

## Research Campaign: Space Environmental Effects on Microbial Growth and Survival

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Abstract: As humanity moves from earth to the moon and beyond, there are many challenges, both known and unknown, which will complicate our path. One unknown variable is how microorganisms will be affected by the space environment, and how this may impact mission success. There is a serious need for greater research to be conducted on how space environments alter microbial organisms genetically and physiologically, and to understand the threats that potential microbial changes may present to future missions.

The international space station (ISS) has been a workhorse for scientific research conducted off world for over fifteen years. While the value of the data and knowledge gained from the ISS cannot be overstated, the environment of the ISS does not provide an accurate proxy for what life will encounter on extraterrestrial surfaces like the moon or Mars. For instance, the ISS operates in low earth orbit (LEO) which, while still delivering significant radiation exposure, is protected from much of the harmful radiation that exists in space. The moon and Mars have no such protection. Indeed, radiation is the single greatest challenge for moving life off earth. Furthermore, the microgravity experienced on the ISS is far less than the  $\sim 1/6$  and  $\sim 1/3$  gravity found on the moon and Mars, respectively. Not only are these significant challenges to expanding and sustaining human life at these locations, they are also almost wholly unexplored. While we know and can predict some of the impacts that such environments will have on human life, we can predict very little about how these factors would affect the microbes that would inevitably go with us.

Microbes have existed for millions of years adapting to the environments around them. They are experts in surviving, and we should expect nothing different from the microbes that journey into space with us. Indeed, where we go, microbes will follow. This means that they will be on, in, and around humans, being deposited on surfaces and floating in the air. Microbes will also be associated with food grown on the moon. Aside from general cleaning to remove microbes from surfaces, filtration to remove them from air, and hygiene to maintain cleanliness of humans and food, there is an added consideration that microbes may adapt to the space environment in unknown ways. This includes becoming resistant to cleaning methods, increasing virulence, losing viability, or mutating. These changes, while seemingly small, could have catastrophic impacts on crew health, crew survival, equipment function, food quality, or other unknown effects.

While there have been a number of studies performed on microbes in the context of the ISS, for the reasons listed above, the environment of the ISS is not adequate to predict the behavior of microbes in lunar and Martian environments. While there is no replacement for conducting experiments on the moon or Mars itself, more research is necessary to recapitulate certain aspects of extraterrestrial environments here on Earth and aboard the ISS or satellites to examine their effects on microbes individually as well as in combination. While these experiments may be costly, they are critical to understanding a major risk that could impact mission success on the moon.

Microbial research conducted aboard the ISS to understand microbial changes in space has provided varying results, indicating both that microbes may become more virulent or less virulent as a result of experiencing the space environment (1 – 4). However, these are short duration studies that, while fundamental in the field, do not provide a long-term view of how microbes may change as a result of living in space, nor are they expansive in their breadth. Additional studies need to be done in order to evaluate how microbes change over many generations aboard the ISS. This would not be a trivial undertaking as it would be in many ways related to the long-term evolution experiment (LTEE) by

Professor Richard Lenski (5). Importantly, it would provide a critical foundation for understanding microbial adaptation in a spacelike environment.

However, more must be done aside from long duration experiments aboard the ISS or automated experiments on satellites or new stations in LEO (e.g. Biosentinel, Space Tango). There is also a need for ground-based experiments where microbes are grown under high radiation for extended periods, perhaps in a bioreactor where the survival and nutrient sources can be prolonged. This would require experts from radiation physics, planetary science, and microbiology to design and conduct experiments using methods that most closely resembles the radiation that would be experienced on the moon or Mars. It would also require multiple different microbes (i.e. pathogens; skin-, gut-, plant-, and surface-associated microbes), and multiple sources of radiation (i.e. particle, UV) with and without relevant shielding. Following such long duration experiments, or exposure to certain space environments, whole or partial genomes will need to be sequenced to determine what mutations have occurred and in what genes.

Furthermore, mutated microbes would then need to be assessed for changes in physiology or behavior. Pathogenic or mammalian-associated microbes would need to be analyzed for increases in virulence, particularly in light of any immune changes or susceptibilities induced in the human host as a result of spaceflight. Plant-associated microbes would need to be grown with plants to determine if their beneficial effects on plant growth are compromised. And microbes associated with surfaces would need to be studied to determine if they may become less resistant to the methods of cleaning proposed on these missions.

In addition to ground-based *in vitro* studies, and those conducted on the ISS or satellites, studies must be done aboard missions to the moon. Such experiments should include monitoring and sampling to collect specimens which can be analyzed for mutation and change, both from astronauts, plants and food, and habitable environments. Studies on the moon or the Lunar Gateway should also include long term *in vitro* evolutionary experiments with a select group of microbes. Finally, samples should be analyzed in real time, but also return missions should be planned to bring samples to Earth for in depth analysis. Including such experiments at the start of these lunar missions is essential for developing a baseline from which we can compare future experiments, particularly in the event of losses due to microbial factors.

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