Erath based life forms to be sent into microgravity environment of space. These early investigations established that bacteria and fungi remained viable and capable of reproducing while also setting a precedent for conducting research in the space flight microgravity environment. Although more than 100 spaceflight experiments involving microorganisms have been conducted over the past 50 years, significant gaps in our knowledge as to how this environment impact microbial ecology, microbial genotypic and phenotypic characteristics, ad host microbe interaction remain (L. Castro et al).

II. THE ISS AS MICROBIAL OBSERVATORY

The original concept of microbial observatories as stated by United States of National Foundation was to

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study and understand microbial diversity over time and across environmental gradients. A key element of diversity studies is to seek out information about previously unidentified microbes in the various environments in which an observatory is established(Coris et al). The ISS is an excellent experimental system for studying changes in diversity over time under controlled conditions. The establishment of a microbial observatory program on ISS to conduct long term, multigenerational studies of microbial population dyanamics (Burge et al).The development of a research program aimed at demonstrating the roles of microbial plant system in long term life support systems. This program, previous space flight and ground based space flight analogue researches has established that even microorganisms, the smallest earth based life forms, are intrinsically able to respond to changes in this force(Dickson et al 1991). Microgravity as a research tool, coupled with current molecular technology provides researchers the opportunity to establish how variations in this physical force affect microbial life at cellular, molecular and evolutionary levels (Apollo, medical concerns and results,1969).

II. 1 Multiputpose Facilities Available on the ISS: There are many multipurpose facilities available on the ISS which helps in microbial observatory program. Their short explanation are given below:

Biological Experiment Laboratory (Bio Lab): **Bio lab Includes** an incubator, microscope, spectrophotometer, glove box, freezer units, and two centrifuges to simulate the effects of gravity. A variety of hardware existing inserts and containers for microbiological experimentation are available through the primary European Space Agency (ESA) contractor Astrium (www.astrium.eads.net).

Bio Culture System (BIOS): The NASA Bio culture System is an advanced space bioscience culturing system capable of supporting variable duration and long duration experiments on the ISS. BIOS provides the ability to culture mammalian and non-mammalian cells and will allow for investigations into host-pathogen interactions.

European Drawer Rack (EDR): EDR supports seven Experiment Modules (EMs), each with independent cooling, power, and data communications as well as vacuum, venting, and nitrogen supply, if required.

European Modular Cultivation System (EMCS): EMCS allows for cultivation and stimulation of biological experiments under controlled environmental conditions (e.g. temperature, gas, water supply and light). The EMCS has two centrifuges that can spin at 0 to 2 times Earth's gravity.

Expedite the Processing of Experiments for Space Station Racks (EXPRESS Racks): EXPRESS Racks is a multipurpose payload rack systems that provide structural interfaces, power, data, cooling, water, and other items needed to operate microbiological experiments in space.

Image Processing Unit (IPU): The IPU receives, records, and downlinks experiment image data for experiment processing. The IPU is housed in the Ryutai (fluid) experiment rack.

Light Microscopy Module (LMM): The light imaging microscope that takes digital images and videos across many levels of magnification using standard Leica objective lenses. It is capable of high resolution black and white microscopy, bright f ield, epifluorescent and fluorescent techniques.

Microgravity Science Glove box (MSG): The MSG is a contained work environment for research with liquids and hazardous materials. It is equipped with a front window, built-in gloves, video system and data downlinks allow for monitoring enclosed experiments from the ground.

Nano Racks: Nanoracks contains optical and reflective microscopes with digital image retrieval for ISS experiments. A NanoRacks Plate Reader is also available to monitor samples in micro titer plates with 96 wells with controls for temperature and stirring. Saibo Rack: Saibo rack contains the

Cell Biology Experiment Facility (CBEF) and Clean Bench (CB): CBEF is an incubator with a micro-G compartment and a1G compartment equipped with small centrifuge. CB is a glove box with a HEPA filter and high-performance optical microscope.

III. PLANETARY EXPLORATION

The search for extraterrestrial microbial life have focused mostly on Mars due to trhe promising environment and close proximity ;however, other astro biological sites include the moons Europa, Titan and Encela dus(Davila et al). All of this sites currently have or had a recent history of processing liquid water, which scientists hypothesize as the most consequential precursor for biological life. Europa and Encedalus appear to have large amounts of liquid water hidden beneath the layers of ice that covers theirs surfaces. Titan , on the other hand, is only planetary body besides Earth with liquid hydrocarbons on surface(Burdge et al). Mars is the main area of interest for the search for life primarily (Astronomic biology,10th Sep,2010).

III. 1. Discoveries: So far, the search for microbial life in extra terrestrial locations have been less than successful. The first of such attempts, occurred through Viking program (NASA-1970s), in which two Mars landers were used to conduct experiments that searched for bio signatures of life on Mars. The landers utilized robotic arms to collect soil samples into sealed containers that were brought back to earth. The results were largely inconclusive, although some scientists still dispute them.

In 2008, Russian cosmonauts reported findings of sea plankton living on the outside sea surfaces of the ISS windows. They have yet to find explanations for the discovery, but it seems to have been a result of human contamination, though this may never been proven.

III. 2. Experiments: Many experiments have been done on earth and space.

***Earth*:** Many studies on earth have been conducted to collect data on the response of terrestrial microbes to various stimulated environmental conditions of outer space. The response of microbes such as viruses,bacterial cells, bacteria and fungal spores and lichens, to isolated factors of outer space were determined in space and laboratory stimulation experiments. In general microorganisms tended to thrive in the stimulated space flight environment – subjects showed symptoms of enhanced growth and uncharacteristic ability to proliferate despite the presence of normally successive levels of antibiotics. The mechanisms responsible for explaining this enhanced responses have yet to discovered (Burge et al).

***Space*:** The ability of microorganisms to survive in an outer space environment was investigated to approximate upper boundaries of the biosphere and to determine the accuracy of the interplanetary transport theory for microorganisms. Among the investigated variables solar UV radiation had the most harmful effect on microbial samples. Among all the samples, only lichens (Rhizocarponsp and Xanthoria sp) fully survived the 2 weeks of exposure to outer space(Horneck et al, Space microbiology).

Microorganisms tested in outer Space: The survival of some microorganisms exposed to outer space has been studied using both stimulated facilities and low earth orbit exposures. Bacteria were som of the first organisms investigated , when in 1960s A Russian satellite carried E.coli,Staphylococcus sp,Emterobacter sp imto orbit(Love et al,2016). It is possible to classify the microorganisms into two groups :

- The human borne
- Extremophiles

● ***The Human Borne:*** These microorganisms is significant for human welfare and future crewed missions in space(Space flight alter bacterial social network-NASA-2013). NASA has pointed out that normal adults have ten times as many microbial cells as human cells in their bodies(Olsson et al). They are also nearly everywhere in the environment. And although normally invisible, can form slimly biofilms.

● ***Extremophiles:*** They have adopted to live in some of the most extreme environments on earth. This includes hypersaline lakes, arid regions, deep sea, acidic sites, cold and dry polar regions, and permafrost(Rothschild et al). The existence of extremophiles has led to the speculation that microorganisms could survive the harsh conditions of extra terrestrial environments and be used as model organisms to understand the fate of biological systems in this environments. Because of their ubiquity and resistance to space craft, decontamination bacterial spores are considered likely potential forward contaminants on robotic missions to Mars. Measuring the resistance of such organisms to space conditions can be applied to develop adequate decontamination procedure(Nicholson et al).

There are some microbes which are tested given below:

- Actinomyces sp(Dublin et al)
- Aeromonus sp(Taylor et al)
- Anabaena sp(Olsson et al)
- Aspicilia sp(Raggio et al)
- Canine sp(Hotchin et al)
- Tolypthrix sp(de Vera et al)
- Symploca sp(de Vera et al)
- Deinococcus sp(Dose et al)
- Synechococcus sp(Mancinelli et al)
- Circinariasp (Sanched et al)
- Buelliasp (Meeben et al)
- T7 bacteriophage (Koike et al)
- Influenza PR8(Hotchin et al)

IV. CHANGES IN BACTERIAL INVASION

Many space-based experiments have proved that the virulence of the bacteria would be changed. Nickerson et al. found that Salmonella grown during space-shuttle mission STS-115 in2006 underwent major changes in the expression of 167 genes (Wilson JW et al). When administered to mice back on Earth, the bacteria proved more deadly than an equivalent strain grown on the ground (Wilson JW et al,2007). They reported that spaceflight-induced increases in Salmonella virulence are regulated by media ion composition (Wilson et al,2008). Space research projects on S. pneumoniae were carried out in 2008 by the US NASA (Streptococcus pneumoniae expression of genes in space) and found that the state of low-shear modeled microgravity increased the capacity of Streptococcus pneumoniae to adhere to and infect respiratory epithelial cells; in a mouse-infection model, the median lethal dose 50 of a microgravity-induced strain significantly decreased when compared with the wild-type strain(Allen CA). The genetic ana lysis demonstrated that low-shear modeled microgravity can cause some changes in the gene expression of S. pneumoniae, but that it does not influence the genes that encode the main virulence factors(Allen CA et al). Our preliminary studies on K. pneumoniae that were transported on the Shen Zhou VIII spacecraft demonstrated that hemolytic mutations and biochemical profiles of expression significantly changed in the bacterium as a result of space environment exposure.

V. CHANGES IN ANTIBIOTIC RESISTANCE

It has been observed that reversible increases in antibiotic resistance occured during short term space flight (Taylor et al). MICs of E. coli, which were obtained from the commensal flora of an astronaut during the Salyut 7 Mission, and were isolated against both colistin

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and kanamycin increased significantly (Tixador R et al). In addition, the Challenger experiments established that in-flight-cultivated E. coli ATCC 25992 grew more rapidly in subinhibitory concentrations of colistin than cells cultured in a terrestrial environment (Lapchine L et al). In our study, we found that drug sensitivity of four species of bacteria showed obvious changes. E. coli isolate with resistance to ampicillin, cefazolin, ceftazidime, ceftriaxone and azithromycin increased significantly; K. pneumoniae isolates with resistance to cefazolin, ceftazidime and ceftriaxone increased significantly; E. faecium isolates with resistance to azithromycin and meropenem increased significantly; and B. cereus isolates with resistance to amikacin increased significantly.

VI. THE BACTERIA CONTINUE TO ALTER THEMSELVES TO ADAPT TO ENVIRONMENTAL CHANGES

In outer space, the lag phase of the microbial growth curve was observed to be shorter, whereas growth and reproduction rates increased along with the potential to increase the production of secondary metabolites (Horneck G et al). Fang et al. found that the degree of b-lactam antibiotic production by Streptomyces clavuligerus was markedly inhibited by simulated microgravity, which could be relevant to stimulatory effects of phosphate and l-lysine(Fang A et al). Phenotype microarrays are a high-throughput phenotypic testing method that carry out gene function analysis. Therefore, we used the phenotype microarrays to demonstrate the bacterial changes in metabolism (Bochner BR et al). Compared with the ground control group, all space bacteria demonstrated significant changes in glucose metabolism, amino acid metabolism and production of several antibiotic-associated secondary metabolites. As with K. pneumonia, there are many metabolic differences in the mutant strain LCT-KP182 in fusidic acid, d-serine, troleandomycin, minocycline, lincomycin, guanidine HCl, niaproof 4, vancomycin, tetrazolium violet and tetrazolium blue when compared with the ground control LCT-KP214.

VII. ADVANTAGES ON MARS

Modern technology has already allowed us to use microbes to assist us in extracting materials on Earth, including over 25% of the our current copper supply. Similarly, microbes could help serve a similar purpose on other planets to mine resources, extract useful materials, or create self-sustaining reactors. The most promising of these candidates known to date is cyanobacteria. Billions of years ago,cyanobacteria originally helped us create a habitable Earth by pumping oxygen into the atmosphere, and manage to exist in the darkest corners of the Earth. Cyanobacteria, along with some other rock-eating microbes, seem to be able to withstand the harsh conditions of the vacuum of space without much effort. On Mars, however, cyanobacteria will not even have to endure such harsh conditions(Wilson JW et al). Scientists are currently working on the possibility of installing bioreactors or similar facilities on Mars, which would run entirely on cyanobacteria and provide material for the creation of fuel cells, soilcrust formation, regolith amelioration, extraction of useful metals/elements, nutrient release into the soil, and dust removal; a variety of other potentially useful functions are also in the works.

VIII. ANTIBIOTICS PRODUCTION FROM SPACE

Test tubes of bacteria produce more antibiotics in space than they do on Earth. Experiments sponsored by Bristol-Myers Squibb in the mid-1990's revealed that microbes grown in test tubes or gas-permeable bags aboard the space shuttle produced more antibiotics than did microbes on the ground. In one case the improvement was as much as 200%.Antibiotic production is an important part of the pharmaceutical industry on Earth, so this result caught the attention of scientists and business people alike. Is it time to move antibiotic factories to space? Not yet. Sophisticated bio-reactors on Earth still yield more antibiotics than simple test tubes or bags do on orbit. The value of space -- for the moment -- is as a laboratory. Ongoing research is supported by BioServe Space Technologies, a NASA Commercial Space Center (CSC)at the University of Colorado, industry partner Bristol-Myers Squibb, and NASA's Space Product Development program. Their goal is simple: to find out why microbes yield more antibiotics on orbit, and apply those findings to increase yields on Earth. It's possible that the increase occurs simply because of the way microgravity alters the motions of fluids surrounding the bacteria, says BioServe associate Director David Klaus, who co-heads the study. On Earth, gravity causes the fluid -- that is, the medium -- to circulate. Heavier fluids fall and lighter ones rise. Within the medium, cells and the molecules they produce mix and move around. "But in a zero-g environment," points out Klaus, "there is no convection, or buoyancy, or sedimentation." Less of the mixing normally caused by such factors could change the metabolic activities of these one-celled creatures. For example, when bacteria are introduced into a new environment, they don't start multiplying immediately. First, they have to 'condition' themselves or their surroundings. That is the reason that you can leave food out for a while before it begins to spoil. Researchers speculate that bacteria produce vitamins, enzymes or other "cofactors" either inside or around the cell. Cells will begin to multiply only when enough of those substances have accumulated. In microgravity, the bacteria seem able to achieve this conditioning and begin growing sooner than they can on the ground -- perhaps because of reduced mixing. If a cell excretes a certain

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type of molecule, those molecules stay closer, and their concentration increases faster. The same kind of change, Klaus suggests, might account for the increased production of antibiotics.

IX. FOOD PRODUCTION BY BACTERIA IN OUTER SPACE

Sunlight on Mars is less intense than on Earth. So we can use cyanobacteria for food production. To use cyanobacteria as a raw material and energy for the next step of synthesis is a good idea. But thechallenge will lie in finding a suitably fast production process. Cynobacteria produce sugar from sunlight and CO2, and Bacillus subtilis then use it to produce whatever we want. The bacteria can alsobe genetically modified versions of a special type of bacteria, the photo synthetic cyano bacteria Synechococcus elongatus, and another common bacteria, Bacillus subtilis. The two species work together in a so-called co-culture, where one depends on the other. These genetically modified bacteria can be used for diseases resistant grain production.

X. PRODUCTION OF ALCOHOL BY BACTERIA IN OUTER SPACE

Space research with microbe fermentation might help improve this process. Yeast are tiny single celled fungi important for brewing beer and baking bread. Understanding the puzzling behaviour of such cells in space will benefit pharmaceutical research here on earth. We should try to do now is to find the specific mechanism of that (increased fermentation efficiency in space), and then we can ask whether we can modify the fermentation process on earth to take advantage of that. A more efficient fermentation process, even by a small percentage save millions of dollars in production costs. For beer, of course, increased fermentation efficiency means a more alcoholic brew-not necessarily good news for crew members who need to remain sober in the dangerous environment of space. The space brews would need to be adjusted and of course, consumed in moderation.

XI. BENEFITS OF THE MICROBIAL OBSERVATORY PROGRAM IN OUTER SPACE

There are numerous benefits from enabling and expanding microbial diversity research on the ISS. From a microbial ecology perspective, research in this area has the potential to play a major role in the development of the microbial ecology of indoor environments. Humans in the developed world spend more than 90 percent of their lives indoors where they are exposed to airborne and surface microorganisms. These microbial communities might be intimately connected to human health. Examples include the spread of acute respiratory disease (Cohen et al. 2000, Smith 2000, WHO 2007, Glassroth 2008) and the increase in the occurrence of asthma symptoms (Ross et al. 2000, Eggleston 2009, Schwartz 2009). The relative abundance of human-associated bacteria, including those that could potentially cause disease, is higher indoors than outdoors. As the ISS is relatively closed, the microbial diversity is relatively stable throughout the interior of the station, such that the dispersion of new microorganisms can be tracked and impact of their addition on the "station" community can be evaluated. This premise may also be possible for investigations into changes in the astronaut microbiome. The use of the ISS as a microbial observatory would drive experiments that could decrease infectious disease risk during the human exploration of space, advance the application of beneficial purposes for microorganisms (e.g., waste remediation, probiotics), and provide unique insight into basic microbial functions and interactions that could be translated to studies for scientists and commercial entities on Earth. Translation of spaceflight findings has already begun to take place as scientists and corporations are investigating the use of ISS microbial findings to better understand virulence profiles, antibiotic and disinfectant resistance, biofilm formation, and biodegradation properties.

As NASA travels beyond low-Earth orbit to planets such as Mars, insight from an ISS Microbial Observatory will impact our approach to exploration. Understanding how spaceflight and gravity alter microbial responses, their exchange of genetic material, and their expected concentrations and distribution will be vital in our search for extraterrestrial life and concurrent planetary protection.

CONCLUSION

Although a variety of bacteria have demonstrated changes following space flight, the mechanism of this alteration still remains unclear. For those with limited production capacity and expensive drugs, we can use the spacecraft equipped with microorganisms to improve their drug producing ability. This will become an important issue for aerospace technology used in the pharmaceutical industry. In recent years, with the large-scale application of antibiotics, levels of multidrug resistant and pan-resistant bacteria have significantly increased, and antibiotics are becoming less effective. Therefore, it is important to explore new anti-infective strategies and techniques. Space flight provides a new platform for the development of space microbiology. The influence of space environment on bacterial mutations may lead to some new risks for humans. For example: pathogens with increased virulence and antibiotic resistance in the space environment may be a threat to the health of astronauts; with the expansion of the scope of human space exploration, mutated bacteria transported by humans may pollute the space environment; and after the spacecraft has returned to Earth, mutants with high virulence and resistance may also be a threat to human health on the ground. Therefore, studying the effects of the space environment on different pathogens and its relevant mechanisms are very important and necessary for protecting the health of astronauts and humans on Earth. That research will use advanced molecular biology, genomics, bioinformatics, and cultivation technologies to understand spaceflight microbial community fundamental properties, interactions with humans, and adaptation to other planets or interplanetary space. It is critical that we develop a better understanding of the changes that may be induced in the various types of microbial relationships with humans in the unique space environment as humans prepare to spend increasing periods of time in space. The study of the effects of the space environment on microorganisms and their mechanisms will continue to be a vital part of manned space exploration, one that will benefit the study of the origin of life and biological evolution.

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