

Survival of *E. Coli* in the Rhizosphere and Phyllosphere of Leafy Greens Grown in Controlled Environment Chambers under International Space Station Conditions

Mary E. Hummerick Christina L. M. Khodadad, Lashelle E. Spencer, Anirudha R. Dixit, Cory J. Spenn, Aaron B. Curry, Jennifer L. Gooden, Jason A. Fischer, Gretchen J. Maldonado, Gioia D. Massa, Matthew W. Romeyn
NASA, Kennedy Space Center, FL

Abstract

NASA's mission for manned long-duration space exploration drives the research for crop selection to provide a nutritious and safe supplement to an astronaut's diet. Understanding plant growth, health, and the associated microbial communities in closed environments will be critical to the success of this mission. Cultivation of crops in closed controlled environment agricultural systems may limit microbial colonization and reduce diversity of the microbial communities. Furthermore, practices like seed and growth medium sanitization may impact microbial communities in the mature plant and the capacity to limit the growth of food borne pathogens through competition.

To determine the persistence of a potential human pathogen in plants cultivated using International Space Station plant growth protocols and environmental conditions, sanitized and unsanitized seeds from, mizuna (*Brassica rapa* var japonica) and red romaine lettuce (*Lactuca sativa* cultivar 'Outredgeous'), were inoculated with *Escherichia coli*, ATCC 21445 and grown in controlled environment chambers. Leaves and roots were harvested and processed for analysis every 7 days until harvest at day 28. The presence of *E. coli* in the phyllosphere and rhizosphere was determined by plating on selective media and quantitative PCR. *E. coli* was detected on plates in the crops' roots and leaves for several weeks post germination and in some plants through harvest. At day 28 plants grown from sanitized seeds had higher counts of *E. coli* on the roots than those from unsanitized seeds. *E. coli* was also detected on a few uninoculated plants indicating air borne cross contamination among plants in the same growth chamber. These results suggest an influence of plant cultivation conditions and the natural microbiome on human pathogen survival and persistence in leafy greens.



Figure 1. Veggie chamber on ISS and first crew consumption of 'Outredgeous' romaine lettuce after harvest from Veggie. (Photo Credit NASA)

Results



Figure3. Average *E. coli* detected by qPCR and enumeration on EMB on mizuna leaves and roots. Error bars indicate standard error. Missing bars indicate values below detection limit.

Methods

- ❖ **Sanitized and unsanitized seeds** of mizuna (*Brassica rapa* var japonica) and red romaine lettuce (*Lactuca sativa* cultivar 'Outredgeous'), were inoculated with *Escherichia coli*, ATCC 21445 by submerging overnight in broth culture.
- ❖ **Whole community sequencing of 16S rRNA** was performed on seed extracts to identify genera present. No *E. coli* was detected.
- ❖ **Grown in controlled environment chambers** at 50% RH, 3000ppm CO₂ and 23° C under fluorescent lighting at 300 μmol per m² s⁻¹
- ❖ **Shoots and roots were harvested** and processed for analysis every 7 days until harvest at day 28.
- ❖ **The presence of *E. coli*** in the leaves and roots was determined by plating on selective media and quantitative PCR.
 - ❖ PCR was run using primers specific for *E. coli* ycfR gene on the LightCycler® 480 instrument with the LightCycler® 480 SYBR® Green I Master mix (Roche Diagnostics).
 - ❖ Cp values were evaluated based on a standard curve for *E. coli* DNA with known concentrations.
 - ❖ Copy number and CFU were calculated per gram fresh weight of plant tissue.

Conclusion

- ❖ *E. coli* was detected on plates in the crops' roots and leaves for several weeks post germination and in some plants through harvest.
- ❖ At day 28 plants grown from sanitized seeds had higher counts of *E. coli* on the roots than those from unsanitized seeds.
- ❖ *E. coli* was also detected on a few uninoculated plants indicating cross contamination among plants in the same growth chamber.
- ❖ These results suggest an influence of plant cultivation conditions and the natural microbiome on human pathogen survival and persistence in leafy greens

