Curating a Collection of Seed Bacterial Community Members Enriched Under Maternal Plant Stress

Joanna Colovas[1], Abby Sulesky-Grieb[1], and Ashley Shade[1]
[1]Department of Microbiology and Molecular Genetics, Michigan State University

Plant-Microbe Interactions

Plants, like humans, have many members of their microbiota that serve to help them:[1]

- Gain Essential Nutrients
- Fight Pathogenic Infection and Colonization
- Withstand Environmental Stressors (Nutrient, Drought)

Members of the microbial community can be acquired both vertically and horizontally. Vertical transmission entails microbes inherited from the parental lineage, whereas horizontally transmitted microbes are acquired from the environment.[2]

Seed microbes are hypothesized to aid in plant resilience to environmental stressors.

The ability to determine what effects specific microbes have on plant resilience to stress could provide new methods for improving plant health.

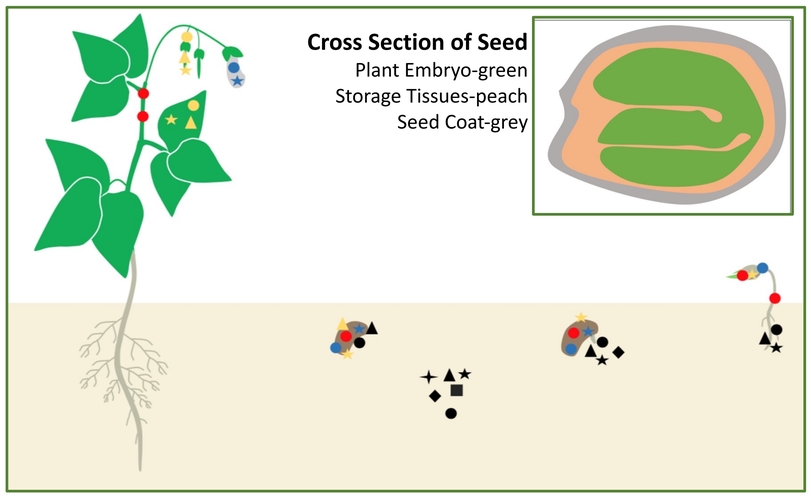

Figure 1. Methods of Microbial Colonization of Plants[3]
The parent plant (left) transmits its microbes (various colored shapes) vertically to the seed offspring. Once in the ground (moving right), the seedling can also horizontally acquire microbes from the soil (black colored shapes).

Goals

- We will use the common bean, *Phaseolus vulgaris L.,* to study the effects of abiotic stresses (excess nutrient and drought) on the microbial community inherited from the parent to the daughter plant.
- **We will develop a protocol to isolate and culture members of the seed microbiome from plants that experience abiotic stress.**
- Cultured members of the seed microbiome will be used to inoculate plants to determine their effects on plant growth with and without fertilizer and during drought stress.

Preliminary Work

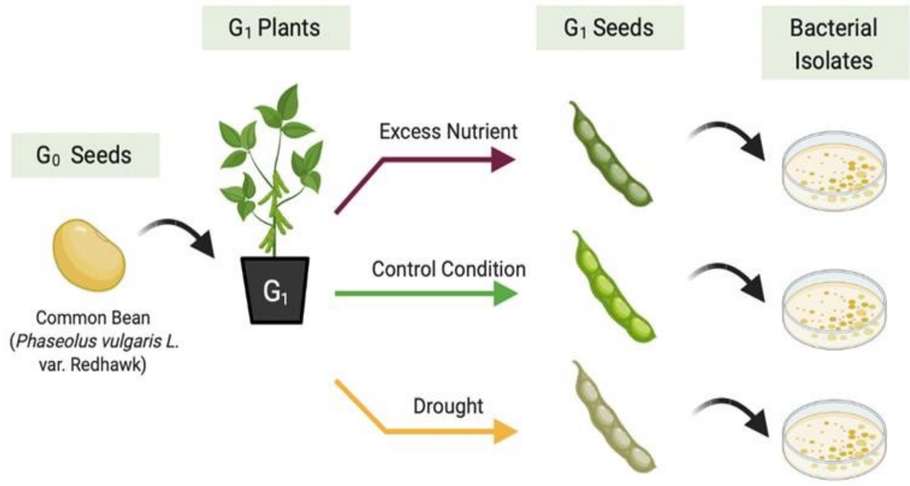

Figure 2. Experimental Design Schematic
(Generation 0 - G0) seeds were planted and grown as G1 plants and experimental conditions of control or abiotic stress were applied. **Control:** Ample water and nutrient solution. **Drought:** Reduced water but maintained nutrient levels. **Nutrient:** Excess nutrient solution applied. Once plants were mature, we collected seeds for microbial community isolation.

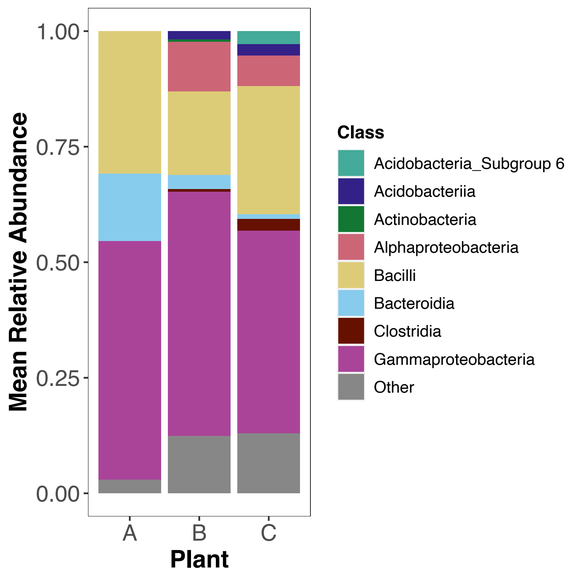

Figure 3. Preliminary Data on Relative Abundance of Seed Microbes[4] (Left)
Our preliminary data gives us an estimate of the relative abundances of the lineages and functions of bacteria found in the seed microbial community. Each bar represents one plant and visually shows the pooled average relative abundance of several microbial lineages.

Table 1. Media Choice
We used lineage data from Figure 3 to inform the media types investigated and chosen to isolate seed bacteria. Final choices in **bold.**

Table 1. Media Choice
Trypticase Soy (TSA)
Brain Heart Infusion (BHI)
Jensen's Media
Nutrient Agar (NA)
(Reasoner's 2A) R2A
Rhizosphere Induction Media (RIM)
Mannitol Salts Agar
Moderate Halophile

Challenges of Isolation

- Seeds are notoriously low microbial biomass systems.
- When bean seeds are ground, grown, or activated, they release antimicrobials which kill/inhibit microbial members.
- Current methods to extract microbes from the seed use sequencing methods to determine the identity of microbial members, and cells are not needed intact.[5]

Tables 2. Experimental Isolation Techniques
Indicates different growth conditions and methodology that were investigated for this project. Final choices in **bold.**

Growth Temperature	Additives
22ºC	Tween-20 (Cell Lysis Agent)
28ºC	**Cycloheximide (Antifungal)**
32ºC	Nicotinamide (Antifungal)
37ºC	

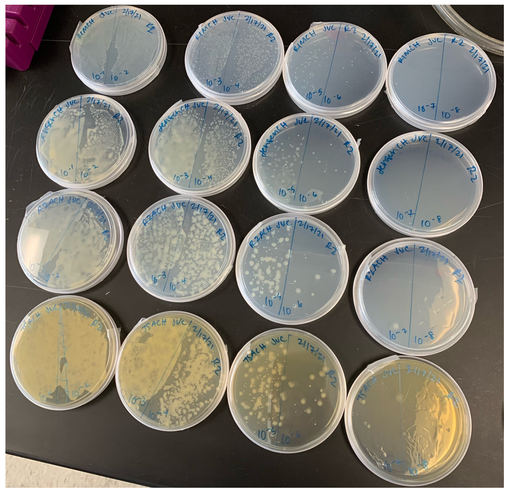

Figure 4. Serial Dilutions
Serial tenfold dilutions were performed to 10^{-8} to attempt to determine the concentration of bacteria in the sample.

Next Steps

1. Continue to develop a working protocol to isolate microbial members of the inherited seed microbiome.
2. Apply protocol to seeds produced from treated G1 plants to identify and cultivate a representative isolate collection.
3. Bio-inoculate future plants to determine what effect isolates have on protecting plants from abiotic stress.

In the future, adding microbes to seeds may be an important mechanism to increase plant tolerance and resilience to environmental stress.

References

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2. Li, H., Parmar, S., & Sharma, V. K. (2019). Seed Endophytes and Their Potential Applications. In 1272071279 939437083 S. K. Verma & 1272071280 939437083 J. F. White (Authors), *Seed Endophytes: Biology and Biotechnology.* Cham, Switzerland: Springer.
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