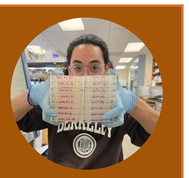
Microbiome Mediated Disease Protection of Tomato Seedlings Against *Pseudomonas syringae pv. tomato*

The Latinxs & the Environment Initiative, NSF Bay Area RaMP



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Introduction

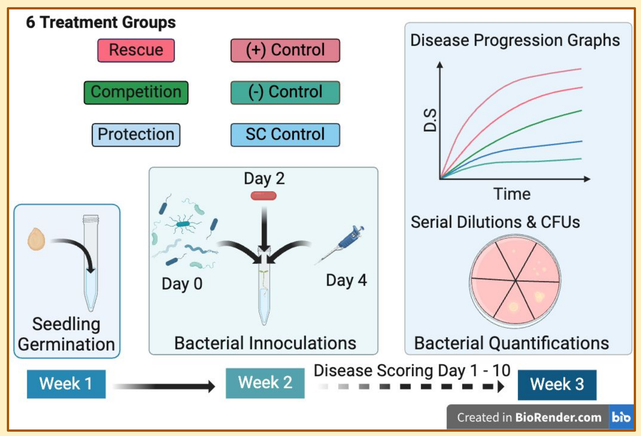
- Until around 2010, most research done on plant-bacterial interactions was done on their root sections. While there is crossover, the environments above and below ground promote the growth of different beneficial and harmful bacteria on plants.
- A notable disease-causing bacteria that exists primarily above ground is ***Pseudomonas syringae pv. tomato*** (*Pst*). *Pst* generally grows on the underside of tomato leaves and flowers before entering the host through natural openings or wounds to cause bacterial speck infection.
- We've previously seen the lab's **Synthetic Community** (SynCom), a group of 16 bacteria, protect juvenile plants (~4+ weeks old) against infection from ***Pst* P23,** a strain of the pathogen. Plants at seedling age (~2 weeks) have naïve immune systems and may be more susceptible to disease.
- Arrival order of bacteria onto the host can also be a determining factor due to bacterial adaptation and evolution. Mutations occur at such a rapid pace that the niche a specific bacteria occupies can vary depending on numerous factors.

Hypotheses

1. The **SynCom can protect** seedlings from disease caused by *Pst* DC3000, our pathogen.

2. There will be **less disease** in seedlings when the **SynCom is established earlier** than *Pst* DC3000.

Methods



Treatment Groups

Treatment Group	Treatment Description	Day 0 Inoculation	Day 2 Inoculation	Day 4 Inoculation
Negative Control	control for no bacterial inoculation	MgCl2 (saline buffer solution)	MgCl2 (saline buffer solution)	MgCl2 (saline buffer solution)
Positive Control	control for DC3000 disease symptoms	MgCl2 (saline buffer solution)	DC3000 (pathogen)	MgCl2 (saline buffer solution)
SynCom Control	control for SynCom symptoms	SynCom (microbiome)	MgCl2 (saline buffer solution)	MgCl2 (saline buffer solution)
Protection	SynCom before DC3000	SynCom (microbiome)	DC3000 (pathogen)	MgCl2 (saline buffer solution)
Competition	Syncom and DC3000 together	MgCl2 (saline buffer solution)	SynCom & DC3000	MgCl2 (saline buffer solution)
Rescue	DC3000 before SynCom	MgCl2 (saline buffer solution)	DC3000 (pathogen)	SynCom (microbiome)

Table 1: Inoculation Order for each Treatment Group

Results

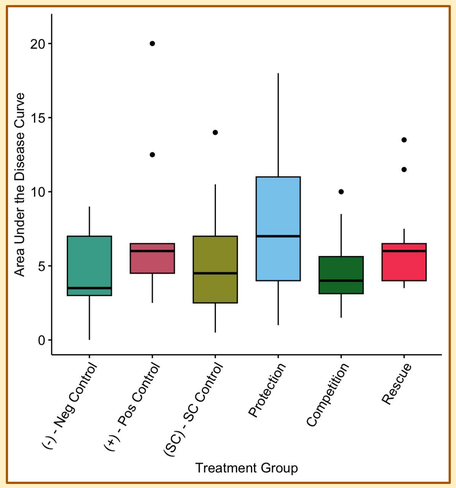


Figure 1: Area Under Disease Curve per Treatment

Figure 1: Starting Day 1, each seedling leaf was scored on a disease severity scale between 0-3. Each day was averaged by seedling, graphed, and analyzed in R with the agricolae package.

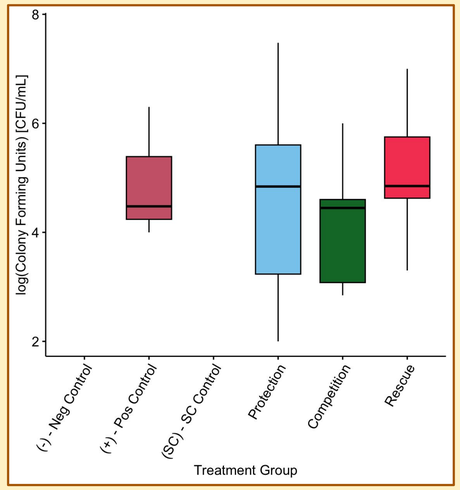


Figure 2: *Pst* DC3000 Colony Forming Units per Treatment

Figure 2: On day 11 after inoculation, seedlings were homogenized, diluted and plated on Rifampicin plates. Plates were incubated @ 28.2 °C and colonies counted after 3 days.

Preliminary Results

There were **no significant differences** of disease progression or DC3000 growth **across the six treatment groups**. Some main contributors to the variation could be:

Seedling Variability

There were three separate seedling trials, each with their own amount of variability across treatment groups. The last set introduced high amounts of noise/variability into the comparisons.

***Pst* P23 vs *Pst* DC3000 Virulence**

Each Pst strain has different capacities for causing disease in plants. The initial concentration of bacteria placed on the seedling may have let P23 infect but not DC3000.

Initial Inoculation

Human errors lead to inoculating the seedlings at a lower initial amount than planned. Together with the difference in *Pst* virulence, the lack of significance between groups is supported.

Next Steps

Additional Seedling Sets

More seedlings are currently growing and will be inoculated in the coming weeks to **repeat the experiment at an elevated inoculum** based on Optical Density. Once the experiment and further analysis are complete, the QR will be updated to reflect the updated results.

Future Directions

I'm currently working on another project that aims to describe indirect interactions between all members of the SynCom and DC3000 through nutrients they consume. If any notable interactions are captured in the data, the seedlings can be **run again with different community compositions** to see the effect on DC3000 virulence.

Acknowledgments & More

Thank you to Alexis, Tiffany, and Britt for all their guidance and support throughout the experiment! Scan the QR code for more about what I do and additional information about the project.

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