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Metagenomic analysis of the microbial community of an experimental hydroponic system growing leafy greens

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Introduction

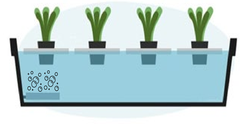
Hydroponics is a growing industry for food production, particularly in the area of leafy greens. As leafy greens are among the leading sources of food-borne illness, hydroponics are thought to be a tool reducing pathogen outbreaks. While hydroponic and other controlled environment agricultural systems remove many sources of contamination that occur in soil-based and outdoor growing systems, the risks of contamination are not eliminated. Previous studies on hydroponics have shown that bacterial pathogens (STEC, *Salmonella*, and *Listeria*) grow in and rapidly spread throughout hydroponic systems and that hydroponically grown produce show higher rates of bacterial pathogen internalization than soil-grown plants[1-3]. Indeed, several recalls of hydroponically grown leafy greens have occurred in recent months due to contamination with *Salmonella* and *Listeria*. Still, microbiological information about these systems is limited compared to soil-based systems.

In this project, we developed an experimental hydroponics system and characterized the microbiomes of the hydroponics system and its inputs using metagenomics. This work provides a foundation for future work hydroponic food safety microbiology.

Hydroponic system design

Deep water culture hydroponics

- Plants are grown on rafts on a pool of nutrient solution, with the roots submerged in the nutrient solution
- Aeration stones maintain oxygenation of the nutrient solution and root zone



Rockwool used as a rooting medium

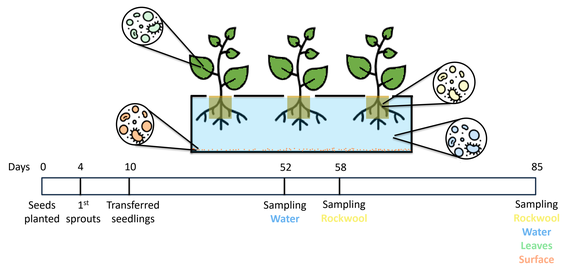
Seeds planted in rockwool and transferred as seedlings to net cup

MaxiGro commercial nutrient solution in DI water, pH adjusted to 5.5

Butterhead and romaine lettuce grown in separate trays of nutrient solution

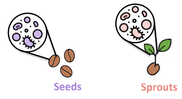
Materials and Methods

Hydroponic system microbiome sample collection



- Swabs, rockwool (including roots), and leaves were shaken in 1xPBS to remove attached cells

Seed and sprout sample collection



Varietal	Lettuce Type	Seed Type	Est. No. Seeds Extracted	Est. No. Seeds Sprouted
Rex	Butterhead	Pelleted	70	50
Chicarita	Romaine	Pelleted	70	50
Milagro	Butterhead	Naked	200	50
Monte Carlo	Romaine	Naked	200	50

- Lettuce seeds included pelleted and raw seeds
- Seeds sprouted in petri dishes with sterile filter paper and water for 7 days
- Seeds and sprouts were shaken in 1xPBS + 0.05% Tween for 2 hours to remove attached cells

Sample processing and sequencing

- Sample supernatants were filtered through 0.22µm filters or pelleted
- DNA was extracted using the Qiagen PowerWater kit and cleaned with Zymo Clean and Concentrator kit
- Shotgun sequencing using the Illumina NextSeq 500/550

Metagenomics methods

- Host reads were filtered out using Kraken2
- Kraken2 was used for taxonomic identification of the metagenomic reads
- Bracken was used for relative abundance determination

Results and Discussion

Taxonomically diverse communities in system and inputs

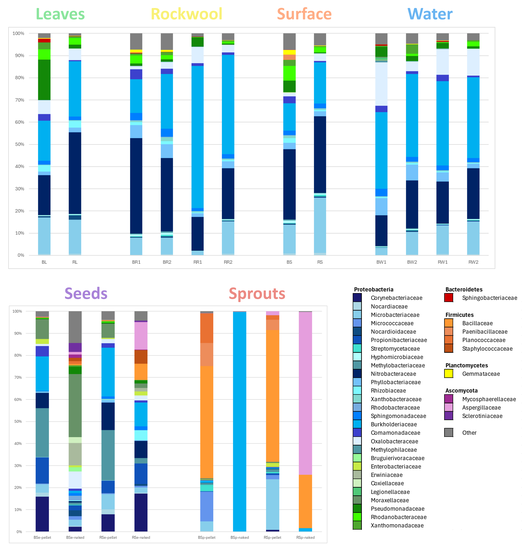


Figure 1. Bar graph showing taxa at the family level with at least 1% relative abundance in at least one sample.

- Hydroponics system and seeds are dominated by Proteobacteria
- *Cupriavidus metallidurans*, a highly metal resistant member of the Burkholderiaceae, is a prominent member of the hydroponics community, particularly the rockwool
- Fungi make up a large proportion of the seed and sprout microbiomes
- Pelleted seeds are more similar to each other than to other seeds of the same lettuce type
- Sprouts have the most divergent microbiomes, reflecting the drastic chemical and microbiological changes that occur during seed sprouting

Distinct communities by sample type

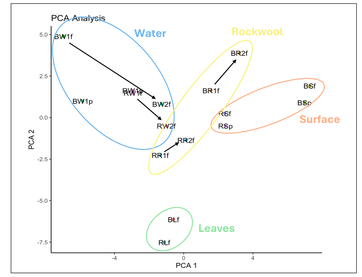


Figure 2. PCA plot of the Bray-Curtis dissimilarity comparing the microbiota of samples from the hydroponics system

- Samples cluster first by sample type, then by lettuce type
- There is a temporal shift in the microbiota as the plants and system develop

Strong influence of seed microbes on hydroponic microbiomes

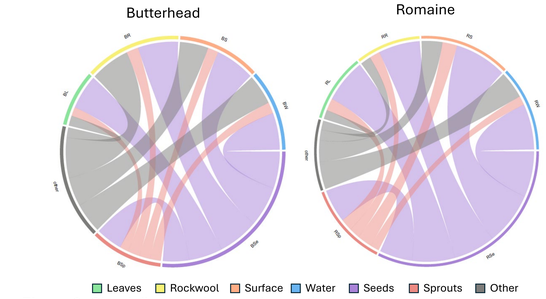


Figure 3. Chord diagram showing the relative contributions of taxa (at the genus level) from the hydroponic inputs (seeds, sprouts, and other) to the hydroponic inputs (leaves, rockwool, surfaces, and water solution).

- 90-97% of seed genera remained in the hydroponics system at the end of the trial
- 41-50% of hydroponics and sprout genera were found in the seeds

Conclusions

- Lettuce can be grown on benchtop hydroponics systems and microbial DNA can be extracted from several sample types
- Hydroponic microbiomes differ by sample type, plant type, and time, each of which may influence how well a hydroponics system supports a bacterial food-borne pathogen
- Seeds are an important source of microbial input into hydroponics systems and should be considered as sources of contamination with food-borne pathogens

Upcoming work

- Scaling up in-house hydroponics system, including the addition of new leafy greens
- Improving DNA yield (especially from seeds and sprouts) and exclusion of host DNA
- Combining metagenomics work with inoculations of bacterial food-borne pathogens to correlate the microbiota with pathogen persistence
- Including physical and chemical measurements of the nutrient solution
- Microbiome functional analyses including virulence, resistance, and secondary metabolic profiles

References
1 Shaw *et al* (2016) Growth of *Escherichia coli* O157:H7, Non-O157 Shiga toxin-producing *Escherichia coli*, and *Salmonella* in water and hydroponic fertilizer solutions. *J Food Protect* 79:2179-2183.
2 Ilic *et al* (2022) Lettuce contamination and survival of *Salmonella* Typhimurium and *Listeria monocytogenes* in hydroponic nutrient film technique systems. *Foods.* 11(21):3508.
3 Saldinger *et al* (2023) Hydroponic agriculture and microbial safety of vegetables: promises, challenges, and solutions. *Horticulturae.* 9(1):51.