Chapter 8: Microbial Life in Space

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Abstract

Outer space is a harsh environment harbouring multiple forms of stress like cosmic radiation, space vacuum, extreme temperature and pressure, UV radiations, and altered gravity. Earth's atmosphere has several layers that expose microbial and terrestrial life to harsh external environments. In order to study the limits of survival of microbial life in extremes, it is imperative to study the response of micro-organisms to space-related stress. The present chapter summarizes the various balloon and flight experiments performed to investigate the presence and response of microbial life in space. Studying the microbiome in the ISS is important as pathogenic bacteria can present a major risk to astronaut health in a closed environment. Hence, studying occurrence, ecology, diversity, response, and adaptations of microbial life in space is crucial to understanding the limits of organismic survival in inhospitable conditions. Studying microbial life in space also helps predict the plausible survival and endurance of microbial travel between planets, crucial to lithopanspermia theories and planetary protection.

Keywords: Space, radiation, stress, International Space station, microbial life, microbiome

8.1. Introduction

Microbial life has been known to survive in harsh and extreme environments on Earth. Though Antonie van Leewonhoek discovered microorganisms or “animalcules” in 1675 (Leewenhoeck, 1677), it is estimated that microbial life may have evolved in the Archaean era (~2500-million years ago) (Cavalier-Smith et al., 2006). The origin and evolution of microbial life on Earth can be traced using microfossil traces, biomarkers, and biogenic isotope ratios unique to varied metabolisms. The presence of steranes and hopanes found in Archaean shales dating to 2.7 Gyris considered as the most reliable ancient biomarkers for the presence of microbial life (Brocks et al., 1999; Summons, 1999). The discovery of Archaea by Carl Woese and his colleagues and their addition as the third domain in the Universal tree of life using universal small subunit ribosomal RNA (SSU rRNA) (Woese & Fox, 1977)has ushered a new era of microbiology, evolutionary biology, and comparative genomics. Most archaea are extremophiles as they inhabit and thrive in extreme environments like hot springs, soda lakes, salt beds, hydrothermal vents, and permafrost (Rothschild & Mancinelli, 2001; Thombre et al., 2020). Dr. Thomas D. Brock defined an ‘extreme environment’ as an environment where life forms find it difficult to survive or where microbial diversity may be lower than elsewhere (Brock T., 1969; Moissl-Eichinger et al., 2016). Extremophiles survive in extreme environments with lethal concentrations and doses of acids, alkalis, metals, radiations, and toxic compounds. Extremophilic life has been reported from hydrothermal vents at 122 °C to frozen seawater, pressures of up to 110 MPa, the salinity up to 25-30 % NaCl, from extreme acid (pH 0), and depths of 6.7 km inside the Earth’s crust (Merino et al., 2019; Pabulo Henrique Rampelotto, 2013; Thombre et al., 2016). Extremophiles are classified as thermophiles (survive at high temperature, halophiles (survive in a high concentration of salt, acidophiles (survive at low pH), alkaliphiles (survive at basic pH above 8), barophiles (survive in high pressure), oligotrophic (survive in low concentration of nutrients), psychrophiles (survive in low temperature), xerophiles (survive in low concentration of water) and radiophiles (survive in high doses of radiations).

Over the past few decades, extremophiles have pushed the boundaries for the survival of life and helped define new normal parameters for the growth of micro-organisms. In the search for life beyond Earth, scientists have often speculated about the survival of microbial life (from Earth) in space and other planetary environments. For studying questions related to the origin of life, it is essential to investigate the *“Limits of life”* or the *“Limits of survival”* for microbial life. Extremophiles have been crucial to defining these boundaries for survival or limits of life (“Polyextremophiles: life under multiple forms of stress,” 2014).

Outer space can be used as an ideal test environment for studying the limits of survival for microbial life (Gerda Horneck et al., 2010). The space environment is extremely harsh and has numerous stress factors and environmental parameters affecting microbial growth. It is imperative to investigate the survival of microorganisms in space and how space environments with parameters like space vacuum, altered gravity, solar and cosmic radiations, UV radiation, extreme pressure, and temperature can affect microbial life in space. Louis Pasteur conducted the first experiments to study micro-organisms in air and higher

altitudes (Pasteur, 1860; Dassarma et al., 2020). Since the 1800s, numerous experiments have been conducted to detect microbial life in space. The earliest record of microbiology experiments in the stratosphere dates to 1935, when the US manned high-altitude balloon was used to isolate microorganisms from the stratosphere (Roger and Meir, 1936). Since then, many micro-organisms and especially extremophiles have been tested and known to survive extreme space conditions, and numerous experiments have been conducted over the last 50 years to study the response of microbial life to the space environment (Saffary et al., 2002; Sancho et al., 2007; Gerda Horneck et al., 2010; C. S. Cockell et al., 2011; Moeller et al., 2012; Wassmann et al., 2012; Kawaguchi et al., 2013; Selbmann et al., 2015; Milojevic & Weckwerth, 2020).

Extremophiles and extremotolerant organisms have been isolated from Low Earth Orbit and the International Space Station (ISS) (Checinska Sielaff et al., 2019). The ISS is an enclosed habitat and has application as a 'microbial observatory' to study the effect of space conditions on microbial life. In Astrobiology, studying microbial life in space is crucial to answering the primary questions related to the 'origin of life.' The study of Microbial life in Space is imperative in space exploration and planetary protection. Numerous missions have been planned for Mars and other planarity bodies in the future. Studying the survival and resilience of microbial life in space is key to developing planetary protection protocols and guidelines to mitigate backward and forward contamination. A wide range of experiments have been performed to study the survival of micro-organisms in simulated Mars conditions, and it is crucial to investigate if terrestrial organisms could affect future space explorations by forward contamination. Besides, many micro-organisms have been studied for their applications in space. The present chapter summarises the microbial experiments conducted in the stratosphere and space. We have also highlighted the applications of micro-organisms in space, especially as microbial fuel cells, in planetary protection, and bio-mining. Finally, we discuss the potential applications of synthetic biology, systems biology, and CRISPR in space microbiology.

8.2. Space and Low Earth Orbit (LEO) environment

The mixture of gases that forms a protective layer around Earth is called the atmosphere. The atmosphere is a thin layer of gases approximately 150 km above sea level, with an average radius of Earth being 6370 km (Poulopoulos, 2016). The border that separates the atmosphere and outer space environment is called the Karman line situated around 100 km from the surface of the Earth (Gerda Horneck et al., 2010). The atmosphere forms a part of the biosphere, and its component gases like nitrogen, oxygen, carbon dioxide (**Table 1**) interact with the hydrosphere and lithosphere in various geo-chemical cycles. The atmosphere is stratified into various layers depending on composition, pressure, temperature, and density (Speight, 2017). Vertically upward from ground level, the layers are the troposphere, stratosphere, mesosphere, thermosphere, and exosphere (**Figure 1**). Exosphere finally fades into Interplanetary Space (Poulopoulos, 2016; Speight, 2017). There are many variations between the Earth's atmosphere and the Space environment. The key variations in the space

environment are relativistic heavy ions of Galactic cosmic rays (GCR), ionizing radiation, energetic protons from solar particle events, UV radiation, cold and hot plasma, neutral thermosphere, particle radiation, and meteoroid debris. The earth's magnetosphere is a space formed by Earth's magnetic field and solar wind (Barth, 2003). The Earth's magnetosphere also contributes to space-related stress. Spatial and temporal variations exist in Earth's atmosphere and magnetosphere. The space environment also differs from the Earth's environment in parameters like pressure, temperature, gravity, and cosmic ionizing radiation. A comparative analysis of the key parameters on Earth, Low Earth Orbit, and Interplanetary space have been depicted in **table 2** (Olsson-Francis & Cockell, 2010). Micro-organisms have been exposed in different space missions, and the experiments conducted in space can be divided into categories like Low Earth orbits (LEO), middle Earth Orbits (MEO), geosynchronous (GEO), geosynchronous transfer orbits (GTOs), interplanetary, and mission's other planets (Barth, 2003). The Low Earth Orbit is a unique area around 200-1,000 km above Earth's surface. The LEO comprises solar cosmic radiation (SCR), galactic cosmic radiation (GCR), and radiation belts (Gerda Horneck et al., 2010). The LEO environment is characterized by extreme temperature and pressure, UV rays, SCR, GCR, space vacuum, and desiccation. In the following section, we have discussed the space microbiology experiments conducted in LEO.

8.3. Microbial Experiments conducted in LEO

Since the 1960s, many organisms have been exposed to LEO to study the effect of space-related stress on the survival and growth of organisms (John Hotchin et al., 1965; J. Hotchin et al., 1967; Lorenz et al., 1969; Baglioni et al., 2008; Yamagishi et al., 2009; Olsson-Francis et al., 2010; Gerda Horneck et al., 2010; C. S. Cockell et al., 2011). Earth orbiters, Space shuttles, facilities on the ISS, and Russian crewed spacecraft MIR have been used for exposure to LEO. The earliest experiments on exposure of microbial cells to space were conducted in 1968 (J. Hotchin et al., 1967; Lorenz et al., 1969). The experiment consisted of exposure of *B. subtilis* spores, *E. coli*, bacteriophage T-1, and type III poliovirus in space at an altitude of 155 km. Similarly, dried suspensions of an organism like *Penicillium roqueforti*, *Coliphage T-1, Bacillus subtilis, and Tobacco Mosaic Virus* (TMV) were exposed on-board Gemini IX A and XII earth satellites and Agenda-VIII space rocket (J. Hotchin et al., 1967; Lorenz et al., 1969). Only protected samples survived the exposure of space in this experiment (J. Hotchin et al., 1967; Lorenz et al., 1969). The European Space Agency has constructed various LEO facilities (BIOPAN and EXPOSE onboard ISS) and conducted microbial exposure experiments in LEO(**Figure 2**)(Gerda Horneck et al., 2010; Olsson-Francis et al., 2010). Phototrophic biofilms have been exposed to LEO and a unique species of cyanobacteria *Gloeocapsa* OU_20, has been isolated after exposure to LEO (Olsson-Francis et al., 2010). Cockell et al. (2011) have studied the effect of long-term exposure (548 days) on *A. cylindrica* akinetes and extreme-tolerant vegetative cells of *Nostoc commune* and *Chroococcidiopsis* in LEO.

The Japanese have constructed a special exposure facility called "TANPOPO" in the ISS for LEO exposure (YAMAGISHI et al., 2009). The TANPOPO" mission derives its name from dandelion, whose seeds are spread by wind. This is Japan's first Astrobiology experiment in space in the ISS-Kibo facility, which aims to study microbial life in space stress. One of the most detrimental stress factors for microbial life in space is desiccation by space vacuum (Ott et al., 2019). The mutagenic effect of space vacuum on *Bacillus subtilis* (exposed to (1.2×10^{-4} Pa) was first reported in 1984 in the Spacelab1 experiment in 1984 (G. Horneck et al., 1984). Since then, numerous experiments have been conducted to expose bacteria and spores to space vacuum.

However, the molecular mechanisms of survival of microorganisms in the space environment are still poorly understood. Ott et al. (2019) studied the effect of simulated LEO vacuum on *Dienococcusradiodurans. The* proteomic analysis revealed the increased presence of ROS scavenging proteins, e.g., peroxidases and catalases in stress. A summary of microbial experiments conducted in LEO by NASA (National Aeronautics and Space Administration), ESA (European Space Agency), and other agencies is depicted in **table 3**.

8.4. Microbial Life in Stratosphere

Studies related to the exploration of microbial life in the stratosphere (5-20 km) have been conducted since the 1800s (Gerda Horneck et al., 2010). Louis Pasteur used the classical swan neck experiment to show that the occurrence of microbes decreases with higher altitudes (Pasteur, 1860; Dassarma et al., 2020). H. Dyar reported the presence of micro-organisms like *Micrococcus, Bacillus,* and *Sarcina*at moderate elevation in 1890 (DYAR, 1894). One of the earliest microbiology experiments in the Stratosphere were conducted in 1935. A US-based manned high-altitude balloon called Explorer 2 was one of the first air sampling missions to isolate micro-organisms from the stratosphere (up to 21 km ASL) (Rogers & Meier, 1936; Dassarma et al., 2020). The organisms isolated in this mission were from the genera *Bacillus, Macrosporium, Aspergillus, Penicillium,* and *Rhizopus.* Since then, numerous balloon experiments have been conducted to isolate microbial flora from the stratosphere (Table 8.6). These experiments were previously referred to as Aerobiology experiments. Some missions conducted the air sampling up to mesosphere (up to 48-85 km) (Imshenetsky et al., 1976; Imshenetsky et al., 1977; Imshenetsky et al., 1978; Imshenetsky & Murzakov, 1979). A list of the various microbiology experiments conducted in the stratosphere is depicted in **table 4**.

The previous experiments laid the foundation for future studies and missions for studying microbial life in the stratosphere, and many experiments were conducted using balloons, planes, and rockets (Narlikar et al., 2003; Wainwright et al., 2003; Griffin, 2004; Shivaji et al., 2006; DeLeon-Rodriguez et al., 2013; Smith et al., 2010). India conducted its first balloon experiment to study the microbiology of the Earth's upper atmosphere in 2001 and later in 2005. Cryogenic air samplers and Millipore filters were used to collect samples from an altitude of 24, 28, and 41 km above the surface of the Earth. In this experiment, four

species of *Bacilli, Bacillus aerius* sp. *nov*., *Bacillus aerophilus* sp. nov., *Bacillus stratosphericus* sp. nov., and *Bacillus altitudinis* sp. nov. were isolated (Shivaji et al., 2006).

The key stress factors for microbial growth in the stratosphere are radiation, low temperatures, low pressures (hypobaric, 0.1-10 kPa), desiccation, and nutrient deficiency. Due to these conditions, the most common microbial population present in the stratosphere are spore-forming bacteria and fungi. The experiments with balloons are extremely useful for studying the effect of stress on microbial life in the stratosphere. The key advantage of balloon experiments is that they have inexpensive landing strips during the sample return process to Earth, can be maneuvered to many diverse sites and positions, and can carry large payloads (Dassarma et al., 2020). Studying microbial life can provide information for developing guidelines for policies to minimize forward- and backward- contamination and strengthen planetary protection.

8.5. Effects of microgravity on microorganisms in space

Hypothetically, life may have emerged independently throughout the universe14 billion years ago, shortly after the Big Bang. The space dust, meteoroids, asteroids, comets, and planetoids, could be responsible for the spread of life between habitable planets, a process called panspermia (P H Rampelotto, 2010). Earth's biosphere has evolved for more than 3 billion years, shielded by the protective blanket of the atmosphere protecting terrestrial life from the hostile environment of outer space (Gerda Horneck et al., 2010). Recent developments in space technology offer researchers the opportunity to study the extra-terrestrial microbial life or life beyond Earth's atmosphere under substantially reduced gravity conditions than on Earth. Microorganisms or simply microbes can be classified into two groups, the human-borne and the extremophiles. The study of the human-borne microorganisms is important for human welfare and human-crewed space missions, while the extremophiles are vital for studying the physiological requirements of survival in extreme environment like space (Olsson-Francis & Cockell, 2010). Microorganisms are thought to make up more than 60% of the earth's biomass ("Fundam. Sp. Biol.," 2006). They have been found in almost every environment. The diversity and range of environmental adaptations exhibited by microbes make them a natural choice for studying how microbes adapt to change in gravity conditions. Microbes are known to survive in many extreme environments, including outer space, excellently reviewed by Gerda Horneck et al. (2010). Earlier there has been a long debate on whether gravity can affect microorganisms due to small size and weight. However, spaceflight and ground-based microgravity experiments have proven microorganisms as an ideal model life forms for microgravity research because they are lightweight, small, and relatively easy to handle in space, and have short generation times (Nickerson et al., 2004). Consequently, numerous experiments have been performed on microorganisms in orbit and Earth-based clinostats that simulate microgravity.

Immediately upon entry into space, organisms experience two most important physical parameters, viz., low gravity or near weightlessness condition created by the vehicle's free-

fall trajectory and radiation exposure as a consequence of being outside Earth's protective atmosphere. The spaceflight effect on microorganisms originated during the 1960s when countries like USSR and USA were planning to conduct manned spaceflight programs. The first successful recovery of directly exposed unprotected terrestrial microorganisms in space was carried out in 1968 (Lorenz et al., 1969). In this experiment, spores of *Bacillus subtilis*, type III poliovirus, and *Escherichia coli* bacteriophage T-1 were exposed for 500 s at an altitude of 155 km. Since then, space flight experiments have been performed on various organisms, as documented in numerous review articles (Taylor, 1974; Tixador et al., 1981; Cioletti et al., 1991). Many cellular processes and functions in microorganisms, such as cell growth (Kacena, Merrell, et al., 1999; Baker et al., 2004; Lawal et al., 2013), gene expression (James W. Wilson et al., 2002; J. W. Wilson et al., 2007), cell morphology and development (Van Mulders et al., 2011)(Vukanti et al., 2012), virulence and resistance (James W. Wilson et al., 2002; Lynch et al., 2004; Crabbé et al., 2010; Lawal et al., 2010), secondary metabolism (Lam et al., 1998; Demain & Fang, 2001; De Gelder et al., 2009) are found to be affected by spaceflight and ground-based simulated microgravity (SMG).

8.5.1. Ground-based microgravity and hypergravity techniques

Scientific experiments in space (real microgravity), including spaceflights, are particularly challenging because of certain constraints involved with these experiments. These constraints mainly include limited availability of spaceflight opportunities, high costs, and the requirement of specialized equipment to perform experiments aboard spacecraft or space stations. Experiments with biological systems such as microorganisms require additional facilities to maintain environmental and cultural conditions. Thus, there exists a basic need for ground-based microgravity simulation facilities, which provide an opportunity for microbiologists to study the effects of extra-terrestrial conditions with controlled environments. Simulation of microgravity facilities is important for preparing future space missions and understanding life in space (Nickerson et al., 2004; Olsson-Francis & Cockell, 2010). Some ground-based microgravity simulation techniques are described below.

8.5.1.1. Clinostats

Clinostat is a widely used simulated microgravity device designed and developed in the late 1700s mainly to rotate plants or biological specimens around a single horizontal axis. Clinostats exist in 1-D or 2-D forms depending on whether the dimension of the rotated axial line or the whole experimental area is considered. Clinostats with two axes are called three-dimensional (3-D) clinostats or Random Positioning Machine (RPM). The principle of clinostat is based on redistributing the gravity vector in a circle (2D-clinostat) using mechanical devices that force the sample to rotate around an axis. The basic 2-D clinostat has been defined as a tool to obtain a vector-averaged gravity (Sarkar et al., 2000; Nakamura et al., 2003) or to provide the nullification of the gravity stimulus (Dedolph et al., 1967). To obtain good quality microgravity simulation, the rate of rotation of sample around horizontal

axis must be uniform, rapid enough to prevent geotropic responses, and slow enough to avoid the development of significant accelerative forces.

8.5.1.2. 3-D Clinostat/Random Positioning Machine (RPM)

Based on the hypothesis that the quality of microgravity simulation might be increased by rotating around two axes, especially for larger objects, 3-D clinostats have been developed and also commercialized by Japan and Netherlands (van Loon, 2007). It contains two independently rotating frames. The term 3-D clinostat is appropriate as long as the device is running with constant speed and constant direction. However, both frames can also be operated with different speeds and different directions, termed as "random positioning machine" (RPM) mode (Hoson et al., 1997; Borst & Van Loon, 2009). Thus, in a random positioning machine (RPM), the gravity vector is redistributed in a sphere, and the sample is rotated around two axes (3D). Depending on the rotational speed and the distance from the center of the clinostat to the external edge of the sample container, good quality microgravity simulation can be achieved without too much residual gravity or shear forces as long as the sample is placed close to the rotation center (Raúl Herranz et al., 2015). Randomization of the gravity vector requires time; therefore, only processes that require a certain lag-time phase can be studied using RPM. For this reason, clinostats or RPMs cannot properly simulate microgravity for relatively fast molecular and cellular processes. Increasing the speed of rotation strongly increases the quality of the simulated microgravity and can even provide near weightlessness (Briegleb, 1992). However, fast rotation also strongly reduces the area along the rotation axis in which the omnilateral stimulation prevents gravity sensing. Further away from the rotation axis, centrifugal forces dominate over the randomization effect. Therefore, fast clinorotation provides simulated near weightlessness for only small samples that are positioned along the rotation axis. For RPM experiments in which a relatively large liquid volume is used, one should note liquid movement and shear forces within the volume (Leguy et al., 2011). Though these are used to carry out experiments on various biological samples, their use is limited due to temperature fluctuations and sometimes vibrations which can cause alterations in the g value.

8.5.1.3. Rotating wall vessel

Rotating wall vessels (RWVs) which are also called rotating bioreactors, have been designed and developed by NASA, especially for cell cultures (Schwarz et al., 1992) and aquatic organisms such as zebrafish eggs/embryos (Moorman et al., 1999). Another submersed version of the RWV was designed and constructed at the German Aerospace Center (Brungs et al., 2011). The major components of RWVs include a Plexiglas cylinder (diameter of 10 cm) with a 5 cm wide central core, mounted on a horizontal plane and a shaft connected to a variable speed motor. Cells grown in RWV bioreactors develop in a low fluid-shear environment and provide necessary oxygenation and nutrients, which enable cells to form complex 3D tissue-like aggregates (Gardner & Herbst-Kralovetz, 2016). Since its development, the RWV bioreactor has been utilized for the study of cellular and microbial gene expression in microgravity, cellular differentiation, host-pathogen interactions, and tissue engineering (Navran, 2008; Grimm et al., 2014). Figure 3 adapted from Soni et al. (2014) gives the principle of rotating wall vessel (RWV). **Figure 3a)** shows the rotating wall

vessel (RWV). F**igure 3b)** gives two operating orientations of RWV. In the LSMMG orientation, the axis of rotation of the RWV is perpendicular to the direction of the gravity force vector, while in the normal gravity, or 1 g orientation, the axis of rotation is parallel to the gravity vector. **Figure 3c)** shows the effect of RWV rotation on particle suspension. When the RWV is not rotating or is rotating in the 1 g orientation, the force of gravity will cause particles in the apparatus to sediment and eventually settle on the bottom of the RWV. When the RWV is rotating in the LSMMG position, particles are continually suspended in the medium. The medium within the RWV rotates as a single body, and the sedimentation of the particle due to gravity is offset by the upward forces of rotation. The result is a low-shear aqueous suspension that is strikingly similar to what would occur in true microgravity (Hammond & Hammond, 2001; Nickerson et al., 2004).

8.5.1.4. Diamagnetic levitation

The levitation facility uses the diamagnetic properties of water, which is the major component of biological objects. A diamagnetic force is developed due to a strong magnetic field applied to biological material, which has the same magnitude as that of gravity but in the opposite direction, capable of effectively compensating the weight of the sample, producing the levitation phenomenon (Beaugnon & Tournier, 1991; Valles et al., 1997). The constant diamagnetic effect applies at the molecular level thus, it is not the result of averaging the forces in the system with time but is linked to the diamagnetic properties of each material. One can achieve stable levitation by this technique; a diamagnetic material can be made to levitate at a particular point in space in stable equilibrium (Berry & Geim, 1997), with no mechanical means of support. The diamagnetic force balancing the force of gravity on a levitating object acts throughout its volume, just as the centrifugal force acts to balance the force of gravity on a weightless body in Earth orbit. Thus, various ground-based facilities are available nowadays for microgravity simulation. The choice of appropriate microgravity analog is very important while designing any biological experiment. None of them is absolutely optimal, the final choice will depend on the biological system, its response time, sample size, type of tissue and cells, and the experimental parameters to be analyzed (Raul Herranz et al., 2013). The following table describes the comparison of biological responses in microgravity to real microgravity in spaceflight (**Table 5**).

8.5.1.5. Centrifuge

During launch and re-entry of a space vehicle, biological samples such as organisms, insects, animals, including humans, are exposed to gravity, more than 1 g called hypergravity. Aground-based technique called centrifugation, which works on the principle of sedimentation, is widely used to study and understand the effects of hypergravity. Centrifuge is equipment driven by a DC motor, puts an object under study in rotation around a fixed vertical axis, causes more dense substances to separate and sediment along the radial direction. The particles are separated from a solution according to their size, shape, density, the viscosity of the medium, and rotor speed. The magnitude of gravity depends on the distance of the sample from the axis of rotation and the angular velocity or speed of rotation. The sample experiences a centrifugal acceleration $a_c = \omega^2 r$, where ω is the angular velocity

(rad sec^{-1}) and r the centrifuge arm radius (in cm) (van Loon, 2016). There are various types of the centrifuge devices which are the tabletop centrifuges (such as a 1-foot diameter centrifuge device) connected with a tissue culture incubator, the large diameter centrifuge system at the European Space Agency (ESA) , and a custom-made gondola-type centrifugal device with 1.5 m long-arm (Tominari et al., 2019).

8.5.2. Effects of microgravity on microorganisms

8.5.2.1. Cell growth

Each phase of bacterial growth is governed by unique factors. The length of the lag phase when cells are inoculated into a fresh medium is dependent upon changes in nutrient composition and on the age and size of the inoculum. The exponential phase is characterized by a population doubling period in which the cells consume nutrients and excrete waste by-products. The stationary phase is initiated as nutrient and/or toxic by-product concentrations achieve values that can no longer support the maximum growth rate, representing maximum cell population density (Bailey & Ollis, 1986). The effect of space flight on bacterial growth, presumed to be primarily that of weightlessness, may result in uniquely altered growth kinetics during any or all of these phases (Klaus et al., 1997).

The bacterium *Bacillus subtilis* flown in one of the experiments of ESA's multi-user facility ‘Biorack’ showed an increase in growth while reducing lag phase (Mennigmann & Lange, 1986). The effect of space flight on each phase of microbial growth (lag, exponential and stationary) was studied by a series of experiments using in vitro suspension cultures of *Escherichia coli* aboard seven US Space Shuttle missions. As a result of space flight, the lag phase was shortened, the duration of exponential growth was increased, and the final cell population density was approximately doubled (Klaus et al., 1997). Flight experiments performed aboard Space Shuttle Missions STS-63 and STS-69, with simultaneous 1g static, agitated controls, and clinorotated controls, revealed that both *E. coli* and *B. subtilis* cultured in space flight grew to significantly higher final cell densities than static 1g controls. The final cell concentration of *E. coli* cells cultured under agitation was 43% higher than in static 1g cultures and was 102 % higher in clinorotation. However, instead of the direct influence of gravity, changes in the external fluid environment due to the gravity-induced density-driven gradients could be responsible for the enhanced bacterial proliferation reported in these studies (Klaus et al., 1997; Kacena, Manfredi, et al., 1999). Increased microorganism proliferation in space has been reported by a majority of researchers (Tixador et al., 1985; Moatti et al., 1986; Lapchine et al., 1986). However, several investigations reported controversial results (Bouloc & D’Ari, 1991; Gasset et al., 1994).

8.5.2.2. Secondary metabolism

Besides growth, secondary metabolite production was also affected by microgravity. The production of β-lactam antibiotics by *Streptomyces clavuligerus*, production of rapamycin by *Streptomyces hygroscopicus*, and production of microcin B17 by *E. coli* were suppressed during culturing in simulated microgravity (Fang et al., 1997). Changes in metabolism caused by simulated microgravity were reported previously by Lam et al. (1998). He reported higher

production of the antibiotic monorden by *Humicolafuscoatra* in the Space Shuttle compared to the ground control samples, while Demain & Fang, (2001) observed changes in levels of several secondary metabolites induced by SMG. The effect of low-shear microgravity using a rotating wall vessel on the metabolism of *Cupriavidusmetallidurans* LMG 1195 was studied with Raman spectroscopy. Results showed higher oly-β-hydroxybutyrate (PHB) production in SMG after 24 h of culturing while the reduction in PHB concentrations in SMG compared to the control after 48 hours. The changes in carbon metabolism remain the same for both durations. However, interfering effects of the SMG environment, different oxygen demand, and different growth characteristics may be responsible for the difference in Raman patterns (De Gelder et al., 2009).
The individual effects of magnetic field and the gravitational force on the morphology and secondary metabolism of *S. avermitilis* showed the physiological response of strain PE1 to magnetic field exposure resulted in suppression of sporulation and a reduction in mycelium at 12T. However, results demonstrated that changes in avermectin production could be attributed to the magnetic field rather than an altered gravity environment (Liu et al., 2011).The influence of spaceflight and simulated microgravity on yields of secondary metabolites appears to have caused diverse and conflicting results. Changes in the extracellular microenvironments around microbial cells might play a key role in the diverse responses of microbial growth and secondary metabolisms (Huang et al., 2018).

8.5.2.3. Virulence and resistance

Space and modeled microgravity help understand the mechanisms of pathogenesis and host-pathogen interactions. A common food-borne pathogen, *Salmonella* shown to be more virulent when grown in simulated microgravity. For instance, *Salmonella* enterica serovar Typhimurium grown under modeled microgravity (MMG) were more virulent and recovered in higher numbers from the murine spleen and liver following oral infection than organisms grown under normal gravity. Furthermore, MMG-grown Salmonellae were more resistant to acid stress and macrophage killing and exhibited significant differences in protein synthesis than did normal-gravity-grown cells. Our results indicate that the environment created by simulated microgravity represents a novel environmental regulatory factor of *Salmonella* virulence (Nickerson et al., 2000).

A study conducted in spaceflight showed the enhanced virulence power of *S. entrica* serovar typhimuriumin a murine infection model compared to conditions in normal gravity. These organisms also showed increased resistance to environmental stress, higher survival rates in macrophages and increased levels of protein expressions. 2-D electrophoresis and microarray analysis identified several altered proteins expressions in *S. typhimurium* under microgravity compared to control and found a global RNA binding regulatory protein, *Hfq*(J. W. Wilson et al., 2007). Bacterial virulence is highly associated with biofilm formation. The biofilm formation in *Micrococcus leuteus* was initiated in microgravity to a greater extent than in ground controls. Microorganisms cannot only survive during an altered gravity environment but can display robust proliferative behavior.

8.5.2.4. Proteomics and genomics under microgravity

Although many differences have been reported at the physiological level in space-grown micro-organisms, the regulating mechanisms responsible for the changes remain unknown. The proteome and genetic basis for the various gravity-dependent responses observed are limited (Strauch et al., 2019). Microarray analysis on *Salmonella* cells cultured under simulated microgravity conditions showed significant alteration in the expression of 100 genes, including genes encoding transcriptional regulators, virulence factors, lipopolysaccharide (LPS) synthetic enzymes, and iron utilization enzymes (James W. Wilson et al., 2002). Genome expression of *Escherichia coli* K12 under clinorotation showed significant alteration in 430 genes. Genes involved in the starvation response and redirecting metabolism under starvation; responses to multiple stresses; biofilm formation as well as lipid biosynthesis were significantly up-regulated (Vukanti et al., 2008).
Rosado examined the effect of low-shear RWV growth on protein secretion and gene expression by three *S. aureus* isolates and reported a limited number of changes in gene expression under continuous rotation conditions (Rosado et al., 2012). *M*ycobacteria grown under low-shear modeled microgravity (LSMMG) showed significantly altered transcript levels for 562 and 328 genes under LSMMG after short and long exposure times, respectively. LSMMG induced a reduction in translation, a downregulation of metabolism, an increase in lipid degradation, and increased chaperone and mycobactin expression (Abshire et al., 2016).

8.5.3. Effects of hypergravity on microorganisms

Though enough active research conducted on microbial responses to microgravity, only a few reports are available on microorganisms exposed to partial gravity (hypogravity) such as lunar (0.16 × g) or Martian (0.38 × g) gravity, on bacterial growth (Hemmersbach et al., 2001; Santosh et al., 2011) and greater than 1 x g (Bouloc & D’Ari, 1991; Brown et al., 2002). A decrease of 33% to 40% in final cell numbers with corresponding 29% to 40% lower net growth efficiencies was observed in *E. coli* (Brown et al., 2002). Bouloc & D’Ari. (1991) reported that hyperaccelerations of 3 and 5 × g did not affect the growth of *E. coli*, whereas Brown et al. observed growth suppression at 50 × g. Similar observations were reported for *Paramecium tetraurelia*, which showed no effect at 10 × g but a significantly lower proliferation rate and a lower population density at 20 × g (Kato et al., 2003). At accelerations much greater than ∼102 × g, the effect of sedimentation on microbial cells becomes significant. In a typical example, cultures of bacterial cells subjected to centrifugation at 3,000–5,000 × g for 5–10 min yielded pellets of intact bacterial cells (“Methods Gen. Mol. Microbiol.,” 2007). Hyperacceleration up to 403,627 × g showed enhanced proliferation of *P. denitrificans* and *E. coli*. Under extreme hypergravity, the sedimentation and congestive packing of the cells are thought to induce the changes in cell membrane via mechanosensitive ion channels, which are perceived by the cells (Deguchi et al., 2011).

8.6. Microbial diversity in the international space station (ISS)

Microbes are considered as one of the tiniest organisms that are found on the planet earth. They can evade the earth environments to other planetary environments and space stations by various life-supported systems such as rockets, cargos, and crew members. There these microorganisms are exposed to pressures such as microgravity, high temperature, raised carbon dioxide levels, high vacuum, and radiations called "extreme environments". The International Space Station's (ISS) microbiological inhabitants have been long piqued the public's interest. Additional cargo and crew arrivals have so far brought a variety of other microorganisms. ISS is the biggest space station to date in LEO have been constantly occupied for doing space-related research. The increasing interest in the study of ISS microbial diversity has led to the identification of numerous microorganisms and extremophiles that can thrive in those extreme environments (Gerda Horneck et al., 2010). The study on the survival of microorganisms in space started back in 1967, where the terrestrial microorganisms (*Penicillium roqueforti* mold spores, Coliphage T1, *Bacillus subtilis* spores, and Poliovirus type I) were directly exposed to the space environment conditions via rocket and balloon-borne exposure experiments (J. Hotchin et al., 1967). Some microorganisms have also been subjected to several space flight missions such as Cosmos 110, Biosatellite II, etc. During the Apollo-16 space mission, in total, 9 different microbial species were exposed to the space environment for their survival response (Taylor et al., 1974). Similarly, the examination of UV radiations on microbiological spores in MIR station was carried out under a French mission called PERSEUS (Rettberg et al., 2002) and bacterial SFA experiment on Russian Foton satellite and European Retrievable Carrier (EURECA), respectively (Poletti, 1995).

Various studies on the survival of microorganisms in space environments under extreme conditions have been studied by researchers to discover unique mechanisms exhibited by them. The long-term exposure of *Bacillus subtills* bacterial spores in outer space was conducted on Long Duration Exposure Facility (LDEF) as a part of the NASA mission, and their response to solar ultra-violet radiations, vacuum, and cosmic radiations was recorded, resulting in the survival of 80% spores in space (G. Horneck et al., 1994). *Bacillus subtills* bacterial spores were also exposed to short-term exposure on artificial meteorites by using meteorological rockets to study the hypervelocity atmospheric entry with an implication to lithopanspermia hypothesis (Fajardo-Cavazos et al., 2005). The interactions between people and microorganisms in space habitation settings are essential for the success of long-duration space missions, as they help to decrease possible risks to the crew and spacecraft equipment. Therefore, four years of ISS microbial monitoring in Japanese experiment module "Kibo" with a culture-independent approach was performed by sampling from the different surfaces of incubator parts and other air filters using optimized swabbing methods, leading to the detection of various microbial species using quantitative PCR and pyrosequencing (Ichijo et al., 2016). Similarly, the bacterial isolates of Yellowstone National Park and the Kamchatka Peninsula hot springs under high vacuum and gamma radiations, i.e., *Bacillus sps.* strain PS3D and *Deinococcus radiodurans* were studied under high space vacuum and extreme UV irradiations (10-100 nm) during rocket flight resulting in decreased bacterial survival via DNA damage mechanisms (Saffary et al., 2002). The International Space Station is presently

being utilized as a "Microbial Observatory" to research microbial responses in spaceflight conditions. Seuylemezian et al., (2017), with their colleagues, reported a novel organism *Solibacillus kalamii* ISSFR-015, isolated from HEPA filter on the international space station. Similarly, the four strains of *Methylobacteriaceae*, i.e., IF7SW-B2^T, IIF1SW-B5, IIF4SW-B5, and *Methylorubrum rhodesianum being the fourth organism was isolated from the different locations of craft on ISS using* ANI and dDDH analyses resembling *M. aquaticum* and *M. terrae (Bijlani et al., 2021).* Scientists have used various sequencing approaches such as targeted gene-based amplicon sequencing, shotgun metagenomics, culture-dependent analysis, and whole-genome sequencing for the identification of bacterial species on ISS (Nitin Kumar Singh et al., 2018). However, the first report on metagenomic analysis without using whole genome amplification came in 2018, where 8 different microbial species, i.e., *Haemophilus influenzae, Salmonella enterica*, *Yersinia frederiksenii*, *Acinetobacter baumannii*, *Shigella sonnei*, *Aspergillus lentulus*, *Staphylococcus aureus, and Klebsiella pneumoniae* were isolated from 8 different ISS location using whole genome amplification (WGA) and metagenome sequencing techniques (Nitin Kumar Singh et al., 2018). Further, the analysis of persistence and antimicrobial resistance genes related to these microorganisms revealed them to be vancomycin-resistant with multidrug efflux pump as one of the virulence factors. Evidence of the pathogenic bacteria isolation from ISS drinkable water has been reported with *Acidovorax*, *Afipia*, *Brevundimonas*, *Propionibacterium*, *Serratia*, and other bacterial species using DNA extraction, PCR amplification, molecular cloning, and rDNA sequencing procedure (La Duc et al., 2004). The biosafety level 2 (BSL-2) pathogens isolated from different surfaces of the ISS resulted in the isolation of two MDR *Enterobacter* sp. using Illumina NextSeq WGS from the waste and hygiene compartment (WHC) (Sielaff et al., 2016). Other investigations of the dust samples present in ISS have identified microorganisms such as *Corynebacterium*, *Staphylococcus*, *Coprococcus* , etc. using 16sRNA gene targeting next-generation sequencing method (Mora et al., 2016). The Whole-genome sequence (WGS) analysis of seven different strains found on ISS resulted in the isolation of a novel microorganism with a novel genus named *Kalamiella* belonging *to the Eriwiniaceae* family using metagenome to phenome approach (Nitin Kumar Singh et al., 2019). Further analysis of disc diffusion and genome annotations revealed *Kalamiella piersonii* to be MDR in nature. Similarly, *Mhatre et al. (2020) isolated chloramphenicol resistant Kineococcus rubinisiae sp. nov.* from the spacecraft assembly facility by multi-locus sequence and whole-genome sequence analysis. Some of the pathogenic microorganisms have also been found on ISS. The whole-genome sequencing of 26 bacterial strains retrieved from ISS resulted in the isolation and identification of Enterobacterial strains *Pantoea brenneri*, *Pantoea agglomerans*, *Kalamiella piersonii*, and *Enterobacter bugandensis* using the WGS and NovaSeq 6000 system (Bharadwaj, Daudu, et al., 2020). Likely, *Lactobacillales* (Bharadwaj, Singh, et al., 2020), *Rhodotorula mucilaginosa* (Daudu, Parker, et al., 2020), *Bacillaceae* strains (Daudu, Singh, et al., 2020), *Sphingomonas* Sps. (Bijlani et al., 2020), *Bacillus cereus Sensu Lato* (Venkateswaran et al., 2017), and other bacterial phyla (Simpson et al., 2021) were isolated from ISS using WGS, adding up to the vast microbial diversity in ISS.

Increasing AMR and multi-drug resistant conditions in humans are the dominant contributor to hospital-acquired infections. Various spaceflight investigations on microbial species have revealed mutations and genes related to or unassociated with virulence factors (Avila-Herrera et al., 2020). Recent reports on the isolation of Enterococcus faecalis, a multi-drug resistant microorganism, from the surfaces and air of ISS with a potential pathogenic nature to astronaut's health were recorded (Bryan et al., 2021). Likewise, two different strains of *Fusarium oxysporum,* ISS-F3, and ISS-F4 were reported from the ISS dining table with virulence potential to human health (Urbaniak et al., 2019). Nitin K. Singh et al. (2018) isolated multi-drug resistant *Enterobacter bugandensis* species from ISS, and their comparative analysis revealed their involvement in virulence, disease, and defense with efflux systems. Rapid molecular based tools such as miniPCR bio's miniPCR™ thermal cycler and Oxford Nanopore Technologies' MinION™ sequencer has been used for easy to use and easily portable advantages. Stahl-Rommel et al. (2021) recently reported a swab-to-sequencer method using miniPCR and the MinION for real-time culture-independent microbial profiling in ISS during Extreme Environment Mission Operations (NEEMO) analog NASA missions 21 and 22, resulting in the isolation of gram-positive *Staphylococcus* Sps and gram-negative *Moraxella osloensis*, *Acinetobacter johnsonii*, *Sphingomonas hankookensis,* and *Aureimonas altamirensis*.

Microbes are often discovered on the interfaces of space-system parts and components. Biofilm formation has been seen on these hardware surfaces that can cause damage, costly repairs, and other serious technical issues. Therefore, investigations on biofilm-causing microorganisms such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* etc. have led to the identification of microbial diversity (A. Vaishampayan & Grohmann, 2019). Reports on the design of a NASA-supported biofilm in space project as a goal to describe biofilm within the International Space Station in a controlled environment, measuring changes in bulk, thickness, and shape have been reported by Zea et al. (2018). The first report on the biofilm formation and its persistence of *Pseudomonas aeruginosa* under microgravity conditions on space shuttle flight STS-95 was reported in 2001 (McLean et al., 2001). Further, Lynch et al. (2006) demonstrated the lower shear modeled microgravity (LSMMG) in ground-based systems leading to the formation of *Escherichia coli* biofilms. Further, studies on the microbial biofilm formation ability and adaptations of *Acinetobacter baumannii* after the spaceflight on China Shenzhou 11 spacecraft resulted in overall decreased biofilms after 33 days of spaceflight (Zhao et al., 2019). Sobisch et al. (2019), with their colleagues, isolated biofilm causing antibiotic-resistant microorganisms *Staphylococcus, Bacillus,* and *Enterococcus* sps. from the surfaces of the ISS. Similarly, the study on the comparisons of antibiotic resistance, biofilm formation, and other factors of microorganisms *Staphylococcus* and *Enterococcus* isolated from ISS and Antarctic Research Station Concordia was conducted, resulting in their high biofilm formation ability and gene transfer capacity (Schiwon et al., 2013). Furthermore, as the search for novel microorganisms and their diversity in ISS is implemented, future life exploration in space is paving the way for new opportunities to astrobiology.

8.6. Applications of Micro-organisms in Space

8.6.1. Applications of Micro-organisms as Microbial Fuel cells in Space

Biological systems for life support in space have been used since the 1950s (Myers, 1954). NASA initiated the Controlled Ecological Life Support System (CELSS) program, NASA L/MSTP tests, the European Space Agency's Micro-Ecological Life Support System Alternative (MELISSA) Project (MacElroy & Bredt, 1984). The MELiSSA loop is a system that uses a number of microbial species and plant cells. The concept of utilization of MFC by using human waste generated in spacecraft for microbial electricity generation was conceived in 1960 (Guo et al., 2012). NASA has examined the potential to use MFC as part of advanced life support in long-haul spaceflights during the 1960s (Shukla et al., 2004). These studies were discontinued because the underlying processes were not well understood. MFCs can now be employed in long-term space flight such as a mission to Mars for producing electricity using organic wastes generated onboard a spaceship. It is perused that in the future, a miniature MFC inside a human body fuelled by the nutrients from the body can be used to power a medical implant (Qiao et al., 2007).

8.6.2. Applications of Microbial proteins and molecules in Space

Electrically important molecules and proteins can be further used for enhancing MFC technology for space applications that impact NASA missions. The target molecules that can be genetically engineered for MFC are Piezo Electric Proteins (Prestin), Photo-Active Proteins, Mechanosensitive ion channels (MscL, MscS, MscM), and rhodopsins like bacteriorhodopsin. The most obvious application for these proteins involves biological photovoltaic cells that convert solar energy directly into electricity. InTact Lab LLC (USA) has devised a concept called Power skin. Power skin concept is a thin skin that uses the transduction potential of electroactive proteins like piezoelectric proteins to generate power for sensors. These thin skins could be made of self-assembling biomolecules like lipids or proteins that can be used in spacesuits or Biosuits. Scientists are considering linking power suits to flat MFCs for the generation of power for space applications. This demonstrates that the application of micro-organisms in Space sciences can be a solution to many unsolved problems. Most Spaceflights, Spacecraft's and Robots used for space missions are powered by a battery. These batteries are operated by nuclear or solar energy. Nuclear energy, when used for powering the spacecraft or robots, has major disadvantages such as its high maintenance and limited efficiency (Hsu et al., 2012). Nuclear energy commonly used for powering spacecraft can be hazardous as they use radioactive material like plutonium- 238. Fission reactors are also used in space missions where nuclear fission is utilized to release heat and energy. Energy generated by MFC can be a potential solution for powering spacecraft, Space flights, and Robots in future Space missions (Hsu et al., 2012). BugNRG experiment was conducted in the International Space station in which *Rhodoferax ferrireducens* was introduced for electricity production (De Vet & Rutgers, 2007). These studies indicate that MFC technology has tremendous applications in future space missions, and a better understanding of the molecular biochemistry and use of synthetic biology can

prove to be a potential answer to many problems related to power generation for future space missions.

8.6.3. Microbial diversity in Spacecraft assembly room and Planetary protection

Studying microbial diversity in spacecraft assembly rooms is extremely critical to Planetary protection. Planetary protection encompasses the policies, methods, and practices utilized for the protection of the solar system, including planets, moons, and other bodies from contamination by terrestrial organisms from Earth, as well as protection of Earth from plausible life from outer space (Rummel & Meyer, 1996; Rummel, 2000). The Committee on Space Research is responsible for the development of planetary protection policies for the protection of Earth and other planets from biological contamination (Rummel et al., 2002). Planetary protection is imperative for the maintenance of proper conditions for future space exploration studies in order to avoid terrestrial contamination that may obscure our quest for life on other planets. Protection of our solar system bodies from biological contamination has been the major goal of Planetary protection since the dawn of the Space era in 1957 (Olsson-Francis & Cockell, 2010). The UN Outer Space Treaty was established in 1967, and the Committee on Space Research (COSPAR) maintains a planetary protection policy complying with Article IX of this treaty (Rummel et al., 2002; Hobe, 2019). Several spacecraft and missions have been sent in outer space for the exploration of extraterrestrial life forms. The prevention of planetary cross contamination by Hitchhikers launched via spacecraft on robotic and crewed missions is a major objective of planetary protection. COSPAR describes the Planetary protection policies, regulations, and recommendations for five categories for interplanetary missions. Forward contamination is a significant concern for future missions as it may contaminate and compromise the scientific quest for life in habitable zones like Mars, Europa, and Enceladus (C. Cockell & Horneck, 2004; Crawford, 2005; Rummel et al., 2012). The standard microbiological methods, bioassays, NASA spore assays, Live/Dead staining methods, DNA microarrays, bioinformatics, and 'omics' based high throughput techniques are used for detection of microbial contamination and bioburden on spacecraft hardware (La Duc, Kern, and Venkateswaran 2004; Moissl-Eichinger et al. 2015; Olsson-Francis and Cockell 2010; A. Probst et al. 2010; A. J. Probst and Vaishampayan 2020; Seuylemezian et al. 2018; Vaishampayan et al. 2010, 2013; Weinmaier et al. 2015). Furthermore, as the search for traces of extinct and extant life on Mars continues, the implementation of Planetary protection in the past and future mars missions is inevitable for preserving the integrity of scientific exploration. Planetary protection also ensures that explorations are conducted responsibly for the benefit of science and society. Hence, studying microbial life in space is critical for Planetary protection and is essential for the prevention of jeopardization of future Astrobiological explorations of solar system bodies (Crawford, 2005; Rummel et al., 2012).

8.6.4. Applications of micro-organism in Bio-mining

Bio-mining is the process where minerals are leached from the materials locally present on Mars and the moon, such as Basalt. Microorganisms can be applied for the bio-mining of rare earth elements (REEs) on ISS for studying the bioleaching process, soil formation, and

generation of minerals applications in space. C. S. Cockell et al. (2020) conducted the bio-mining experiment on ISS to investigate the REEs bioleaching from basalt rock under microgravity and stimulated Earth and mars gravities. They used three microorganisms, *Sphingomonas desiccabilis*, *Bacillus subtilis,* and *Cupriavidus metallidurans,* in the biomining reactor that resulted in the enhanced reduction and no difference biomining efficiency, respectively; therefore revealing their potential role in space biomining or mining beyond earth (C. S. Cockell et al., 2020). Similarly, the biomining studies of Vanadium were investigated using microbes under mars- and microgravity on ISS. (C. S. Cockell et al., 2021) conducted an ESA BioRock experiment to investigate the Vanadium mining using *Sphingomona desiccabilis* and Bacillus subtilis under mars as well as microgravity conditions, resulting in 184.92 to 283.22% enhanced bioleaching, revealing their potential role in conducting elemental mining and other bioindustrial processes in space locations for future settlements.

8.6.5. Application of micro-organism for production of secondary metabolites in space

Secondary metabolites are defined as the low molecular mass products that are generated during the stationary/late phase of microbial growth as a survival function in nature that condenses in more complex compounds by various metabolic pathways (Sansinenea & Ortiz, 2011). Secondary metabolites are often thought of as chemical languages that allow bacteria to communicate with one another. From an ecological standpoint, they are always described as a result of evolutionary and environmental forces (Soldatou et al., 2019). Studies on the effect of spaceflight and simulated microgravity on microbial growth and the secondary metabolite have been conducted to study key roles of microbial metabolic processes and their diverse responses to space conditions to explore the effects of weightlessness on secondary metabolism (Gao et al., 2011; Huang et al., 2018). Microbial genome sequencing has revealed Biosynthetic gene clusters (BGCs); nevertheless, connecting them to secondary metabolites producing the major gridlock to chemical discoveries revealing the new chemicals and their processes (Soldatou et al., 2019). Production of secondary metabolites such as antibiotics has been demonstrated on the ISS for studying the specific mechanisms. Benoit et al. (2006) studied the microbial antibiotic production on ISS during 72-day 8A increment using *Streptomyces plicatus,* resulting in enhanced production of actinomycin; hence elucidating the mechanisms responsible for stimulation in space and applying it to commercial production facilities on Earth.

8.7. <u>Conclusion and future outlook</u>

One of the notable aims of astrobiology ventures is to look for the signs of life in outer space, planets, or the moon in our solar system. With various microbial experiments such as BIOPAN-5, EXPOSE, BIOSTACK, and BIOROCK being conducted in LEO to investigate the persistence of life in the international space station (ISS) and stratosphere, additional possible interplanetary habitats may be found in analogy to terrestrial extremophilic environments (high salt, high UV radiation, and cold environments). Studies relating the

effect of radiation and microgravity on the microorganisms in ISS and outer space have revealed the complex ecology, diversity, response, and adaptations of microbial life in space. However, analogues field- and space-based investigations for the identification of potential extremophiles and pathogens, respectively necessitates multidisciplinary collaborations for crucial information in planning the right “search of life” experiments to these solar system worlds.

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8.9. References

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Table 1: Principal composition of gases in the Atmosphere of Earth (Poulopoulos,2016)

Gas	*Formula*	*Volume (ppm)*	*Volume (%)*
Nitrogen	N_2	80,840	78.084
Oxygen	O_2	209,460	20.946
Argon	Ar	9340	0.9340
Carbon dioxide	CO_2	397	0.0397
Neon	Ne	18.18	0.001818
Helium	He	5.24	0.000524
Methane	CH_4	1.79	0.000179

Footnotes: Water vapor is not included in above dry atmosphere and is approximately 0.25% (v/v) over the full atmosphere but does vary considerably.

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Table 2: Comparative analysis of environmental parameters of Earth and Low Earth Orbit (LEO) (Horneck and Rettberg, 2007; Olsson and Cockell, 2010; Horneck et al., 2010)

Parameter	Earth	Low Earth Orbit	Interplanetary Space
Pressure (Pa)	10^{3}	$10^{-4} – 10^{-6}$	10^{-14}
Temperature (K)	Wide range	Wide range	>4
Gravity (*g*)	1 g	$10^{-3} – 10^{-6}$	$<10^{-6}$
Cosmic Ionizing Radiation (Gy/yr)	$<10^{-4}$	400-10,000	$\leq 10^{-6}$

Table 3: List of Experiments conducted in LEO

No	Payload/ Experiment	Mission	Period of Exposure	Experimental Details	Reference
1	Biostack 1	Apollo 16	266 hours	Effect of cosmic rays on several types of biological materials such as *Bacillus subtilis* spores, *Arabidopsis thaliana* seeds, *Vicia faba* radiculae and *Artemia salina* eggs.	(Bucker et al., 1973)
2	MEED	Apollo 16	59.7 minutes	To measure the effects of certain space environmental parameters on the microbial test systems.	(Volz et al., 1974)
3	Biostack II	Apollo 17	304 hours	effectiveness of cosmic HZE-particles on unicellular procaryotic, organisms was studied on Bacillus subtilis spores	(Bucker et al., 1975)
4	Biostack III	Apollo-Soyuz	218 hours	colony forming ability, metabolic mutations, and mutations affecting UV- and x-ray sensitivity in Bacillus subtilis	(Facius et al., 1978)
5	Advanced Biostack/ES 027	Space lab 1	9 days	radiobiological properties of the heavy ions of cosmic radiation by using Biostack, monolayers of biological test organisms sandwiched between thin foils of different types of nuclear track detectors.	(Bücker et al., 1984)
6	UV RAD	Space lab	10 days	responses of terrestrial organisms to the full	(Horneck et. al.,

		II		spectrum of extraterrestrial solar UV radiation; spores of different strains of *Bacillus subtilis*, cells of the radiation resistant bacterium *Deinococcusradiodurans*, and plasmid DNA of Escherichia coli were exposed to space vacuum and/or selected wavelengths and intensities of extraterrestrial solar UV radiation	1995a)
7	BIOPAN	Foton M2	15 days	test the limits of life in the hostile environment of space, and the effects of selected parameters, such as space vacuum and specific wavelength bands of extraterrestrial UV-radiation, on the viability of *Rhizocarpongeographicum* lichens	(de la Torre Noetzel et al., 2007)
8	Exobiology and Radiation Assembly	EURECA I	335 days	spores of different strains of *Bacillus subtilis* and the *Escherichia coli* plasmid pUC19 were exposed to selected conditions of space (space vacuum and/or defined wavebands and intensities of solar ultraviolet radiation)	(Horneck et al., 1995b)
9	Free Flyer Biostack	LDEF	274 days	measuring the biological effects of individual heavy ions from cosmic radiation (HZE particles).	(Heinrich, 1980)
10	Exobiologie	MIR	97 days	samples containing chiralamino acid and peptides, mixed or not with montmorillonite or meteoritic powder were	(Viso, 2015)

				deposited on windows of Magnesium fluoride and exposed directly to the solar UV flux; Spores of *Bacillus subtilis* mixed with meteoritic powder were exposed in cooperation with the DLR.	
11	EXPOSE-R2	ESA	-	more than 600 biological samples of archaea, bacteria (as biofilms and in planktonic form), lichens, fungi, plant seeds, eggs, mosses and 150 samples of organic compounds were exposed to the harsh space environment and to parameters similar to those on the Mars surface.	(Rabbow et al., 2017)
12	EF-JEM	Tanpopo	Up to 3 years	test the panspermia hypothesis; capture any orbiting microparticles, such as micrometeorites, space debris, and terrestrial particles carrying microbes as bioaerosols, b y using blocks of silica aerogel; survival of microbial species in the space en vironment; Possible escape of terrestrial microbes from Earth to space evaluated by investigating the upper limit of terrestrial microbes by the capture experiment.	(Kawaguchi et al., 2016)
13	BIOROCK	CRS-18	21 days	mining experiment on the International Space Station to test hypotheses on the bioleaching of REEs from basaltic rock in microgravity and simulated Mars and Earth gravities using *Sphingomonasdesiccabilis,*	(Cockell et al., 2020)

				Bacillus subtilis and*Cupriavidusmetallidurans.*	

Table 4: Microbial experiments conducted in Stratosphere using Balloons

Experiment	Altitude	Sampler/Method	Micro-organisms isolated	References
Explorer II- CrewedUS high-altitude balloon sampled air in stratosphere	21 km	Balloon with Air sampler using Autoclaved collection tube	*Bacillus, Macrosporium, Aspergillus, Penicillium and Rhizopus*	(Rogers & Meier, 1936)
Balloon Experiment by G.A.Soffen for air sampling	up to 40 km ASL	Balloon with Ethylene oxide sterilized impactor for isolation	*Penicillium species*	(Soffen, 1965)
Collection of samples from stratosphere and the mesosphere by A. A. Imshenetsky and colleagues	48-85 km ASL	γ-radiation sterilizedmedium exposed on meteorological rockets	Mycobacterium sp. *Micrococcus* sp., *Aspergillus* sp., *Penicillium* sp.	(Imshenetsky et al., 1976; Imshenetsky et al., 1977; Imshenetsky et al., 1978; Imshenetsky & Murzakov, 1979)
Balloon experiment with cryosampler	41	Cryosampler with sterile probes	*Bacillus* sp. *Staphlococcus* sp., *Engyotontium*sp.	(Wainwright et al., 2003)
Sampling bacteria using Aircraft	20	Aircraft used for exposure of sterile impactor plates for	*Bacillus* sp., *Penicillium, Micrococcus* sp., *Staphlococcus* sp.,	(Griffin, 2004)

		sampling bacteria	*Brevibacterium*	
ISRO's Balloon experiment	24, 28, and 41 km	Sample tubes	*Bacillus aerius sp. nov*., *Bacillus aerophilus* sp. nov., *Bacillus stratosphericus* sp. nov. and *Bacillus altitudinis*	(Shivaji et al., 2006)
Low- and high-altitude air masses sampled onboard the National Aeronautics and Space Administration DC-8 platform during the 2010 Genesis and Rapid Intensification Processes campaign in the Caribbean Sea.			Bacteria	(DeLeon-Rodriguez et al., 2013)

Table 5. Biological Responses in ground-based microgravity simulators in comparison to real microgravity. Comparative table adapted from (Raul Herranz et al., 2013). Symbols indicate that biological response to simulation is identical (++), similar (+), or different (-) to those of real microgravity experiments, n.a. means not applicable or no data available from spaceflight experiments

Object	**2-D clinostat**	**RPM**	**Diamagnetic levitation**
Paramecium	++	-	-
Euglena	++	-	-
Chara	++	+	-*
Arabidopsis			
Cell proliferation/growth	n.a.	++	+*
Gene expression	n.a.	+	-
Fish			
Behavior	n.a.	n.a.	n.a.
Development	+	n.a.	n.a.
Drosophila			
Behavior	n.a.	+	++
Gene expression	n.a.	++	+
Mammalian			
Adherent cells	+	+	-
Cells in suspension	+	n.a.	-

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Figure 1: Stratification of the atmosphere (Poulopoulos , 2016)

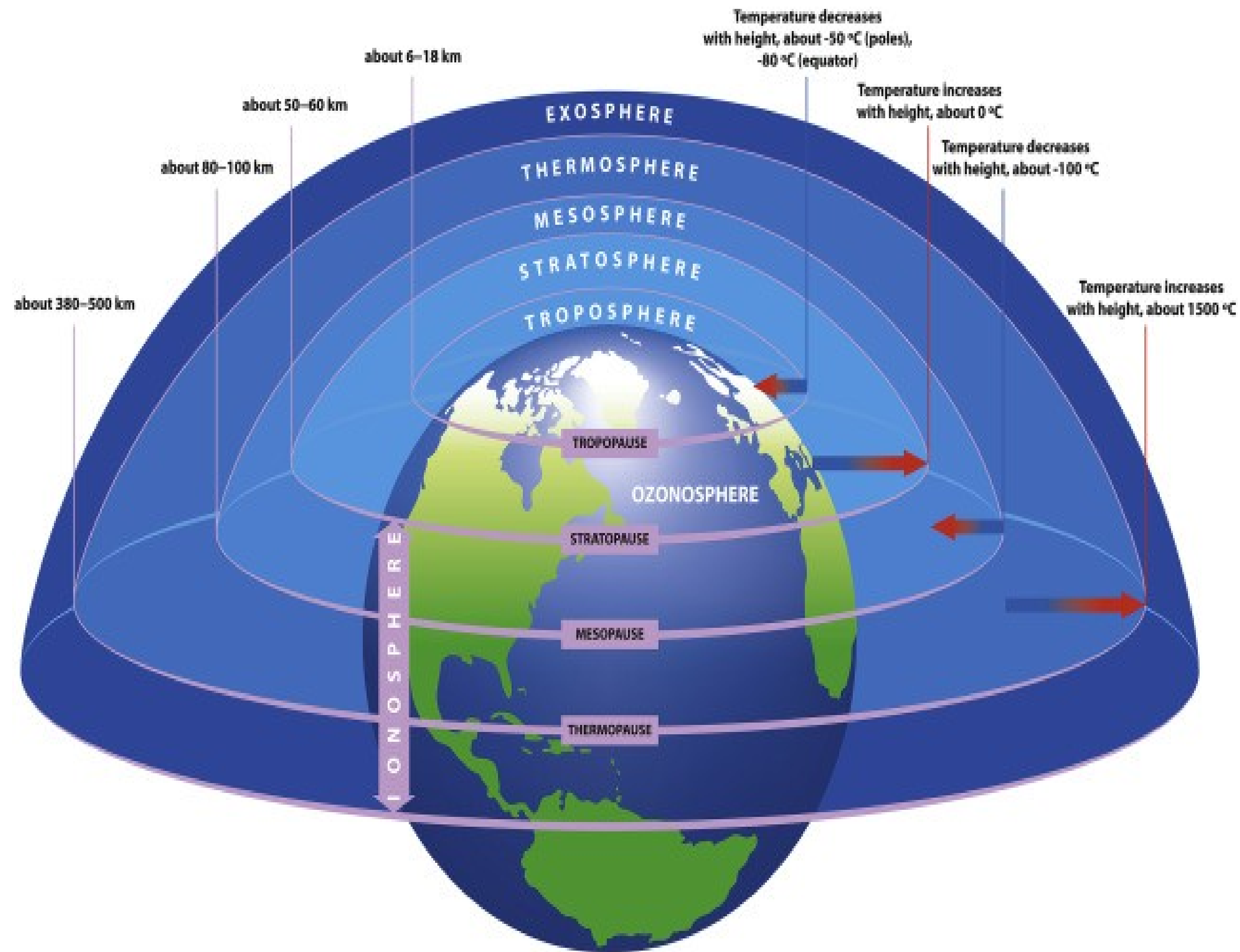
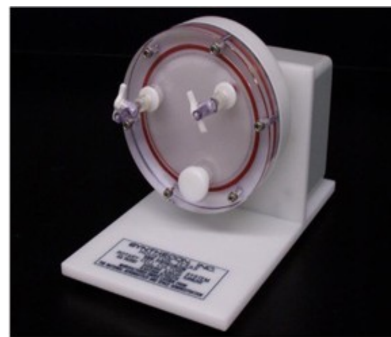
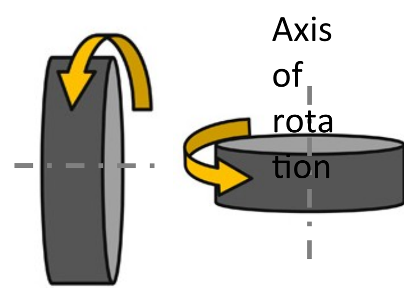
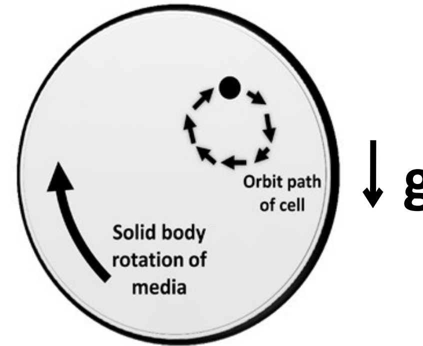


Figure 2: Experiment BIOPAN-5 part of the Foton-M2 mission Olsson and Cockell, 2010)

Figure 3 Principle of Rotating wall vessel (RWV) A) Rotating wall vessel model B) LSMMG and Control orientations, dashed line indicates axis of rotation C) the effect of RWV rotation on particle suspension. Figure adapted from Soni et al. (2014)







A)

B)

C)