Resilient Agriculture in Space: Microgreens and the Microbiome

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Microgreens in Space

Fresh vegetables are needed for adequate nutrition during long-term missions (1). Microgreens are an excellent option because they have a higher concentration of essential nutrients than mature plants and can be grown on a wet cloth without soil and with minimal fertilization which reduces weight (2). Space needs are low due to high biomass density and the time to harvest is 10-21 days. A food production system based on short batches is resilient because if there is a problem with one batch, it can quickly be replaced.

Unknown Microbiome Impact

The microbiome is a critical environmental factor which affects plant growth, health, and stress resilience (3,4,5). During a space mission plants undergo radical changes in microbiome due to sterilization and subsequent colonization by habitat bacteria (6,7). It is unknown what impact microbiome changes have on microgreen growth.

Analog Mars Habitat

The Mars Desert Research Station (MDRS) was used as an analog habitat simulating the microbial community that occurs in the close quarters and isolation of a space habitat.

Figure 1. MDRS habitat and greenhouse. Inhabitants must follow strict simulation protocols.

Experimental Methods

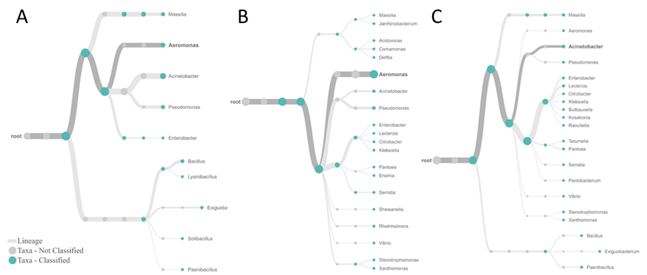
Daikon radish seeds and growing trays were sterilized using sodium hypochlorite solution. Trays were arranged in the MDRS greenhouse in a randomized block design. 80 mL of seeds were spread on each tray. Trays were inoculated with samples of microbes from the MDRS, soil, or a sterile control. Trays were watered twice a day with tap water sterilized by boiling. A low dose of fertilizer was given on the 8th growth day. Microbial communities were analyzed using 16S sequencing.

Plants were harvested on the 10th day. Fresh weight, fresh volume, mean leaf size, and mean stem length were measured by tray.

Figure 2. Tray system for growing microgreens and randomized block design of experimental layout.

Microbiome Composition

Nanopore 16S sequencing analysis was successful for all microbiome treatments. Total number of DNA strands identified was 16,591 for the control, 33,089 for the habitat, and 64,325 for the soil, indicating differences in microbial biomass. The microbial communities in the habitat and soil treatment differed significantly from the control. Figure 3 shows a taxonomic tree of the microbial communities in each treatment along with a table of the number of identified DNA strands from the twelve most prevalent Genus. Several identified Genus are potentially pathogenic and it is still under investigation if pathogens were present.



Acinetobacter	4,222
Aeromonas	2,434
Massilia	1,569
Pseudomonas	1,536
Bacillus	1,295
Exiguobacterium	630
Solibacillus	79
Lysinibacillus	53
Enterobacter	38
Paenibacillus	17
Xanthomonas	13
Citrobacter	12

Aeromonas	17,730
Pseudomonas	2,568
Acinetobacter	1,512
Serratia	448
Xanthomonas	321
Klebsiella	283
Stenotrophomonas	276
Enterobacter	260
Massilia	139
Janthinobacterium	96
Citrobacter	95
Leclercia	63

Acinetobacter	5,219
Massilia	3,232
Leclercia	2,163
Enterobacter	1,168
Pantoea	1,115
Paenibacillus	1,114
Pseudomonas	952
Bacillus	664
Exiguobacterium	644
Citrobacter	391
Klebsiella	313
Aeromonas	159

Figure 3. Taxonomic tree of the microbial communities in control (A), habitat (B), and soil (C) treatments along with a table of identified sequence frequency from the twelve most prevalent Genus.

Microgreen Growth Comparison

The fresh mass, fresh volume, mean leaf size, and mean shoot length of microgreens subject to the habitat, soil, and control treatments are displayed in Figure 4. Each data point represents the value for one replicate. An ANOVA of the randomized block design showed that the microbiome treatment does not have a statistically significant effect on fresh weight, fresh volume, mean leaf size, or mean stem length ($p=0.984$, $p=0.972$, $p=0.4769$, $p=0.6190$).

These p values are so large that the data was analyzed for equivalence using a TOST procedure with the sterile treatment as a reference, equivalence bounds set at 10% of the mean value for the reference, and an alpha of 0.90. The resulting confidence intervals are shown in Figure 5. The means of each confidence interval are well within the equivalence bounds. However, the data does not support a strict statistical definition of equivalence, except for the leaf size in the soil treatment due to high variability.

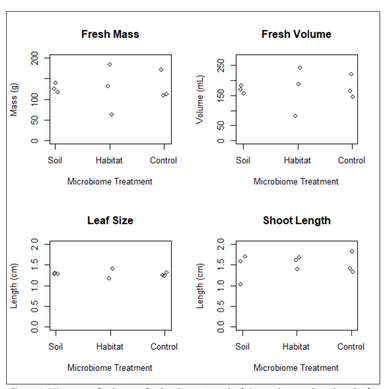


Figure 4. Microgreen fresh mass, fresh volume, mean leaf size, and mean shoot length of trays subjected to the habitat, soil, and control treatments.

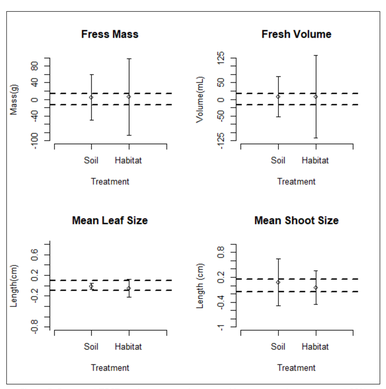


Figure 5. Results of TOST equivalence testing to sterilized control. Intervals are of 90% confidence shown against 10% equivalence thresholds.

Figure 2. Tray system for growing microgreens and randomized block design of experimental layout.

Conclusions

This preliminary study indicates Daikon radish microgreen growth is resilient to the impacts of an evolving microbiome in an analog Mars habitat over a ten day growth period. Sequencing analysis indicates that inoculation had a large effect on microbiome and the three treatments differed significantly in biomass and composition. Microgreen fresh weight, fresh volume, mean leaf size, and mean stem length were very similar across treatments with a very low statistical likelihood of difference. Growth parameter means fall within equivalence bounds, but the statistical equivalence results are inconclusive due to large variance in the data.

Future Research

A larger study should be conducted to obtain statistically conclusive results for equivalence. Confirmation implies that microgreens may be a good choice of fresh food for space missions due to their resilience to unknown habitat microbiomes. Before relying on microgreens for astronaut health, we must better understand if microgreens harbor pathogens, the effects of more extreme microbiomes such as on the International Space station, species specific responses, and effects over longer growth periods.

References

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