

**Topical: A Vision for the Next Generation of Spaceflight Microbiology:
Human Health and Habitat Sustainability**

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Introduction

Microorganisms are critical to maintain the balance between normal homeostasis and dysfunction for the health of astronauts and sustainability of their space habitat. Accordingly, understanding microbial responses that could negatively or positively impact spaceflight operations, onboard life support systems, and crew health and performance is critical for the success of future space exploration missions. In response to the spaceflight and spaceflight analogue environments, microorganisms unexpectedly alter their physiology, gene expression, metabolism, growth kinetics, stress responses, biofilm formation, materials degradation, antibiotic resistance, host-pathogen and host-commensal interactions, microbiome diversity, and virulence in key ways that are *not observed during conventional terrestrial culture conditions*¹⁻³⁰. While these unexpected microbial responses represent potential risks to the crew and their habitat, they also provide beneficial opportunities to enable or enhance spaceflight exploration.

Microorganisms are remarkable in their dynamic adaptive plasticity in response to both short- and long-term environmental changes. The extreme conditions of spaceflight represent no exception to this rule. One of the unique aspects of the spaceflight environment is reduced/fractional gravity and the corresponding secondary effects (*e.g.*, low fluid shear forces, decreased mass transfer). While we continue to learn about dynamic microbial responses to the microgravity environment in Earth's orbit, information on the impact of fractional gravity environments such as lunar and Mars gravity is limited. In addition, negative health effects from other environmental stimuli encountered in space, including exposure to radiation, celestial dusts, and different atmospheric compositions and pressures, may synergistically “stack” to contribute to a higher risk to crew health and environmental sustainability. This consideration reinforces the need to encourage more integrative research between scientific disciplines, including microbiology and radiobiology, to guide future research and development of countermeasures.

Understanding microbial responses to extreme environments has been a cornerstone of microbiological research, such as studying responses to acid and thermal stressors. This has led to advanced mechanistic understanding of biological systems and translational breakthroughs in human health and quality of life. The extreme environment associated with spaceflight platforms provides unique opportunities to study microbial adaptation in low gravity to investigate the impact of various forces on living systems that are often obscured on Earth by the presence of gravity - and to understand how these forces regulate microbial structural and functional processes. Predictably, as with other physical forces, the mechanical unloading experienced by cells in reduced gravity can reveal novel mechanotransduction mechanisms that alter microbial molecular genetic and phenotypic responses, which may influence adaptation to this unique environment. Indeed, the discovery that biomechanical forces (*e.g.*, fluid shear) relevant to those encountered by microorganisms in both spaceflight and *in vivo* during the natural course of infection could regulate microbial virulence was first identified using microgravity analogues⁹ and subsequently validated in separate spaceflight experiments^{9,17}. Moreover, understanding spaceflight-induced microbial responses can be relevant to higher eukaryotic cell types, including mammalian cells, since many human genes have bacterial origins, and several principles of gene network regulation are common to both prokaryotes and eukaryotes.

While previous spaceflight and spaceflight analogue experiments have indicated possible stimuli for unexpected microbial responses observed during growth in these environments, few underlying mechanisms have been identified. Understanding *mechanisms* is critical to predict how microorganisms will respond to the unique environment of spaceflight.

Current Microbiological Operations and Mitigation Approaches

Since many aspects of microbial risks during spaceflight remain poorly characterized, stringent microbiologically-related crew health protocols have been enforced to mitigate the risks of infectious disease and environmental contamination during spaceflight missions, including preflight crew quarantine through the Crew Health Stabilization Program, microbial monitoring of spacecraft, its cargo and food, and biosafety assessments of biological payloads and hardware³¹. Even with these precautions, infectious disease and environmental contamination events (e.g., biofouling and biofilms) still occur^{32,33}. As spaceflight exploration becomes more frequent and commercial vehicles are routinely integrated into future mission scenarios, increased investment in microbiological research will be essential to better characterize spaceflight-associated risks and leverage beneficial aspects of microorganisms for health and habitat sustainability to successfully transition humans to deep space.

Evidence of Health Effects and Corresponding Gaps in Knowledge

As human exploration of space extends toward the Moon and beyond, an improved understanding of the risk due to altered microbial characteristics becomes critical to ensure crew health, safety, and performance. For over 60 years, microbiological research from spaceflight and spaceflight analogue experiments has demonstrated unexpected microbial responses to these unique environments, many of which directly relate to astronaut health and their medical care^{15,34}. For example, previous spaceflight experiments have identified an increase in antibiotic resistance for some bacteria, including *Escherichia coli* and *Staphylococcus aureus*, in response to spaceflight culture¹⁵. Subsequent spaceflight studies confirmed that antibiotic resistance is also increased in other bacteria cultured in microgravity; however, this is not a consistent response, as some species showed either decreased or no change in resistance to antibiotics as compared to ground-based controls. The implications of changes in antibiotic resistance in microbial pathogens during spaceflight was reinforced by independent spaceflight experiments investigating alterations in the virulence and global gene expression of the enteric pathogen *Salmonella enterica* serovar Typhimurium^{17,18}, a leading cause of foodborne illness. *Salmonella* species have been recovered from the Space Shuttle³⁵, the International Space Station (ISS)³⁶, and in spaceflight food destined for the ISS²⁶, and thus are relevant model organisms to understand potential risks to crew health. These studies confirmed that spaceflight-cultured *Salmonella* exhibited increased virulence in a mouse infection model compared to control cultures grown on Earth^{17,18}. Moreover, transcriptomic and proteomic profiling revealed that key genes known to be important for *Salmonella* virulence were not regulated as expected when this organism is grown under conventional terrestrial conditions, suggesting novel mechanisms for the observed spaceflight-associated virulence phenotype^{17,18}. In addition, spaceflight-induced increases in *Salmonella* virulence were shown to be regulated by media ion/salt concentration (especially phosphate) and that modulation of these salt concentrations could be used to turn off the increased virulence. Furthermore, *Salmonella* biofilms were uniquely formed in spaceflight conditions and not in ground controls. The evolutionarily conserved RNA chaperone protein, Hfq, was identified as a global regulator of the *S. Typhimurium* response to spaceflight culture¹⁷. Subsequent studies showed that *Pseudomonas aeruginosa* also used Hfq to globally regulate its gene expression in response to spaceflight culture, identifying the first spaceflight-induced regulator acting across bacterial species^{21,37}. This shared regulation may indicate that mechanical stimuli, like low fluid shear forces experienced by microbial pathogens in both the quiescent microgravity environment of spaceflight and on Earth during their natural life cycles, including

in the infected host¹⁵, may pre-adapt bacteria to be “hardwired” to respond to the microgravity environment. Recently, a second study showed that another bacterial pathogen, *Serratia marcescens*, also exhibited increased virulence during spaceflight culture²⁷. Taken together, these findings indicate the need for additional studies to evaluate spaceflight-induced pathogenesis and virulence changes in other pathogens alone or in the context of mixed microbial co-cultures to improve our understanding of the impact of the spaceflight environment on crew health risk.

Several genomic studies have also reported alterations in crew microbiome diversity and composition throughout spaceflight missions^{38,39}, which could potentially impact a wide range of human physiological conditions, including those associated with immune function, nutrition and behavior as they relate to the gut-brain axis⁴⁰. These insights not only provide a better understanding of the multi-system physiological interactions during spaceflight, but may help lead to countermeasures that address multiple areas of astronaut health and performance synergistically. An interesting question regarding microbiome function in reduced gravity environments is whether microbial homeostasis and ratios of different species would differ from those in terrestrial conditions or whether space-induced alterations in nutrition and/or systemic metabolism would shift microbial dynamics needed to maintain the balance between dysbiosis and homeostasis for crew and habitat health. These findings and considerations also represent an opportunity to address risk mitigation approaches and countermeasures to benefit crew health, including probiotic/prebiotic biotechnologies⁴¹.

Combining functional phenotypic studies with multi-omics approaches is critical to understand how microbial characteristics are altered in response to the environment of spaceflight in ways that differ from those observed on Earth.

Collectively, our limited knowledge of unexpected spaceflight-induced changes in microbial phenotypes represents a critical knowledge gap to the successful transition from short-to-long-duration human spaceflight. This concern is further exacerbated by reports that the human immune system is dysregulated during spaceflight^{26,42,43}. This dysregulation includes alterations in the number and function of immune cell types, such as reductions in T and Natural Killer cell function, altered plasma cytokine profiles, as well as alterations in stress hormones⁴², which may explain the reactivation of latent herpesviruses in many crew members during space missions^{44,45}. Moreover, there is an urgent need to understand the effect of physical and biological causative factors and their interconnections in microbially-induced risks during spaceflight.

Evidence of Effects on Habitat Sustainability and Corresponding Gaps in Knowledge

Just as it is important to understand the interactions between microorganisms and humans during spaceflight missions, it is equally important to investigate the interaction between microbes and their environmental habitats, especially in complex systems in which water and air are recycled. Long duration habitation in the closed, self-contained environment of spacecraft that use regenerative life support systems not only increases human and plant exposure to potential pathogens, but also creates risks to the vehicle systems, including biofouling and biocorrosion, and the habitat itself, due to material degradation. For example, previous spacecraft system failures have identified the need to successfully control microbial growth in Environmental Control and Life Support System (ECLSS) water processing lines during the recycling of wastewater to potable water, where microbially-induced biofilm formation could have profound implications for human habitation in space⁴⁶. Indeed, microbial growth and biofilms have posed

a challenge for several spacecraft, including the water system on the ISS⁴⁷. This type of microbial contamination could be catastrophic for the ISS, as the water system is used for multiple purposes, including potable drinking water, crew hygiene, and irrigation of plants grown for consumption during spaceflight. Accordingly, understanding microbial responses during spaceflight has critical implications for vehicle and life support systems design, materials selection, and performance.

Critical to both human health and habitat sustainability has been research into biofilm formation and associated phenotypic characteristics when microbial communities are grown in decreased gravity. For example, *P. aeruginosa* cultured in spaceflight exhibited a unique “column and canopy” biofilm architecture²³. This formation of novel biofilm architecture provided a new perspective on microbial biofilms and prompted a series of recent spaceflight studies into how polymicrobial species form biofilms on different materials, induce corrosion in vehicle components, and alter resistance to disinfectants for biofilm control in space habitats. Recent studies have also characterized bacterial isolates recovered from the ISS potable water system to understand mixed and single species biofilm formation, composition and stability, as well as metabolism and antibiotic resistance^{28,29,48}. More detailed analysis of the impact of spaceflight on the kinetics, composition and architecture of biofilms is needed.

Over the past two decades, environmental monitoring of ISS air and surfaces demonstrated that the microbiome is similar to that of a terrestrial home, whether culture-based or molecular-based methods were used for analysis⁴⁹⁻⁵¹. While not an immediate concern, the environmental microbiota still contains opportunistic pathogens and biofilm forming organisms that, if left uncontrolled, could negatively impact the vehicle and its systems as well as astronaut health.

Need for advances in spaceflight and spaceflight analogue biological research hardware

As human spaceflight missions travel farther from Earth for longer durations, biological research will increasingly rely on the development of fully integrated, modular, automated spaceflight hardware with a broad range of capabilities. To enable the delivery of dependable scientific information that can be translated for use by spaceflight operations, this hardware must have analytical precision and accuracy equivalent to research quality instruments in terrestrial labs. These criteria will be difficult to meet, as spaceflight resources for biological research (*e.g.*, mass, volume, power, crew time, and funding) are currently still extraordinarily limited. In addition, many of the science requirements of the investigators (*e.g.*, precise temperature and other environmental control, biocompatibility of materials, homogeneous mixing, accurate and reproducible transfer of liquids, long duration performance, safety containment, modularity, and proper controls) are often not met with existing spaceflight biological hardware.

While current spaceflight biological hardware can be used for multiple experiments, this hardware is often a “custom build” or significantly redesigned for each investigator. Moreover, engineers designing the flight hardware often do not seek input from biologists during the actual hardware development process. As a result, lessons learned to optimize and implement more efficient hardware with greater flexibility and standardization are often lost. For example, simple tasks such as accurate and reliable transfer and mixing of known volumes of liquids, which are critical for a wide range of microbiological experiments, remain a major challenge for most current spaceflight biological hardware. Overall, the current approach to the development and implementation of spaceflight biological hardware often creates issues with experimental quality and causes significant time overruns and scheduling delays. The development of such high-

fidelity hardware - that is built with the *simultaneous input* from both engineers and biologists – and could be repeatedly used by multiple investigation teams with minimal-to-no modification is a critical need that should be prioritized over the next decade to advance microbiological spaceflight research.

Spaceflight microbiological research has also greatly benefitted from the use of spaceflight analogue bioreactors, such as the Rotating Wall Vessel, that reproduce many of the environmental conditions (*e.g.*, low fluid shear) that microbes experience during spaceflight^{15,52}. The contribution of these spaceflight analogue systems would be meaningfully enhanced if a new generation of bioreactors were developed that incorporated the effects of the fractional gravity of the Moon and Mars on the fluid dynamics in the vessels. Findings from these advanced ground-based analogues could then be verified on true spaceflight missions. Knowledge from such biotechnological advancements would also enable better assessments to improve our understanding of how microbial risk from multifactorial exposure to radiation, celestial dust, and reduced gravity forces may combine to create larger threats to crew health, habitat sustainability and mission success.

Conclusion

During past spaceflight missions, microorganisms have caused life-threatening illness in crew members^{26,53} as well as failure of life support systems and other essential spacecraft operations⁴⁶. A key objective for future human exploration missions is to understand and control the impact of the spaceflight environment on interactions between microbes, their hosts, and their habitat. This knowledge will advance our understanding of microbial responses to benefit human exploration missions through a myriad of possible biotechnological breakthroughs, including the design of synthetic biology and metabolic engineering approaches that enable the biosynthesis of diverse molecular compounds (*e.g.*, on-demand pharmaceuticals), food production and nutrient availability (*e.g.*, edible plants, pre-/probiotics, gut-brain strategies to maintain health), new methods for waste recovery, sustaining homeostasis of human, plant and environmental microbiomes, *in situ* resource utilization (*e.g.*, biomining, oxygen generation, carbon dioxide recovery), and planetary protection.

New opportunities will also arise in commercial space flights of various durations and destinations, which could allow for more rapid deployment of pilot experiments and testing of new flight hardware. Immediate responses to short durations of altered gravity (seconds to minutes) can be tested on suborbital flights, while orbital flights would provide several days to months or longer in space.

Moving forward, it is critical to learn from past errors and successes to determine what will and will not work in future microbiological space missions. To fully understand microbial responses to the spaceflight environment and translate those findings to mitigate risks and benefit human spaceflight exploration, it is essential that proper resources be dedicated to this effort. *This includes prioritizing consistent and appropriate funding to support cutting edge research and development of technologically advanced spaceflight and spaceflight analogue hardware.* Future microbiological research must also be hypothesis-driven, emphasize causality, have impeccable experimental design with proper controls, and maintain the same criteria for scientific evidence as terrestrial science. Only through this level of scientific scrutiny can spaceflight microbiological data translate into practical products and scientific breakthroughs that can enable human spaceflight exploration and improve our knowledge of microbiology on Earth.

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