INSIGHTS FOR MICROBIOMES IN REGOLITH BASED AGRICULTURE (RBA) FOR FUTURE OFF WORLD HABITATS BASED ON SIMULANT GROWTH EXPERIMENTS

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Introduction: In-situ food production and bioregenerative life support systems are mission critical for sustainable deep space exploration [1-4]. These systems aim to produce fresh, nutrient-rich crops to support the dietary needs of space crews, contribute to oxygen production, and offer psychological benefits. The systems both rely on and produce space resources. The Comparing Hydroponics and Regolith Growth and Evolution (CHRGE) project examined and contrasted the effectiveness of hydroponic and regolith-based agricultural (RBA) systems for creating autonomous extraterrestrial habitats [5]. One of the outcomes of the CHRGE project centers on the improvement of plant response to regolith simulants when reused for multiple generations of plants [6]. This abstract presents results that examine plant and microbiome development for reused regolith simulant.

Materials and Methods: Morphological evaluations were executed via a destructive sampling methodology at five-day intervals throughout a thirty-day cultivation period. Parameters such as plant height, crown diameter, Specific Leaf Mass (SLM), Root Mass Fraction (RMF), Shoot Dry Mass (SDM), Root Dry Mass (RDM), and leaf area were measured during the testing period. Furthermore, stomatal conductance was quantitatively assessed thrice daily at predetermined times (06:00, 10:00, 14:00, and 18:00 hours). Concurrently, environmental variables, including CO_2 concentration, relative humidity, and ambient temperature, were monitored hourly using a HOBO data logger within a precisely controlled environment chamber. Additionally, comprehensive quantification of energy and water consumption for both experimental systems were conducted hourly, encompassing all system interactions and setup durations.

Due to the improvement of plants with multiple generations of regolith use, additional growth studies of micro-tomato cultivars were conducted in regolith simulants over multiple generations both with and without microbial inoculant. Similar plant metrics were collected for tomato plants as described above.

Statistical analysis determined mean values and standard deviations for all measured parameters. The Shapiro-Wilk test was applied to the dataset to validate the assumption of a normal distribution. Given that all data conformed to a normal distribution, ANOVA was employed to identify significant differences between the means of the groups. A Tukey's (HSD) test was used as a post hoc analysis to discern specific group differences.

Samples of regolith simulant were homogenized and subsampled using sterile technique for DNA extraction. DNA was extracted using a Qiagen PowerSoil® Pro kit with an adapted methodology. The adaptation applies a 1 M phosphate,15% ethanol buffer at pH 8 to the cell-lysing step to help increase extraction of DNA from difficult substrates such as regolith simulant [7]. The remaining extraction followed the manufacturers protocol for the Qiagen PowerSoil® Pro kit. DNA quantification was performed using a Qubit DNA HS kit (Cat Q32851, ThermoFisher, Waltham, Massachusetts). The samples were sequenced with Oxford Nanopore MinION® Rapid PCR Barcoding Kit (v14 chemistry). The sequences were then analyzed with Epi2Me Labs metagenomics workflow using Plus PFP 16GB prebuilt Kraken2 database. Output from Epi2Me pipeline was converted using KrakenBiome and taxonomic and diversity analysis completed in Phyloseq.

Results and Discussion: Results indicate that hydroponically grown lettuce plants exhibit superior growth metrics, including an average plant height of 22.43 cm, root length of 14.66 cm, and shoot length of 7.85 cm, significantly outperforming their single generation regolith-grown counterparts with means of 12.21 cm, 7.02 cm, and 5.45 cm, respectively (ANOVA $p<0.001$). Furthermore, hydroponic plants demonstrate a greater biomass yield, with a mean total weight of 4.42 g and a leaf area of 35.07 cm², indicative of an enhanced photosynthetic capacity.

Despite their smaller stature, regolith plants present a higher edible biomass ratio of 85.47% when compared to hydroponically grown plants (ANOVA $p<0.031$), suggesting a potential for greater nutritional efficiency. After a 30-day grow-out, operational analysis reveals that RBA systems require less time investment, totaling 736.15 minutes, compared to 949.24 minutes for hydroponic systems, suggesting a more efficient use of resources. Reuse of the regolith, i.e. second and third generation regolith resulted in increased total growth while the ratio of edible biomass remained consistent, demonstrating a continuance of the efficiency and growth metrics comparable to hydroponic plants.

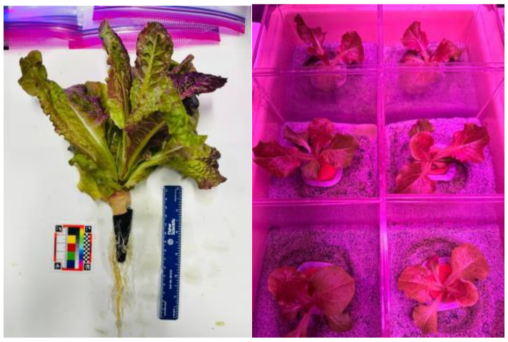

Figure 1: Images of hydroponic (left) and regolith growth (right) lettuce plants.

The combined benefits in reduced energy and water usage as well as crew time support that RBA may prove to be a more efficient management strategy than hydroponics. Stomatal conductance measurements further differentiate the two cultivation methods. Hydroponics exhibited enhanced gas exchange and potential photosynthetic activity, while regolith systems showed lower conductance values, indicating a more stressful environment for plant growth.

The generational study of micro-tomato cultivars demonstrates significant improvement with even one reuse of simulant material; the addition of an inoculant further increased plant metrics in both generations of regolith simulant. ANOVA results indicated significant effects of generation, variant, treatment, and their interactions on all measured variables. The second generation (G2) outperformed the first generation (G1) across all variables, regardless of the variant, with even better results when inoculated with PEP1. For plant height, ANOVA showed $F = 24.3$ (generation), 22.8 (variant), 18.4 (treatment), and 5.2 (interaction), all $P < 0.001$. Tukey tests confirmed that hybrids were significantly taller than other variants ($P < 0.001$), with PEP1-treated plants also showing increased height ($P < 0.001$).

Samples of regolith simulant resulted in very low biomass of extracted DNA. Initial extractions without the modified protocol did not result in usable amounts of DNA for subsequent sequencing, however modifications with the 1 M phosphate,15% ethanol buffer at pH 8 successfully increased extracted DNA, albeit still at low biomass. Taxonomic plots included large portion of samples classified as unknown, but the identified portion consisted of Rhodococcus, Acinetobacter, and Pseudomonas genera.

Conclusions and Future Work: The results of our comprehensive study advocates for a hybridized cultivation approach, integrating the growth efficiency of hydroponics with the resource optimization seen in RBA systems [4]. This strategy proposes a balanced and adaptable life support solution for future space exploration endeavors. Additional data to inform this approach is expected with bioinformatic analysis of the metagenome comparing the microbiome between hydroponic and regolith growth conditions. Further benefits of RBA approaches may also arise as validated methods to ameliorate regolith materials for plant growth are developed. Such advances in RBA must also include consideration for the resources, efforts, and risk required for the ameliorative process and comparisons to equivalent risk and resource requirements for hydroponic systems. Similarly, advances in hydroponic technologies may facilitate adaptive hybrid strategies for space crop production.

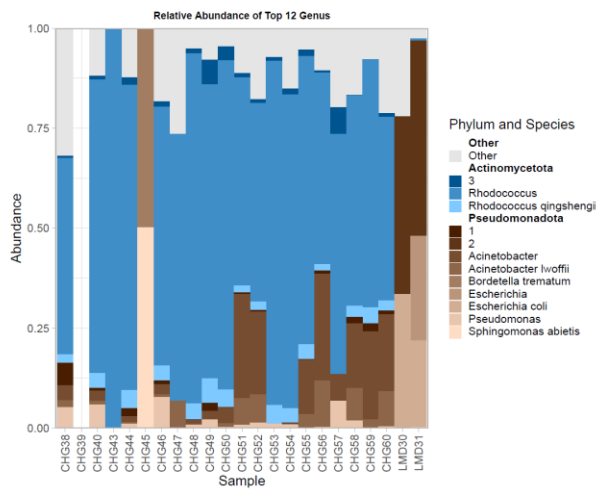

Figure 3: Taxonomic plot of the top 12 ranked by relative abundance at the species level.

By customizing a hybrid model to suit the needs of different crops and balance the strength, weaknesses and costs of each approach, we can optimize sustainability and productivity, ensuring the viability and success of human activities in space.

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