Identifying Novel Genetic Pathways for Toxin-Triggered Immune Recognition

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Abstract

Syringomycin E is a lipopeptide produced by bacterial plant pathogen Pseudomonas syringae. - and causes plants to die when introduced to the toxin. We are interested in examining how Syringomycin can support/exemplify the notion of **Toxin-Triggered immunity**, which is a multi-step process. Our results showed that swapping out the most expensive nitrogen source (Histidine) with another organic source of nitrogen (Yeast extract) proved to be the cheapest and most efficient for inducing our toxin. The next steps are to purify our toxin and then run plant-dependent immune assays to find the genes within mutated plants that are responsible for preventing host (plant) death from the Syringomycin - ultimately supporting the idea of toxin-triggered immunity. Understanding the intricacies of plant innate immunity is not only crucial for unraveling the mysteries of plant-pathogen interactions but also holds significant implications for agricultural practices, biotechnology, and ecological balance.

Introduction

- Microbiomes are shaped by various modes of innate immunity. How immunity distinguishes between pathogenic and beneficial bacteria is poorly understood. **Toxin-Triggered Immune Response** is a novel mechanism for shaping host-microbe interactions.

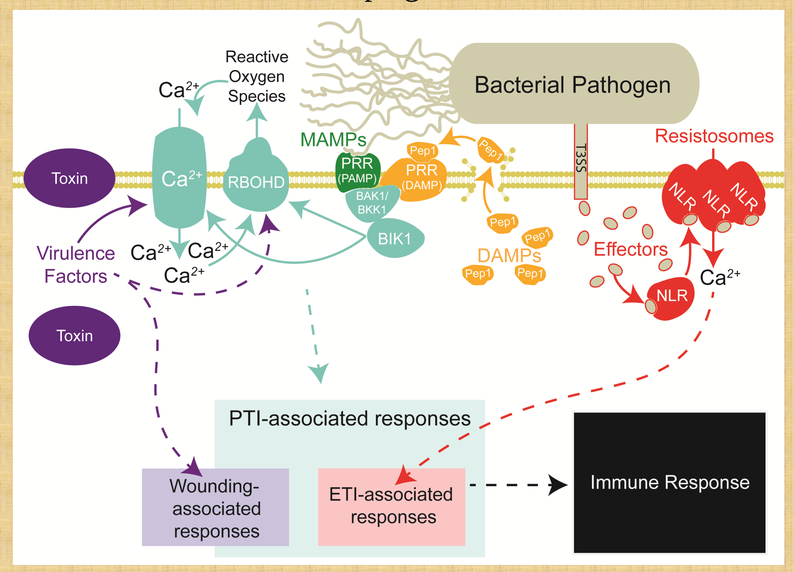

Figure 1. Role of toxins in shaping host-microbe interactions

- Our goal is to identify the gene required for toxin-triggered immune response. We focus mainly on the methods for increasing biosynthesis in the pathogen.

Methods

- Create media using either a Czapek or IMM recipe, with chosen modifications & inoculate with N2C3 and toxin-free Δsyrsyp - shake & incubate at 28°C for 3 days
- Transfer cultures into microcentrifuge tubes and centrifuge at 12,000 RPM
- Separate the supernatant from the pellet (discard the pellet)
- Follow the SN Yeast-Toxicity Protocol (Figure 1) on a welled plate

Table 1

200 uL total volume per well	50% metabolite SN	25% metabolite SN	12.5% metabolite SN	6.25% metabolite SN	3.125% metabolite SN	0% metabolite SN
Toxin-free SN e.g. (syrsyp mutant)	0 uL	50 uL	75 uL	87.5 uL	93.75 uL	100 uL
Toxin SN (e.g. N2C3)	100 uL	50 uL	25 uL	12.5 uL	6.25 uL	0 uL
YPD + antibiotic + yeast inoculation media	100 uL	100 uL	100 uL	100 uL	100 uL	100

Figure 2. Supernatant Yeast Toxicity Protocol

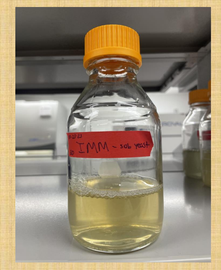

Figure 3. sub-Yeast media

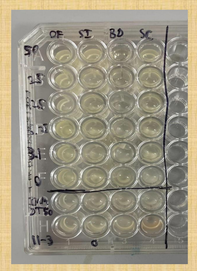

Figure 4. Yeast Toxicity Assay

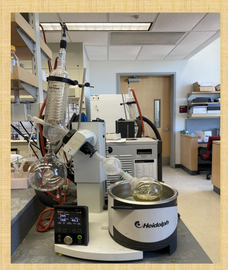

Figure 5. *RotoVap machine*

- Quantify toxin production by measuring the effects of the supernatants on yeast growth.
- Select the media whose bacterial supernatant inhibited yeast growth the most.

Results/Conclusion

- Results indicate that substituting the nitrogen source, L-histidine, with Yeast Extract, and by altering its pH to about 5.5 we were able to make an environment that allowed for Syringomycin induction.
- Our next step is to use acetone which will isolate the toxin from peptides and other compounds in the supernatant ultimately reversing phase chromatography to purify the toxin.
- This purified toxin will be used to screen for genes that are responsible for toxin triggered immunity.

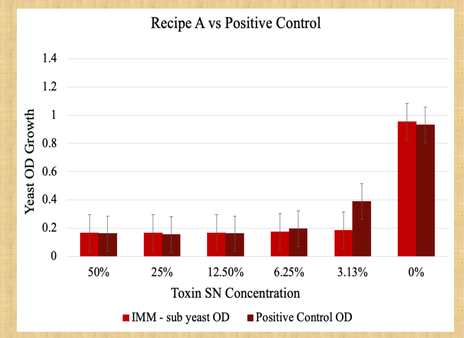

Figure 7. Recipe A (IMM - sub yeast)

	Sub Yeast OD	Positive Control OD
50%	0.166	0.162
25%	0.168	0.157
12.5%	0.167	0.162
6.25%	0.174	0.197
3.13%	0.186	0.389
0%	0.954	0.932

Figure 6. Average yeast growth over 42-hours for 3 trials of the experimental and positive control

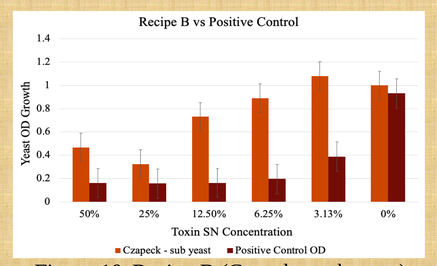

Figure 10. Recipe B (Czapek – sub yeast)

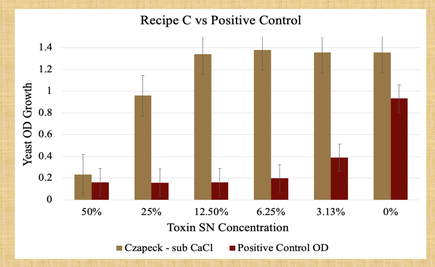

Figure 9. Recipe C (Czapek – sub CaCl)

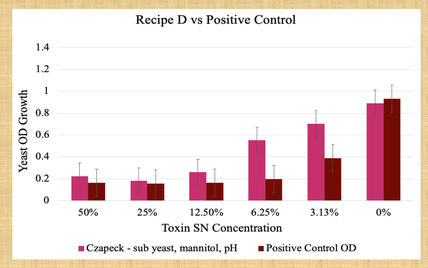

Figure 8. Recipe D (Czapek - sub yeast, Mannitol & pH alteration)

Acknowledgements

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References

Thoms, D., Chen, M. Y., Liu, Y., Moreira, Z. M., Luo, Y., Song, S., Wang, N. R., & Haney, C. H. (2023, January 1). *Innate immunity can distinguish beneficial from pathogenic rhizosphere microbiota*. bioRxiv. https://www.biorxiv.org/content/10.1101/2023.01.07.523123v1.full