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. 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]. Although 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 habitat sustainability. This consideration reinforces the need to encourage more integrative research between microbiology and other scientific disciplines, including radiobiology, geology, medicine, physics, and engineering, to guide future research and development of countermeasures.

Understanding microbial responses to extreme environments has been a cornerstone of microbiological research, such as 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. The discovery that biomechanical forces (*e.g.*, fluid shear) encountered by microorganisms in both spaceflight and *in vivo* during the infection process could regulate microbial virulence was first identified using microgravity analogues [9] and subsequently validated in separate spaceflight experiments [17,18]. Understanding spaceflight-induced microbial responses can be relevant to higher eukaryotic organisms, including mammalian cells, since many human genes have bacterial origins, and many principles of gene network regulation are common to both prokaryotes and eukaryotes.

While spaceflight and spaceflight analogue studies have indicated possible stimuli for unexpected microbial responses in these environments, few underlying mechanisms have been identified. Understanding *mechanisms* is critical to predict how microbes will respond to the unique environments of current and future space exploration.

Current Microbiological Operations and Mitigation Approaches during Spaceflight

Since many aspects of microbial risks from bacteria, fungi, and viruses 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, routine housekeeping, and biosafety assessments of biological payloads and hardware [31]. 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 and astronauts 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.

Health Effects and Knowledge Gaps

As human exploration of space extends toward the Moon and beyond, 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]. Previous spaceflight experiments have identified an increase in antibiotic resistance for some bacteria, including *Escherichia coli* and *Staphylococcus aureus*, in response to spaceflight culture [15]. Subsequent spaceflight studies confirmed that antibiotic resistance is also increased in other species of bacteria cultured in microgravity. However, this is not a consistent response, as some species show either decreased or no change in resistance to antibiotics compared to ground-based controls. The implications of changes in antibiotic resistance in microbial pathogens during spaceflight were 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 Shuttle [35], the International Space Station (ISS) [36], and in spaceflight food destined for the ISS [26], 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 culture [17]. 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 host [15], may pre-adapt bacteria to be "hardwired" to respond to the microgravity environment. Recently, a second bacterial pathogen, *Serratia marcescens,* was also shown to exhibit increased virulence during spaceflight culture [27]. 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 axis [40]. 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 biotechnologies [41].

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 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 hormones [42], which may explain the reactivation of latent herpesviruses in many crew members during space missions [44,45]. While understanding the effect of physical and biological causative factors and their interconnections in microbially-induced risks is challenging, advanced data analysis approaches, such as machine learning, should be encouraged to integrate data and generate predictive models to benefit risk assessment during spaceflight.

Habitat Sustainability and Knowledge Gaps

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 spacecraft 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 the 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 space [46]. Microbial growth and biofilms have posed a challenge for several spacecraft, including the water system on the ISS [47]. This type of microbial contamination could be catastrophic, 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 unique "column and canopy" biofilm architecture [23]. This 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 using culture-based or molecular-based methods for analysis [49-51]. Although not an immediate concern, the environmental microbiota still contains opportunistic pathogens and biofilm forming organisms that, if left uncontrolled, could negatively impact the vehicle, its systems, and 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 fulfilled by 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 high-fidelity hardware built with the *simultaneous input* from both engineers and biologists and which could be used repeatedly by multiple investigators 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 enhanced by a new generation of standardized bioreactors that incorporate 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 operations [46]. 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. The goal is to objectively understand and characterize a "new" spaceflight normal that reflects biological adaptation in this alternate environment. This knowledge will require integrated, multidisciplinary studies to 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 to enable human spaceflight exploration and improve our knowledge of microbiology on Earth.

References

1 Hiebel, T. L. & Volz, P. A. Foreign body reactions induced by fungi irradiated in space. *Phytologia* **35**, 365-372 (1977).

2 Tixador, R. *et al.* Study of minimal inhibitory concentration of antibiotics on bacteria cultivated in vitro in space (Cytos 2 experiment). *Aviat Space Environ Med* **56**, 748-751 (1985).

3 Lapchine, L. *et al.* Antibiotic activity in space. *Drugs Exp Clin Res* **12**, 933-938 (1986).

4 Kacena, M. A. *et al.* Bacterial growth in space flight: logistic growth curve parameters for *Escherichia coli* and *Bacillus subtilis*. *Appl Microbiol Biotechnol* **51**, 229-234 (1999).https://doi.org/https://doi.org/10.1007/s002530051386.

5 Fang, A., Pierson, D. L., Mishra, S. K., Koenig, D. W. & Demain, A. L. Secondary metabolism in simulated microgravity: beta-lactam production by *Streptomyces clavuligerus*. *Journal of industrial microbiology & biotechnology* **18**, 22-25 (1997).https://doi.org/https://doi.org/10.1038/sj.jim.2900345.

6 Fang, A., Pierson, D. L., Koenig, D. W., Mishra, S. K. & Demain, A. L. Effect of simulated microgravity and shear stress on microcin B17 production by *Escherichia coli* and on its excretion into the medium. *Appl Environ Microbiol* **63**, 4090-4092 (1997).https://doi.org/https://doi.org/10.1128/aem.63.10.4090-4092.1997.

7 Fang, A., Pierson, D. L., Mishra, S. K., Koenig, D. W. & Demain, A. L. Gramicidin S production by *Bacillus brevis* in simulated microgravity. *Curr Microbiol* **34**, 199-204 (1997).https://doi.org/https://doi.org/10.1007/s002849900168.

8 Klaus, D., Simske, S., Todd, P. & Stodiek, L. Investigation of space flight effects on *Escherichia coli* and a proposed model of underlying physical mechanisms. *Microbiology* **143**, 449-455 (1997).

9 Nickerson, C. A. *et al.* Microgravity as a novel environmental signal affecting Salmonella enterica serovar Typhimurium virulence. *Infect Immun* **68**, 3147-3152 (2000).https://doi.org/https://doi.org/10.1128/iai.68.6.3147-3152.2000.

10 McLean, R. J., Cassanto, J. M., Barnes, M. B. & Koo, J. H. Bacterial biofilm formation under microgravity conditions. *FEMS Microbiol Lett* **195**, 115-119 (2001).https://doi.org/https://doi.org/10.1111/j.1574-6968.2001.tb10507.x.

11 Wilson, J. W. *et al.* Low-Shear modeled microgravity alters the Salmonella enterica serovar typhimurium stress response in an RpoS-independent manner. *Appl Environ Microbiol* **68**, 5408-5416 (2002).https://doi.org/https://doi.org/10.1128/aem.68.11.5408-5416.2002.

12 Wilson, J. W. *et al.* Microarray analysis identifies Salmonella genes belonging to the low-shear modeled microgravity regulon. *Proc Natl Acad Sci U S A* **99**, 13807-13812 (2002).https://doi.org/https://doi.org/10.1073/pnas.212387899.

13 Lynch, S. V., Brodie, E. L. & Matin, A. Role and regulation of sigma S in general resistance conferred by low-shear simulated microgravity in *Escherichia coli*. *J Bacteriol* **186**, 8207-8212 (2004).https://doi.org/https://doi.org/10.1128/jb.186.24.8207-8212.2004.

14 Lynch, S. V., Mukundakrishnan, K., Benoit, M. R., Ayyaswamy, P. S. & Matin, A. *Escherichia coli* biofilms formed under low-shear modeled microgravity in a ground-based system. *Appl Environ Microbiol* **72**, 7701-7710 (2006).https://doi.org/AEM.01294-06 [pii]10.1128/AEM.01294-06.

15 Nickerson, C., Ott, C. M., Wilson, J. W. & Pierson, D. L. Microbial responses to microgravity and other low shear environments *Microbiology and Molecular Biology*

Reviews **68**, 345-361 (2004).https://doi.org/https://doi.org/10.1128/mmbr.68.2.345-361.2004.
16 Nauman, E. A. *et al.* Novel quantitative biosystem for modeling physiological fluid shear stress on cells. *Appl Environ Microbiol* **73**, 699-705 (2007).https://doi.org/https://doi.org/10.1128/aem.02428-06.
17 Wilson, J. W. *et al.* Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. *Proc Natl Acad Sci U S A* **104**, 16299-16304 (2007).https://doi.org/https://doi.org/10.1073/pnas.0707155104.
18 Wilson, J. W. *et al.* Media ion composition controls regulatory and virulence response of *Salmonella* in spaceflight. *PLoS One* **3**, e3923 (2008).https://doi.org/https://doi.org/10.1371/journal.pone.0003923.
19 Allen, C. A., Niesel, D. W. & Torres, A. G. The effects of low-shear stress on Adherent-invasive Escherichia coli. *Environ Microbiol* **10**, 1512-1525 (2008).https://doi.org/10.1111/j.1462-2920.2008.01567.xEMI1567 [pii].
20 Crabbe, A. *et al.* Use of the rotating wall vessel technology to study the effect of shear stress on growth behaviour of *Pseudomonas aeruginosa* PA01. *Environ Microbiol* **10**, 2098-2110 (2008).https://doi.org/https://doi.org/10.1111/j.1462-2920.2008.01631.x.
21 Crabbé, A. *et al.* Transcriptional and proteomic response of Pseudomonas aeruginosa PAO1 to spaceflight conditions involves Hfq regulation and reveals a role for oxygen. *Appl Environ Microbiol* **77**, 1221-1230 (2011).https://doi.org/AEM.01582-10 [pii]10.1128/AEM.01582-10.
22 Crabbé, A. *et al.* Spaceflight Enhances Cell Aggregation and Random Budding in *Candida albicans*. *PLoS ONE* **8**, e80677 (2013).https://doi.org/10.1371/journal.pone.0080677PONE-D-13-21954 [pii].
23 Kim, W. *et al.* Spaceflight promotes biofilm formation by *Pseudomonas aeruginosa*. *PLoS ONE* **8**, e62437 (2013).https://doi.org/https://doi.org/10.1371/journal.pone.0062437.
24 Foster, J. S., Khodadad, C. L., Ahrendt, S. R. & Parrish, M. L. Impact of simulated microgravity on the normal developmental time line of an animal-bacteria symbiosis. *Sci Rep* **3**, 1340 (2013).https://doi.org/10.1038/srep01340srep01340 [pii].
25 Mastroleo, F. *et al.* Modelled microgravity cultivation modulates N-acylhomoserine lactone production in Rhodospirillum rubrum S1H independently of cell density. *Microbiology* **159**, 2456-2466 (2013).https://doi.org/10.1099/mic.0.066415-0mic.0.066415-0 [pii].
26 Nickerson, C. A., Pellis, N. R. & Ott, C. M. in *Effect of Spaceflight and Spaceflight Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms* 301 (Springer, New York, NY, 2016).
27 Gilbert, R. *et al.* Spaceflight and simulated microgravity conditions increase virulence of Serratia marcescens in the Drosophila melanogaster infection model. *npj Microgravity* **6**, 4 (2020).https://doi.org/10.1038/s41526-019-0091-291 [pii].
28 Thompson, A. F. *et al.* Characterizing species interactions that contribute to biofilm formation in a multispecies model of a potable water bacterial community. *Microbiology (Reading)* **166**, 34-43 (2020).https://doi.org/10.1099/mic.0.000849.
29 Yang, J. *et al.* Longitudinal characterization of multispecies microbial populations recovered from spaceflight potable water. *NPJ Biofilms Microbiomes* **7**, 70 (2021).https://doi.org/10.1038/s41522-021-00240-5.

30 Barrila, J. *et al.* Evaluating the effect of spaceflight on the host-pathogen interaction between human intestinal epithelial cells and Salmonella Typhimurium. *NPJ Microgravity* **7**, 9 (2021).https://doi.org/10.1038/s41526-021-00136-w.
31 Oubre, C. M., Pierson, D. & Ott, C. M. in *Space Physiology and Medicine: From Evidence to Practice* (eds A. E. Nicogossian *et al.*) 155-167 (Springer Nature, New York, 2016).
32 Risin, D. in *Human Health and Performance Risks of Space Exploration Missions* (eds J.C. McPhee & J.B. Charles) (NASA SP-2009-3405, 2009).
33 Pierson, D. L. *et al.* in *Environmental Monitoring: A Comprehensive Handbook* (ed J. Moldenhauer) (DHI Publishing, LLC, 2012).
34 Ott, C. M. *et al.* in *Stress Challenges and Immunity in Space* (ed A. Chouker) 327-356 (Springer, 2020).
35 Kish, A. L. *et al.* Biostability and microbiological analysis of shuttle crew refuse. *International Conference on Environmental Systems* **02ICES-113** (2002).
36 Singh, N. K., Wood, J. M., Karouia, F. & Venkateswaran, K. Succession and persistence of microbial communities and antimicrobial resistance genes associated with International Space Station environmental surfaces. *Microbiome* **6**, 204 (2018).https://doi.org/https://doi.org/10.1186/s40168-018-0585-2.
37 Ott, C. M. *et al.* in *Stress Challenges and Immunity in Space: From Mechanisms to Monitoring and Preventive Strategies* (ed Alexander Choukèr) Ch. Microbial Stress: Spaceflight-Induced Alterations in Microbial Virulence and Infectious Disease Risks for the Crew, 327-355 (Springer International Publishing, 2020).
38 Voorhies, A. A. *et al.* Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. *Sci Rep* **9**, 9911 (2019).https://doi.org/10.1038/s41598-019-46303-8 [pii].
39 Garrett-Bakelman, F. E. *et al.* The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight. *Science* **364**, eaau8650 (2019).
40 Bohorquez, D. V. & Liddle, R. A. The gut connectome: making sense of what you eat. *J Clin Invest* **125**, 888-890 (2015).https://doi.org/10.1172/JCI81121.
41 Turroni, S. *et al.* Gut Microbiome and Space Travelers' Health: State of the Art and Possible Pro/Prebiotic Strategies for Long-Term Space Missions. *Frontiers in Physiology* **11** (2020).https://doi.org/10.3389/fphys.2020.553929.
42 Gueguinou, N. *et al.* Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond Earth's orbit? *J Leukoc Biol* **86**, 1027-1038 (2009).https://doi.org/https://doi.org/10.1189/jlb.0309167.
43 Crucian, B. *et al.* Alterations in adaptive immunity persist during long-duration spaceflight. *npj Microgravity* **1**, 15013 (2015).https://doi.org/10.1038/npjmgrav.2015.13.
44 Pierson, D. L., Mehta, S. K. & Stowe, R. P. in *Psychoneuroimmunology* Vol. II (ed Robert Ader) 851-868 (Academic Press, 2007).
45 Pierson, D. L., Stowe, R. P., Phillips, T. M., Lugg, D. J. & Mehta, S. K. Epstein-Barr virus shedding by astronauts during space flight. *Brain Behav Immun* **19**, 235-242 (2005).https://doi.org/https://doi.org/10.1016/j.bbi.2004.08.001.
46 Yang, J. *et al.* in *Methods in Microbiology: Microbiology of Atypical Environments* Vol. 45 (eds V Gurtler & J. T. Trevors) 3-26 (Academic Press, London, 2018).

47 Diaz, A. M., Li, W., Irwin, T. D., Calle, L. M. & Callahan, M. R. Investigation of biofilm formation and control for spacecraft –An early literature review. *49th International Conference on Environmental Systems* (2019).

48 O'Rourke, A., Lee, M. D., Nierman, W. C., Everroad, R. C. & Dupont, C. L. Genomic and phenotypic characterization of Burkholderia isolates from the potable water system of the International Space Station. *PLoS ONE* **15**, e0227152 (2020).https://doi.org/10.1371/journal.pone.0227152 PONE-D-19-24668 [pii].

49 Pierson, D. *et al.* in *Environmental Monitoring: A Comprehensive Handbook* (ed J. Moldenhauer) pp. 1-27 (DHI Publishing, 2012).

50 Lang, J. M. *et al.* A microbial survey of the International Space Station (ISS). *PeerJ* **5**, e4029 (2017).https://doi.org/10.7717/peerj.4029 [pii].

51 Blaustein, R. A. *et al.* Pangenomic Approach To Understanding Microbial Adaptations within a Model Built Environment, the International Space Station, Relative to Human Hosts and Soil. *mSystems* **4** (2019).https://doi.org/10.1128/mSystems.00281-18.

52 Wolf, D. A. & Kleis, S. J. in *Effect of Spaceflight and Spaceflight Analogue Culture on Human and Microbial Cells: Novel Insight into Disease Mechanisms* (eds C. A. Nickerson, N. R. Pellis, & C. M. Ott) Ch. 2, 39-60 (Springer, 2016).

53 Taylor, G. R. Space microbiology. *Annu Rev Microbiol* **28**, 121-137 (1974).https://doi.org/https://doi.org/10.1146/annurev.mi.28.100174.001005.