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# Current Research in Life Sciences

# 22

# Microbiology/Astrobiology

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Since the advent of space flight and the establishment of long-duration space stations in Earth's orbit, such as Skylab, Salyut, Mir, and the ISS, the upper boundary of our biosphere has extended into space. Such space missions expose humans and any other organisms to living conditions not encountered on Earth.

## 22.1 Radiation Environment

Life on Earth, throughout its almost four billion years of history, has been shaped by interactions of organisms with their environment and by numerous adaptive responses to environmental stressors. Among these, radiation, both of terrestrial and of cosmic origin, is a persistent stress factor that life has to cope with [7]. Radiation interacts with matter, primarily through the ionization and excitation of electrons in atoms and molecules. These matter–energy interactions have been decisively involved in the creation and maintenance of living systems on Earth. Because it is a strong mutagen, radiation is considered a powerful promoter of biological evolution on the one hand and an account of deleterious consequences to individual cells and organisms (*e.g.*, by causing inactivation or mutation induction) on the other.

In response to harmful effects of environmental radiation, life has developed a variety of defense mechanisms, including the increase in the production of stress proteins, the activation of the immune defense system, and a variety of efficient repair systems for radiation-induced DNA injury. Radiobiologists have long believed that ionizing radiation, such as gamma rays, kills cells by shattering DNA. Recently, Daly [12] and Frederikson [17] showed that proteins—not DNA—are the most sensitive targets, at least in some radiation-sensitive bacteria. In cells, oxidatively damaged DNA repair enzymes generated by sublethal ionizing radiation doses would be expected

to passively promote mutations by misrepair. Oxidized proteins, however, might also actively promote mutation by transmitting damage to other cellular constituents, including DNA [16, 36].

## 22.2 Change in Gravity Environment

Microbes have the ability to sense and respond to mechanical stimuli. The response of microbes to certain mechanical stimuli has profound effects on their physiology [19, 38, 39, 46, 52]. The response of a cell to mechanical stimulation, such as stretch or shear force, is called mechano-adaptation and is important for cell protection in both prokaryotes and eukaryotes [19, 24, 25]. A great deal of progress has been made in understanding certain aspects of microbial mechano-adaptation, for example, mechanisms used by bacteria to respond to changes in osmotic gradients [9, 19, 41]. Studies have also documented that microbes can sense and respond to changes in culture conditions when grown in the buoyant, low-fluid-shear environment of microgravity [14, 26, 38, 39, 52]. It has been hypothesized that cells sense changes in mechanical forces, including shear and gravity, at their cell surface [24, 46].

Mechanical culture conditions in the quiescent microgravity environment of space flight are characterized by significant reductions in fluid shear [20]. This is because convection currents are essentially absent in microgravity [27].

The most commonly used microgravity simulator is the rotating wall vessel (RWV) culture apparatus (Synthecon; Texas, USA) developed by the NASA biotechnology group at Johnson Space Centre in Texas. This apparatus consists of a rotor, a culture vessel, and a platform on which the vessel is rotated. The RWV has separable front and back faces; the front face contains two sampling ports, and the back is provided with a semipermeable membrane for aeration. The assembled vessel is filled to capacity (zero headspace) with medium and inoculum, and air bubbles are removed to eliminate turbulence and ensure a sustained low-shear environment ($<0.01$ Pa).

In the vessel rotating around a horizontal axis, the liquid moves as a single body of fluid in which the gravitational vector is offset by hydrodynamic, centrifugal, and Coriolis (circular movement) forces resulting in the maintenance of cells in a continuous suspended orbit. In fact, this system "confuses" the biosystems (e.g., cells growing culture) perception of gravity's direction. By placing cells along the axis of rotation and spinning them perpendicular to the gravity vector, they rotate through the vector. Because the cell spins at a constant rate and gravity remains constant, the gravity vector is nulled from the cell's perspective [21]. Thus, the RWV does not generate microgravity as

on the ISS, rather it randomizes gravity vectors and mimics the low turbulence of a space environment. Since the RWV apparatus provides a low-shear culture environment that simulates the aspects of space (and therefore "models microgravity"), Nickerson et al. [39] have adopted the terminology low-shear modeled microgravity (LSMMG) to refer to the RWV culture environment.

Albrecht-Buehler [1] suggested that reduced gravity suppresses buoyancy-driven convection and thus limiting the mechanism of mixing of fluid to diffusion. Along similar lines, McPherson [34] suggested that the lack of convective mixing under reduced gravity conditions created a quiescent environment that resulted in a "depletion zone" around a growing protein crystal, which favored the formation of a crystal with better quality. Based on these studies, it was hypothesized that this same type of phenomenon might occur around a growing bacterial cell under reduced gravity conditions [28, 45]. Similarly, a few studies have speculated that bacteria may indirectly respond to reduced gravity conditions because of changes in their immediate environment resulting either from changes in mass diffusion or from other chemical alterations, such as accumulation of toxic by-products [28], or limitations in the availability of nutrients [3–5, 52]. Gene expression studies performed on *Escherichia coli* K12 under modeled reduced gravity conditions support the hypothesis that bacteria are actively responding to the changes in nutrient availability, imposed by the altered mass transport under these conditions [49, 52]. Creation of zones of nutrient depletion over-time in their immediate surroundings makes these bacteria respond in a way that is similar to their entrance into stationary phase (starvation). Stationary phase cells are generally characterized by the expression of starvation inducible genes and genes associated with multiple stress responses. Microgravity-exposed bacteria appeared, for example, better able to handle subsequent stressors including osmolarity, pH, temperature, and antimicrobial challenge. More recently it was reported that the fluid quiescence and reduced mixing could enhance the accumulation of quorum sensing (QS) molecules in the bacterium's surroundings and thus promoting QS-related gene expression, independently of change in cell concentration [22, 31].

Another microgravity simulator the random positioning machine (RPM) or three-dimensional clinostat is a laboratory instrument to randomly change the position of an accommodated (biological) experiment in three-dimensional space [23]. The layout of the RPM consists of two frames and experiment platform. The frames are driven by means of belts and two electromotors. Both motors are controlled on the basis of feedback signals generated by encoders, mounted on the motor-axes, and by "null position"

sensors on the frames. On the RPM, the samples are fixed as close as possible to the center of the inner rotating frame. This frame rotates within another rotating frame. The RPM has been extensively use to study cytoskeleton structure and motility of human cells [35, 53] and plant gravitropism [6, 23] and more recently the RPM has been used to study bacterial cultivation [8, 13, 30–33]. Comparing LSMMG to RPM cultivation, the two simulators appeared to induce a similar response in *Rhodospirillum rubrum* [31] while *Pseudomonas aeruginosa* only responded to cultivation in LSMMG compared to the control conditions [11] and *Cupriavidus metallidurans* proteome was highly affected only by RPM cultivation [30]. Therefore, one should be cautious to conclude which of the two simulators induced the higher response when cultivating bacteria.

The use of magnetic levitation has also been introduced to balance the force of gravity on a levitating object [10, 50]. However, a major constrain in using diamagnetic levitation is the requirement for large magnetic fields gradients at the levitation point that may influence biological systems. In addition, oxygen dissolved in the liquid culture medium is similarly attracted by the magnetic field [2]. Since oxygen in the liquid is consumed by the bacteria and replaced at the liquid–air interface (from the oxygen in the air above the liquid), an oxygen concentration results, producing a corresponding gradient in the magnetic force density that can cause convection in the liquid medium. Therefore, for diamagnetic levitation to be a useful model of space-related microgravity, where density-driven convective transport is absent, paramagnetically driven convection of oxygen should be prevented. This could be achieved by performing experiment in anaerobic conditions or in nonliquid culture [15]. Beuls et al. [8] compared 3 microgravity simulators, LSMMG, RPM, and diamagnetic levitation, and found no differences in the capacity of *Bacillus thuringiensis* to perform plasmid transfer compared to the control condition. In this case of Gram-positive bacterium, this ability to exchange plasmids in microgravity, as efficiently as occurring on Earth, could be seen as highly relevant in the frame of potentially increasing antibiotic resistances and bacterial virulence in space [8].

## 22.3 Space Flight Experiments and Related Ground Simulations

In general, one could conclude that space flight has been shown not to hinder bacterial growth, on the contrary, it can enhance the growth of planktonic bacterial cultures, possibly through its influences on fluid dynamics [28].

Biofilm formation can also occur in microgravity [33, 42], an issue that, for example, must be addressed during the design of air and water recycling systems for long-term space flights [40].

Various experiments suggested that antibiotics are less efficient in space flight conditions [29, 48]. Bacteria exposed to space flight stresses may become more resistant to antibiotics over a short, introductory period, while losing most but not all of that resistance over the long term [29]. For the future, this possibly changing response of bacteria to antibiotics in space flight may imply that disinfection may be problematic. In addition, it may be difficult to treat an illness or injury with antibiotics on short-term missions, due to the tendency of bacteria to resist them. On long-term missions, such as periods spent on space stations or trips to other planets, it may be difficult to predict the response of bacteria to a certain antibiotic. While most bacteria seem to become more susceptible to antibiotics after long-term space flight exposure [29], a few may retain resistance, leading to potential hazard for the all crew.

Recently, Wilson et al. [54] provided the first direct evidence that growth during space flight can alter the virulence of a pathogen; in this study, *Salmonella enterica* serovar Typhimurium grown in space flight displayed increased virulence in a murine infection model compared with identical ground controls. Importantly, these results correlate with previous findings in which the same strain displayed increased virulence in the murine model after growth in the low-shear microgravity-like conditions of the RWV bioreactor [37, 55]. In agreement with the increased virulence observed for the space flight samples, bacteria cultured in flight exhibited cellular aggregation and extracellular matrix formation consistent with biofilm production. Moreover, several *Salmonella* genes associated with biofilm formation changed expression in flight [54].

Very few attempts were made to mimic space-ionizing radiation on ground and compare it to actual space flight experiment involving microorganisms. Rea et al. [43] studied the effect of ionizing radiation on photosynthetic organisms including the cyanobacterium *Arthrospira platensis* that appeared to maintain the highest photosynthetic efficiency in flight experiments. The authors concluded that in space, the effect of ionizing radiation is enhanced compared to that observed in ground facilities with a single beam of radiation. Our group had a more complete approach trying to match the actual dosimetry measurements inside the ISS meaning about 2 mGy over 10 days [18] at the time of our space experiment involving *R. rubrum* on agar plates and combining it with ground simulation of microgravity using the RPM [32].

We could put forward the importance of medium composition and culture setup on the response of the bacterium to space flight-related environmental conditions but low overlap was obtained for both the microgravity simulation and the ionizing radiation experiments compared to the space flight experiments.

One must be aware that space experiments are always subject to the inconveniences of access to space. Space biology researchers face many limitations; include sample preparation long before the flight with prolonged storage, a strictly limited number of samples and repetitions, strong acceleration during take-off, and a second storage period before recovery and analysis of the samples. In addition, during space flight cells are exposed to many more changing factors than just the reduced gravity (e.g., increased gravity/acceleration during launch and landing, increased radiation doses, different electromagnetic fields, pressures changes, enclosed environment) and ionizing radiation. These constraints always impose a certain degree of caution when drawing conclusions on the effects of space on cells and organisms [45]. It could be therefore difficult to detect the subtle effects caused by the low dose of space radiation inside the ISS while drastic effects on liquid samples due to change in gravity conditions could be easily put forward. As a consequence, these studies did indicate that the effects observed in space flight experiments are partially (potentially even largely) due to the low-shear environment typical of the space environment.

Contrary to open environments, confinement conditions can influence the prevalence, ecology and diversity of the microbial communities via unusual conditions of atmospheric humidity, water condensation, or accumulation of biological residues [51]. Confined habitats such as Antarctic Concordia Station are used as a model environment for long-duration space flights to study human adaptation to isolated and confined extreme environmental situations as they allow to map and monitor the dynamics of airborne bacteria over a certain period of time. In a recent study, Shiwon et al. [44] detected resistances of up to five antibiotics in several *staphylococcal* and *enterococcal* strains from ISS and Concordia. On the other hand, Timmery et al. [47] identified putative pathogens able to perform horizontal gene transfer and potentially able to acquire new DNA and sharing genetic material in Concordia. Because most of the microorganisms originate from the crew, continuous evaluation of the bacterial ecological status in such confined environment was highly recommended [47].

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