

GROUND AND FLIGHT-BASED PLANT MICROBIAL INTERACTION RESEARCH AND RELATED SPACE CROP PRODUCTION APPLICATIONS



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Problem statement to be addressed by content in this whitepaper:

How can the plant associated microbiome and associated chemistries be monitored, selected, assessed, engineered, and applied in the spaceflight environment?

Introduction

Plants can both support crew diets and contribute to biological life support systems in spaceflight and future habitats. To ensure optimal, consistent, healthy, and safe growth of plants in the spaceflight environment there is a need to develop standard practices to manage the plant-microbial interaction within this closed, controlled, and highly derived environment. Current research is building the foundational understanding of these complex interactions and will further provide the ability to engineer these interactions. Here we provide recommendations as to how the space biology community (academic, government, and industry) can work together to conduct research designed to 1. monitor, 2. select, 3. assess, 4. engineer, and 5. apply plant-relevant microbes in the spaceflight environment.

Role of microbes in crop health

The analysis of plant-microbe datasets has demonstrated and underscored the importance of microorganisms (Müller et al. 2016; Bulgarelli et al. 2015; Hacquard et al. 2015) and their chemistries (Walter, Britton, and Roos 2011; Gilbert, Sapp, and Tauber 2012) in the healthy functioning and growth of plants. Plants harbor microbes within their tissues constituting what is known as the *endosphere*, or source microbes from the soil and water surrounding the root system to form what is termed the *rhizosphere*, or through the stems, leaves, flowers, and fruits to form the *phyllosphere*. Microbes within these niches can promote plant growth through nutrient availability or the modulation of phytohormones and associated signaling pathways. To access nutrients, plants enter a symbiosis with microbes that possess the metabolic machinery to depolymerize and mineralize organic forms of N, P, K, S, and Fe (Lambers et al. 2008). Microbes can indirectly support plant health by outcompeting both human and plant pathogens for resources and space, and through support during environmental stress such as salt, temperature, and toxin stress (Mendez, Robert 2013). These plant-microbe interactions are determined by both biotic (plant and microbe genetics, microbiome composition and dynamics, microbe interactions) and abiotic factors (environment, management, physical properties).

The need to understand the plant microbial interaction

Plants can regulate different root exudate signalling molecules, attractants, or stimulants for the purpose of establishing selective symbiotic relationships with different microbes, such as the plant growth promoting (PGP) bacteria and microbes, and for defense against pathogens (Kobayashi et al. 2004; Baetz and Martinoia 2014). For this purpose, plants have evolved recognition mechanisms to discriminate between beneficial and pathogenic microorganisms (Saad, Eida, and Hirt 2020). The environment and the host genome both play a role in the structure of the plant microbial community. Plant hormones (phytohormones) play diverse roles in plant physiological processes including mutualistic interactions with soil microbiota (Shigenaga and Argueso 2016). For example, plants use ethylene as a regulator of stress responses, such as extreme temperatures, water, UV light, and wounding, as well as in interactions with fungi and bacteria (Abeles, Morgan, and Jr 2012). However, high levels of volatile organic compounds (VOCs) such as ethylene are hazardous to health in the ISS closed environment and must be scrubbed from cabin air. As we begin to tease apart the complexities of the plant-microbial interaction on Earth and look toward the use of plant growth promoting microbes (PGPM) in the spaceflight environment, we understand that the monitoring of this environment becomes critical to ensure plant health, human health, and overall ecosystem health. In the spaceflight environment, there is a need to develop fast, reliable sensor systems for monitoring the microbiome.

Monitoring the plant-microbe interaction in space crop production.

Space crop production efforts on the International Space Station (ISS) utilize growth platforms such as the Advanced Plant Habitat (APH), Veggie, and in the future, a crop production platform named

Ohalo III. Seeds for growth on orbit and the arcillite substrate they are grown in are sanitized prior to launch; therefore, the resulting microbiome is a likely a result of any remaining endophytic microbes or habitat acquired contaminants. An overlap has been observed for the microbial species represented on ISS surfaces and those from the Veggie unit (Khodadad et al. 2020). Furthermore, suspected microbial transmission of microbes from ISS surfaces to the astronaut microbiome and *vice versa* (Lee et al. 2021; Avila-Herrera et al. 2020) has been observed in the closed environment of the ISS, highlighting the importance of monitoring the microbiome of both to build a stable and safe environment. To this end, a Hazard Analysis and Critical Control Point (HACCP) plan, a management schema for the analysis and control of any biological, chemical, and physical hazards from the production of the raw material to consumption of the finished product will need to be established and tailored to the spaceflight environment. A strategic and well-informed HACCP plan can be developed using research tools focused on monitoring the plant-microbe-environment interactions in space crop production systems. Systems level monitoring can include automated sensing through gas and soil/nutrient solution sensors, imaging (hyperspectral, multispectral), -omics (e.g., genomics, transcriptomics), spectrometry (metabolomics, nutrients, volatiles), and biosensors capable of detecting microbes of interest directly. Surveillance in combination with correlative techniques will allow for the detection of stress indicators, elements that pose food safety concerns, reduced nutrient content, health-hazardous volatiles, and microbial signals that indicate optimal and stable operation.

Methods to monitor the microbe/plant interaction

Data collection and analysis of space crops exposed to space crop production environmental stressors using environmental sensing, advanced imaging, multi-omics, and machine learning techniques can lead to the development of predictive models of the biological responses of plants and shifts in their associated microbiomes. The goal is to achieve near real time measurement of plant stress and the plant/microbial interaction that is relevant to minimizing crop failure and maximizing crop yields/quality. This predictive ability will promote the advanced monitoring of space crop production in closed environments on an array of spaceflight platforms and inform horticultural requirements and automated system design considerations to facilitate optimal operation and contamination intervention and in turn sustain human exploration.

Imaging: detect wavelengths to characterize the plant/microbial interaction.

Multispectral imaging of plants under defined space crop production stressors in combination with environmental monitoring and artificial intelligence (AI) approaches will lead to the development of models diagnostic of what stress is affecting the plant even when no human-perceptible altered phenotype is present. The AI model will provide a reduced and discrete wavelength list with associated reflectance profiles to allow for the categorization of the stressor phenotypes. This provides an autonomous avenue for the early detection of crop stress via scanning with a specific set of wavelengths and subsequent analysis of the reflectance intensities to identify stress for timely and automated intervention. Imaging can also detect desirable traits, such as high antioxidant content, that are associated with microbial relationships

Enabling technology for imaging: A small and versatile hyperspectral or multispectral camera with a high signal to noise ratio.

Omics: detect gene sets or metabolites to characterize the plant/microbial interaction.

Analysis of metatranscriptomics and/or metabolomic datasets from plants and their associated microbiomes (Havemann, Natasha 2021.) under defined space crop production stresses will provide the ability to predict what stress is affecting the plant. Characterizing the differential expression patterns within the phyllosphere and rhizospheres and plant transcriptome will provide a list of biomarkers whose relative gene expressions, and which plant-microbe interactions, are most descriptive of the observed phenotype. Additionally, such technology can assay the microbial content of any hydroponic or aeroponic or irrigation solutions. Ultimately, these biomarkers, when looked at

in isolation across a set of specimens will reveal the underlying plant health and can be correlated to imaging datasets to further define the stress phenotype. In the context of space exploration this would lead to the autonomous monitoring of space crops, which will not require labor and sequencing intensive analyses previously used to train the models, but instead call for a quick biomarker assay using a quantitative qPCR type set up similar to the NASA developed RAZOR device used on the ISS (<https://www.nasa.gov/feature/new-microbial-monitoring-technology-a-candidate-for-iss-and-beyond>), or the development of antigen-based assays that can detect proteins of interests, similar to current COVID-19 tests. A third possibility is direct detection of a biomarker (e.g. a VOC) to identify the microbe (Maurer et al. 2019)

Enabling technology for genomics/metatranscriptomics: A fully automated pipeline of the Oxford nanopore platform for example Voltrax+Minion.

Nutrient analysis: nutrient content outcomes to characterize the plant/microbial interaction.

Nutrient analysis of the crop will provide an assessment of nutritional status for both optimal production and human nutrition. This dataset when correlated to imaging and omics assessments can further characterize the stress phenotype and guide nutrient management according to desired outcomes such as enhanced nutritional content and nutrient ratios that favour PGPMs.

Enabling technology for nutrient analysis or metabolomics: A miniaturized Inductively coupled plasma mass spectrometer (ICP-MS) or portable Raman spectrometry device (size requirement maximum 6U, U=10cm³) and robust ion-selective electrodes.

Machine learning: to identify the wavelengths, gene sets and nutrient outcomes indicative of a nominal or non-nominal plant/microbial interaction.

Machine learning approaches will allow for the correlation of datasets and the prediction of outcomes. Using ground and flight datasets, it can be determined how good a proxy ground-based simulated space crop environments are for the actual spaceflight, Lunar or Martian environment. Use of ground-based experimentation and subsequent testing of the most robust hypothesis in spaceflight will greatly reduce mission costs by limiting negative results. NASA's Genelab may benefit from expanding their spaceflight dataset to include spaceflight and planetary surface analog datasets and provide standardized resources for machine learning on such datasets.

Enabling technology: A centralized repository with experimental standards. Standard requirements are developed for the research community, so datasets created among separate investigations are amenable to centralized machine learning analyses and model development.

Selecting space relevant plant-microbe consortia

The phenomena of human faecal transplants have illustrated the successful application of donor bacteria in restoring a recipient microbiome from a diseased state, but unfortunately, also the danger of getting it wrong. The occurrence of a death in one clinical trial has shifted the paradigm of faecal transfers to a more defined and controlled approach (DeFilipp et al. 2019). Here individual bacterial strains are isolated, and mixtures are tailored to the individual's microbiome needs by providing strains to fill metabolic functional deficiencies while excluding any potentially harmful microbes. This concept can provide a level of control necessary for the closed environment of the spaceflight setting. Similarly, each crop type will have a most beneficial microbial consortia and will benefit from this tailored approach. We outline two possible approaches to arriving at such tailored mixtures and ultimately synthetic microbial communities. The first option is to start out with a few PGPMs from existing literature accounts, mix these together in a factorial design, then apply to plants in a high-fidelity space crop environment and observe the phenotypic outcomes by the above monitoring techniques. The second is to start with a larger microbial community and determine those microbial

isolates to keep after iterative metagenomic monitoring in combination with phenotype monitoring to determine key players and exclude functional redundancies. The first approach allows for greater strain control such as strain engineering opportunities to prevent contamination of the strain throughout the closed environment (extending to planetary protection) but may miss important microbial synergies. The second approach ensures that all important metabolic functionalities are present but may prove too time consuming and lead to bacterial strains that are difficult to culture and store or genetically engineer. This approach can also lend to outbreaks of pest and pathogenic bacterial strains and thus should be used as a tool to understand community dynamics in the laboratory. Any consortia introduced in a spaceflight setting should have their full genomes sequenced for each strain, full annotation for all genes, and a pathogenicity screen conducted to assure no harm to human cell lines. The strains should be properly contained, and the growth environment should be closed off from the cabin atmosphere. The community dynamics and plant physiology effects which result from the introduction of a microbial consortia can be tracked *in situ* using microbial reporter strains, rootzone sensors, imagery, omics (metagenomics, transcriptomics, metabolomics), and stable isotope experiments.

Assessing the use of microbial consortia in the space crop environment.

It is our recommendation that the first step in determining how to use microbial consortia in the space crop environment is to build a high-fidelity space crop production environment and to work closely with Commercial controlled environment agriculture (CEA) in this endeavour. Such a facility should have the capacity to simulate a diversity of environmental factors experienced in the low-Earth orbit (LEO) and beyond low-Earth orbit (BLEO) such as enhanced radiation, micro-, partial-, and hyper-gravity, as well as altered atmospheric pressures. This high-fidelity environment will allow for the testing of: 1. selective environment parameters that shape and constrain microbial consortia and plant microbiome chemical dynamics; 2. how to grow plants for the best microbial outcomes (substrate-based, hydroponic, or aeroponic, nutrient/environment recipes); 3. longitudinal experiments to understand markers of a healthy community (gene expression, small molecules, macromolecular dynamics, co-association network models); 4. pathogen effects on the microbial community and intraspecies gene transfer of virulence mechanisms; 5. the balance between microbial function and stability as it relates to the design/management of constructed communities for promoting plant health; 6. how to integrate the plant/microbial compartment into a larger bioregenerative life system (BLSS); 7. resistance against disease: any employed approach needs to account for some level of genetic diversity (redundancy) in order to counter pathogen infection and avoid the problems associated with monocultures. In this high-fidelity environment, various experimental scenarios and monitoring techniques can be conducted and only the highest priority hypotheses are then tested in the actual spaceflight environment using platforms such as CubeSats, the International Space Station, Commercial Lunar Payload Services (CLPS). It is also important to consider high-priority hypotheses for partial gravity settings given that Lunar and Martian mission planning is already underway by both NASA and American space companies. The successful addition and monitoring of important symbiotic partners, or the chemistries derived from these partners, has the power to increase the nutritional content of indoor/closed environment crops. If synthetic microbial communities or use of the associated chemistries for plant growth promotion are to be used in the closed environment, their effects must be assessed, and a biosafety containment plan should be established prior to use in any crewed environment application to avoid disease manifestation through opportunistic pathogenicity.

Engineering microbial communities

Microbes exhibit a diversity of mechanisms for adapting to their environment, through immediate control by means of phenotypic plasticity through transcription and translation, and evolutionary mechanisms, such as mutation, epigenetic modification, and horizontal gene transfer by way of conjugation, transformation, or phage-mediated transfer. Microbes in the spaceflight environment of the ISS have been reported to demonstrate enhanced growth, virulence, and ability to form biofilms, although not all to the same degree. It is expected that the spaceflight environment will also affect the

members of a synthetic microbial community. Experimentation in a high-fidelity ground chamber as outlined above should shed light on these dynamics; however, further manipulation of the synthetic microbial communities by way of biological engineering techniques may be necessary to achieve intrinsic biocontainment in spaceflight environments. Biocontainment will ensure that plant-associated microbes do not become a biohazard to crew or spaceflight or other planetary systems. The field of biocontainment began in order to avoid the spread of potentially hazardous biological entities beyond the environment in which they were intended and include the use of emerging technologies in synthetic biology such as genetic circuit engineering, genome editing, and gene expression regulation. The Department of Energy (DOE) and Defense Advanced Research Projects Agency (DARPA) has invested considerable funds to this avenue of research, and we recommend that NASA pairs with this government agency to provide funding opportunities to explore intrinsic biocontainment approaches. Through experimentation in high-fidelity analogues, it is also possible to develop strategies that require little to no microbial addition using primarily manipulation of impactful environmental parameters such as pH, redox potential, and temperature, to bolster PGPMs and provide unfavourable conditions to microbes that pose health or production concerns.

Application of microbial consortia or their chemistries.

Commercial agriculture companies have pioneered the use of agricultural biological agents. Currently, there are many companies developing plant beneficial microbial consortia. Indigo Ag, for example, applies beneficial microbial consortia to seeds and has demonstrated by GFP-tagged microbes that beneficial bacteria applied to seeds can spread throughout the mature plant and be cultured from the leaves and roots. Among previously established seed coatings are Epic® and Kodiak®, which contain *Bacillus amyloliquefaciens* GB03 (Choi et al. 2014), to counter pathogenic fungal growth. It is our recommendation that NASA pairs with the USDA to provide SBIR/STTR opportunities for commercial agriculture companies and academic institutions to collaborate on the research and development of microbial seed coating or other innovative ways introduce or manipulate beneficial microbial consortia to benefit food production in the spaceflight environment. A few suggested plant phenotypes to promote through the application of microbial consortia in the spaceflight environment include reduced volatile organic compounds production, increased nutrient content and biomass, enhanced pathogen control, and increased shelf-life. Synthetic biology can be used to confer beneficial functionalities generated by microbes directly to the plant itself. Precedence for this approach has been demonstrated as historically, organic farmers used the bacteria *Bacillus thuringiensis* as a biopesticide (Zhu et al. 2015) and as a result the effective gene cluster from this bacterium was later genetically engineered directly into the crop to confer this resistance. Here rather than introducing a microbial consortium, beneficial gene clusters are introduced to the plant genome, further prioritizing crops with tractable genetic systems.

Conclusion NASA looks toward the use of plant growth promoting bacteria in the spaceflight environment. A better understanding of plant-environment-microbe interactions will support safe food production and optimize plant growth and associated life support services in planetary or space-transit agriculture systems. High-fidelity spaceflight environments on the ground and spaceflight platforms such as CubeSat and CLPS landers will allow for hypothesis testing. Such platforms will serve as a testbed for enabling hardware, allow for the performance of relatively complex or long-term studies. These efforts will lead to the formulation of automated approaches and machine learning algorithms designed to provide prediction and control over plant growth habitats and to the development of future controlled environment agriculture off-planet. This experimentation will lend to well-informed HACCP protocols aimed at the monitoring of microbial consortia applied to crops as well as the characterization and prediction of microbially driven plant physiological outcomes in the spaceflight and/or planetary settlement environment, ultimately improving the productivity, stability, and safety of space cropping and BLSS systems.

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