Investigating Microbiome Differences between Red Romaine Lettuce Grown from Sanitized and Unsanitized Seeds

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INTRODUCTION

- The International Space Station (ISS) as an integral component for the discovery and development of advanced robotics, materials, communications, medicine, agriculture, and environmental science due to it currently being the world's only microgravity laboratory of its kind.[2]
- Because the ISS is a contained system with confined quarters, much research has been undertaken to assess and diminish the number of microbiological risks associated with astronauts inhabiting the station for extended periods of time. Notable microbiological risk factors include drinking water, air, and food.[3]
- As an avenue for both mental/emotional respite and a source of fresh produce for astronauts, a vegetable production system has been employed on the ISS.[1]
- In order to understand the microbial risks involved with a "pick and eat" vegetable system on the International Space Station (ISS), this study aims to compare microbial differences between sanitized and unsanitized seeds by tracking and identifying seed-borne microbes throughout the development of red romaine lettuce (*Lactuca sativa*)—a plant species that has already been grown on the ISS.

METHODS

- ISS conditions (excluding microgravity) were simulated in a growth chamber (relative humidity RH 50%, temperature 23 ºC, and CO_2 3000 ppm).
- Leaf and root tissue samples were taken from plants germinated with sanitized and unsanitized seeds at day 7 and 14.
- Heterotrophic plate counts on selective media and genetic sequencing were used to quantify and identify microorganisms present on the developing plants (days 7, 14, 21, and 28).
- Inoculum from leaf and root samples were cultured in duplicate onto tryptic soy agar (TSA) plates for quantification, and each sample was characterized using the 16S rRNA gene and next generation sequencing (NGS).
- Sequencing was analyzed using GreenGenes software and diversity levels were determined.
- Similarities and differences in the community structure were determined by comparing the sanitized and unsanitized seeds and plant tissue throughout the time course study

RESULTS

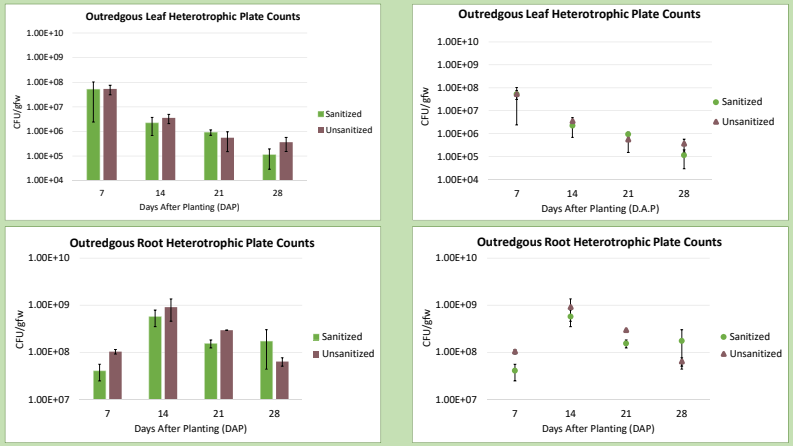
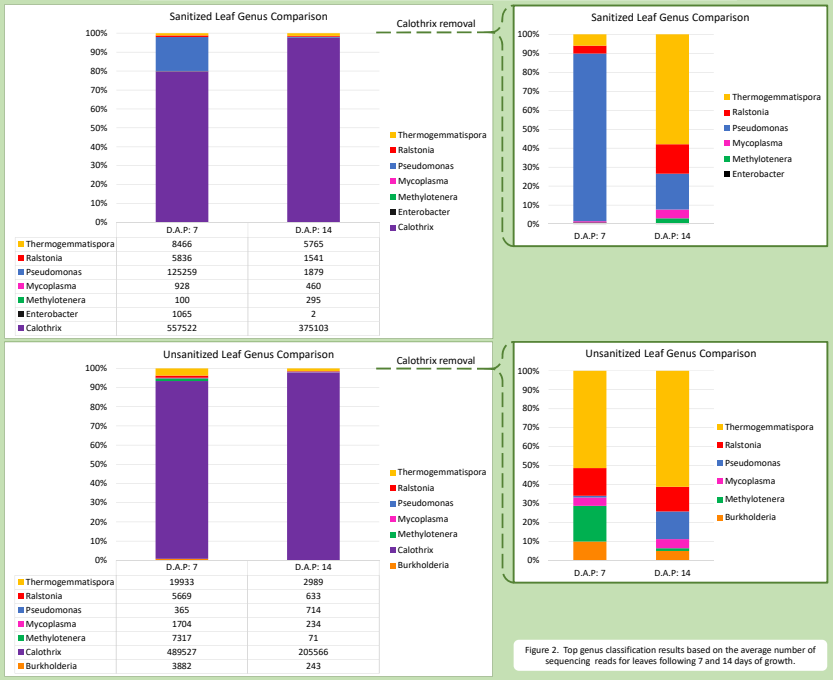


Figure 1. Heterotrophic TSA plate counts following 7, 14, 21, and 28 days of growth. Error bars represent standard errors.



	D.A.P: 7	D.A.P: 14
Thermogemmatispora	8466	5765
Ralstonia	5836	1541
Pseudomonas	125259	1879
Mycoplasma	928	460
Methylotenera	100	295
Enterobacter	1065	2
Calothrix	557522	375103

	D.A.P: 7	D.A.P: 14
Thermogemmatispora	19933	2989
Ralstonia	5669	633
Pseudomonas	365	714
Mycoplasma	1704	234
Methylotenera	7317	71
Calothrix	489527	205566
Burkholderia	3882	243

Figure 2. Top genus classification results based on the average number of sequencing reads for leaves following 7 and 14 days of growth.

DISCUSSION

- Roots display significant increase in bacterial growth from day to day 14 of plant growth, as well as a significant difference in bacterial growth between sanitized and unsanitized roots at d 7 and 21 (Fig. 1).
- *Calothrix* occupied the majority of all genus sequencing reads for both sanitized and unsanitized at day 7 and day 14. This is not entirely surprising as *Calothrix* may be present in a symbi relationship with plants.
- Sanitized and unsanitized samples possessed much of the san bacterial genera—the exceptions being *Enterobacter* in saniti samples and *Burkholderia* in unsanitized samples.
- *Thermogemmatispora* was identified more frequently on day of both treatments, this could be indicative of competition between genera.
- *Pseudomonas* was identified in both treatments. It was identified more frequently on the day 7 sanitized leaves befor dropping on day 14; conversely, *Pseudomonas* identification d day 7 unsanitized leaves was less than on day 14.

REVIEW

- I gained invaluable knowledge, connections, and experience in r time at NASA's Kennedy Space Center, and I can genuinely say th has been the opportunity of a lifetime.
- Participating in this incredible internship has augmented my des to pursue a career in scientific research, as such I have immedia started to explore and apply to equally amazing research experiences.

REFERENCES

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