

A White Paper Submitted on 23 December 2021
to the
Decadal Survey on Biological & Physical Sciences Research in Space 2023-2032

Campaign:

**Microbial reference missions and open science to accelerate Space
Biology investigations**

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Space Microbiology and Long-duration Exploration

Understanding and leveraging microorganisms is critical to safe and sustainable human space exploration. Host-microbe interactions are central to crew health and space horticulture [1]–[3]. The microbiome of the built environment can affect the function of the spacecraft and can serve as a selective environment that allows for persistence of known human pathogens [4]. Microorganisms can be engineered to produce nutrients and contribute to hybrid life support systems [5], and for *in situ* resource utilization [6] necessary for long duration space travel.

The 2011 Decadal Survey highlighted “(m)odel systems, underlying molecular mechanisms, and the need for rigorous experimental design...(to) develop a mechanistic understanding of the responses of microbes (...) to the spaceflight environment” [7]. This statement is equally valid for this upcoming Decadal Survey. Discovering mechanism and then using that knowledge to create predictive models of biological systems in flight is at the core of all of NASA’s Space Biology endeavors.

A Systems Approach to Space Microbiology

Systems level analysis is a powerful tool for generating mechanistic insights. Systems biology views life as a hierarchical series of interconnected networks. Systems analysis of biological networks requires “(a) to comprehensively gather information from each of these distinct levels for individual biological systems and (b) to integrate these data to generate predictive mathematical models of the system” [8]. The predictive modeling is predicated on observational data.

NASA’s GeneLab collects curates and distributes this observational data [9]. GeneLab Analysis Working Groups (AWGs) are communities of researchers from around the world with an interest and passion for space biology research. This White Paper represents challenges and opportunities identified by the GeneLab Microbe AWG. The primary gap currently faced is the lack of observational datasets in the GeneLab database related to microbial research. Of the 350+ datasets, only ~20% are related microbial specimens. From that subset, 16S amplicon sequencing is the dominant data type. While amplicon sequencing provides taxonomic information, it is difficult to extract mechanisms from this data type due to the functional diversity of microbial genomes even within the same species [10]. A recent uptick in whole-genome sequences, transcriptional profiling, and the inclusion of fungal species are important trends in need of continued momentum.

This leads to the fundamental gap at the heart of this White Paper:

Currently, space microbiology lacks sufficient observational data to create robust, actionable hypotheses that can generate the requisite predictive models.

The value of this data cannot be understated as access to previous data enhances hypothesis generation and meta-analysis of future datasets. For example, as a part of NASA SPOCS¹, members of the NASA GeneLab Microbe AWG designed an experiment to study the possible formation of antibiotic resistance within mixed-species biofilms composed of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. While there are no global transcriptomic sequencing datasets available for the latter, the former does have one previous dataset. This

¹https://www.nasa.gov/mission_pages/station/research/experiments/explorer/Investigation.html?id=8728

automatically increases the possible interpretation value of the future dataset since there is a control for direct comparison. This was previously done for *Bacillus subtilis*, which was flown twice and compared between both flights and ground controls [11]. The existence of reference datasets allows both for the comparison of technical variation and continued probing of a “conserved bacterial space flight” response among species.

To date, the primary mechanism to acquire observational data was through the normal grant solicitation process. An example is the Microbial Tracking series of experiments [12]. These studies, now on their third iteration, began by sampling the surfaces of the International Space Station and cataloging the microbial populations present. Over time, these studies incorporated crew microbiome data, spacecraft assembly facilities, cell viability assessments, and whole genome and metagenome assembly [4], [13], [14]. The migration of observation to mechanism has been of immense benefit to the Agency’s exploration efforts.

However, relying on the Space Biology solicitation process can lead to an incomplete set of reference observations. Over time, the desire for mechanistic understanding and predictive modeling can lead to increased depth at the expense of breadth. If we do not support and fund observational data collection activities, hypotheses will be under-powered and unconvincing in new areas of study, while the existing areas of active research will be over-represented. In a zero-sum, resource-limited scenario, such as the traditional Space Biology funding opportunities, simple observational studies will likely not be sufficiently compelling for selection. Over time, this limits the available knowledge to perform risk management assessments and deliver microbiology-based solutions to challenges associated with long-duration missions. The Space Biology community needs to deliver these solutions on operationally relevant time lines.

Thus, we propose the funding of Microbial Reference Missions (MRM) to accelerate the collection, archiving and accessibility of Space Microbiology datasets to drive hypothesis generation in under-served areas of research necessary to achieve NASA’s exploration objectives.

Reference missions

The concept of a reference mission is to generate a series of data under flight-relevant conditions and provide that data freely to the scientific community. All data will be stored in NASA GeneLab² and freely accessible to researchers around the world. The ISS National Laboratories has sponsored Rodent Research Reference Missions to great success³. The primary benefit is a rapid acceleration of flight feasibility and flight readiness reviews. By standardizing the experimental platform within those with robust flight heritage, as well as coordinated ground analog studies and controls, Space Biology can deliver to the community comprehensive datasets in 1-2 years versus what has, in the past, taken up to a decade.

Overall, the recommendation from the previous Decadal Survey stand:

- a) Capitalize on the technological maturity, low cost, and speed of genomic analyses and the rapid generation time of microbes to monitor the evolution of microbial genomic changes in response to the selective pressures present in the spaceflight environment;

2 <https://genelab.nasa.gov/>

3 <https://www.issnationallab.org/iss360/rethinking-rodent-research-concept-design/>

- b) Study changes in microbial populations from the skin and feces of the astronauts, plant and plant growth media, and environmental samples taken from surfaces and the atmosphere of the ISS; and
- c) Establish an experimental program targeted at understanding the influence of the spaceflight environment on defined microbial populations.

We suggest jump-starting these efforts through a set of MRMs that ensures robust, actionable hypotheses towards the development of predictive models.

Scope

The MRMs in and of themselves may not constitute an entire Research Campaign. Instead, they should be viewed as part of a comprehensive Space Biology campaign to increase the applicability and quality of ground analogs as well as Open Science and computational initiatives. We direct the committee to the following White Papers on these topics: “*A New NASA Flagship Facility: PRECISE – Proton Radiation Environmentally Controlled Investigations for Space Exploration*” (lead author: Sylvain V. Costes, NASA ARC); “*Research Campaign: Open Science for the Next Decade of Life and Physical Sciences Research for Deep Space Exploration*” (lead author: Ryan T. Scott, KBR, NASA Ames).

Structure

The MRMs should be responsive to all aspects of Space Biology’s efforts to Thrive In DEep Space (TIDES). The current state, gaps, and needs of this effort will be identified in the upcoming Decadal Survey. Thus, the MRMs will be an emergent solution to the science strategy derived from the 2023 Decadal Survey. We have compiled a list of four candidate topic areas to serve as a starting point: *Ground analog studies of spaceflight stressors*, *Host-microbe interactions*, *Microbiome of the built environment*, and *Bioengineering*. Reference observational data within these topic areas would accelerate the Space Microbiology community’s ability to design actionable hypotheses towards the development of mechanistic, predictive models. Novel isolates arising from these studies should be deposited in an agency-wide open microbiological culture collection to ensure accessibility and continual scientific insights.

Ground analog studies of spaceflight stressors:

Space is a multi-variate stress environment. The abiotic factors of space radiation and reduced gravity typically dominate the hypotheses and, by extension, the experimental design. However, other factors specific to each microbial niche (host-associated, environmental sample, bioproduction vessels, etc.) also contribute to spaceflight adaptation. Disentangling the relative contribution of the multitude of stressors with flight experiments that are consistently statistically underpowered due to constraints on up-mass and crew time fundamentally limits the extraction of mechanistic insights. Ground analogs can contribute to controlled exploration of space stressors towards uncovering fundamental mechanisms.

Recommended Reference Experiments:

- Subject model microorganisms, defined microbial communities, and host-microbe models to include plants, invertebrates, and scale-down tissue models (e.g. tissue-on-a-chip) subjected to chronic, low-dose ionizing radiation and, if applicable, simulated microgravity.

- Combine multiple model organisms into a unified reference experiment to provide new insights into potentially conserved changes (clino-rotations, hypergravity, hypoxia, ionizing radiation, elevated CO₂).
- Have a mock bacterial community which could be repeatedly cultured (with replication) on space flight missions, lunar gateway, ISS and ground controls to create a baseline microbiome from which to validate all space environments, experimental procedures and confounders, and help support data comparison across environments.
- Validate which variables are accurately recapitulated with ground analogs.

Understanding spaceflight-induced changes to community composition and function first require a mechanistic understanding of these interactions on the ground and will provide a strengthened platform for publication for all Space Biology research outcomes in line with expectations of the ground-based research community. Using combined, multi-level systems biology approaches to combine genomics, transcriptomics, and metabolomics towards an understanding of complex microbial interactions on the ground is a prerequisite for understanding how spaceflight perturbs homeostasis. These efforts will also enhance the terrestrial application of this knowledge towards solving problems on Earth.

Host-microbe interactions:

An open question in host-microbe research is where might perturbation be occurring? Is host tissue responding to flight stress, and in turn, the associated microbiome adjusting to a new homeostasis? Does the microbiome respond to the flight environment creating a dysbiosis with the host tissue? If it's a combination of the two, what is the relative contribution of each? To answer these questions at a mechanistic level, we first require an understanding of the functional interaction between the host and microbiome on Earth.

Recommended Reference Experiments:

- Ground analogs and scale-down human tissue and plant models with defined microbial (bacterial and fungal) populations.
- Determine the functional roles for the different microbial community members. Then apply single stressors, such as ionizing radiation, to determine the adaptive response of both the host tissue and microbiome.
- An integrated cross-host-species reference mission, that uses a combination of model organisms where we can monitor how their microbiomes change over time.
- Routine microbial monitoring for crew, spacecraft surfaces, and crops.

Microbiome of the Built Environment (MoBE):

Existing research established that surface-associated microbes are crew-derived [13], [15]–[18]. As the crew members move about the spacecraft, they deposit their associated microbes into the built environment. The resulting MoBE is distinct from indoor homes and human host-associated microbiomes [16]. As the host microbiome is the dominant contributor to the MoBE community, it's necessary to understand the adaptive pressures on microbial populations while still crew-associated and the selective pressures applied to these populations when released into the spacecraft environment. An understanding of the cycle of crew-associated

adaptation, environmental selection, and re-colonization is necessary to quantify risk to crew health [19].

Recommended Reference Experiments:

- Longitudinal sampling of crew-associated (skin, saliva and gut) microbiome with metagenome assembly and metatranscriptomics.
- High resolution spatial environmental mapping, coupled with microbial mapping, of the spacecraft to link abiotic pressures to microbial population dynamics (temperature, relative humidity, CO₂, illumination, nutrient availability, etc.).

This ongoing generation of human and environmental microbiome reference data will create a reservoir of MoBE knowledge which will empower the next decade research into long-duration space flight. This space generated reference data will also provide uniquely powerful resource for MoBE research on earth, such as intensive care units and surgical theaters, where incoming microbiota can be harder to control.

Bioengineering:

Microbial solutions to long-duration spaceflight include nutritional supplementation, *in situ* resource utilization, and hybrid life support. A general requirement for all these applications is the induction and reactivation of microbes from metabolic stasis. Understanding stasis in spaceflight conditions is required to define optimal storage conditions for biomanufacturing strains. These strains need to be stable on the order of years to support long-duration exploration missions and experiments [20].

Recommended Reference Experiments:

- Create microbial storage facilities where space flight stressors can be analyzed on relevant time lines (years).
- Provide transcriptomics datasets of bioproduction strains emerging from stasis to help characterize the molecular mechanisms required for recovery and mitigation of damage occurred due to space flight stress.
- Determine the rate of “retro-mutation” due to spaceflight stress. Retro-mutants are bioproduction strains that lose their capability to produce an engineered product, allowing them to out-compete the production strain.

Creation of a long-duration microbial storage reference can provide a high-quality platform for microbial bioengineering throughout the next decade space research. As such, this long-term approach will be original but also have far-reaching value to space exploration.

Summary

Overall, we hope to provide the community the baseline data necessary to generate actionable hypotheses. These efforts should accelerate space biology contributions to long-duration exploration through proven Open Science mechanisms.

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