Identifying Novel Elicitors of Toxin-triggered Immunity

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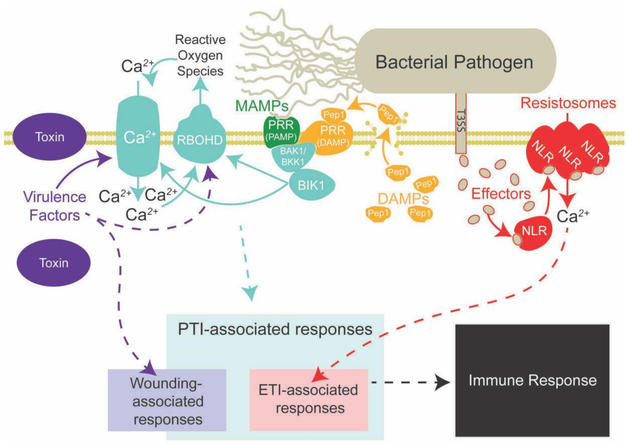
ABSTRACT

A healthy microbiome is essential for the optimal growth and well-being of plants and animals alike. However, beneficial microbes and pathogenic bacteria are often phylogenetically alike and how a host is able to distinguish between the two is not yet understood. In plants, we understand that the pathogenic *Pseudomonas fluorescens* N2C3 strain induces a novel potent immune response while the beneficial *Pseudomonas fluorescens* WCS365 does not. Moreover, this pathogenic immune response is caused by both syringomcyin-dependent and syringomcyin-independent elicitors. To identify the syringomcyin-independent elicitors, an oxidative burst assay with Col-O and BBC triple mutant type plants was performed to measure the ROS (luminescence) response of various Ox-burst treatments via a ROS protocol. The results depict that there is a strong non-syringomycin dependent and MAMP independent immune response being induced by the pathogen suggesting that the novel compound of toxin-triggered immunity is a complex unknown molecule. Looking forward, additional replicates of this experiment will need to be conducted to substantiate these preliminary findings.

INTRODUCTION

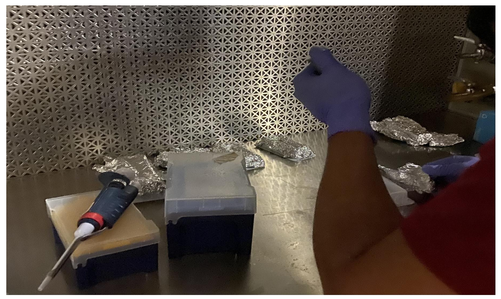


Hypothetical model of plant innate immunity:

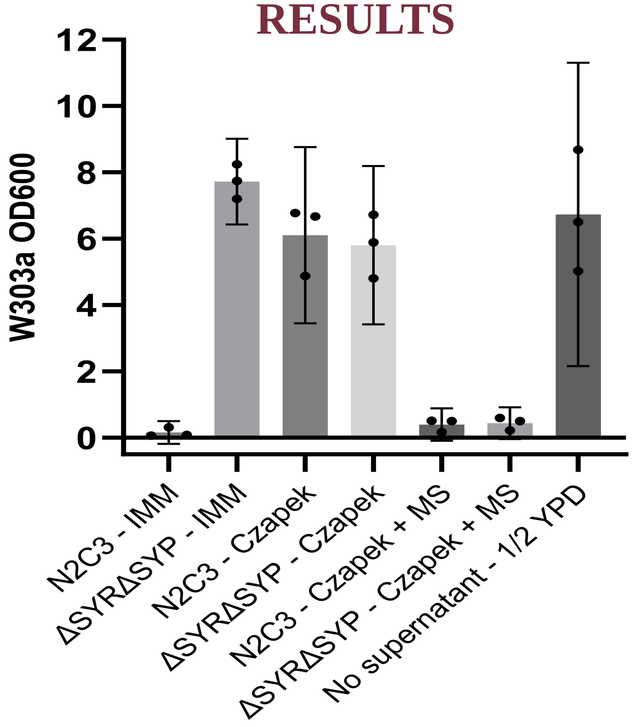
- Pattern triggered immunity uses MAMPs (microbe associated molecular patterns) and plant cell surface PRRs (pattern recognition receptors)
- Intracellular NLRs (nucleotide-binding domain, leucine-rich-repeat-containing receptors) activate immune responses from recognition of effector proteins secreted by pathogens
- New research suggests that plants also possess a novel mode of toxin-triggered immunity
- The underlying mechanism of toxin-triggered immunity is poorly understood.

METHODS

- Used the plant *Arabidopsis thaliana* as the host and *Pseudomonas fluorescens* as the bacterial pathogen
- Used wildtype Columbia-0 and the immune impaired *bak1/bkk1/cerk1* triple mutant
- Grew plants hydroponically in a 96 well tissue culture plates
- Prepared bacterial supernatant from a mutant pathogen strain (impaired for toxin-biosynthesis)
- Used the MAMP elicitor flg22 as a positive control
- Treated supernatants with respective enzyme treatments: pronase, lipase, proteinase, and acetone.
- Prepared a luminol-based oxidative burst assay
- Immediately measured reactive oxygen species via luminescence at 1 min intervals.



RESULTS



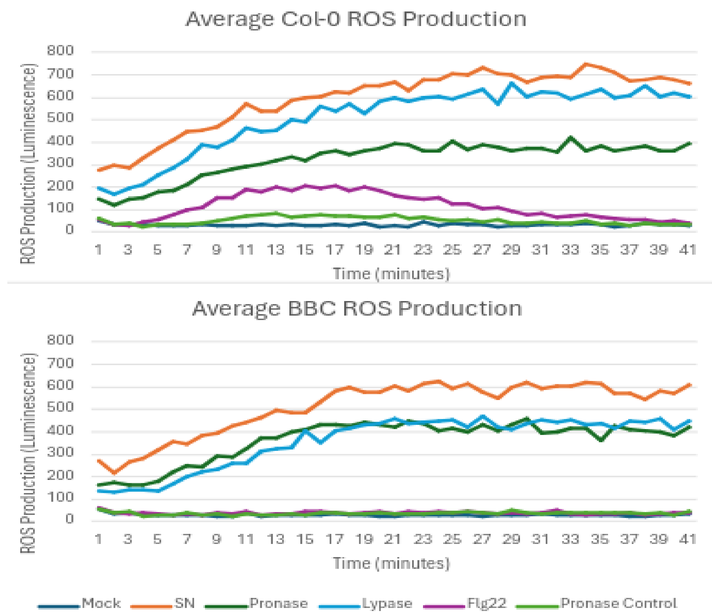


Figure 1: Average ROS response in Col-O and BBC plants from various Ox-burst treatments over a 40- minute time-period.

CONCLUSIONS

In conclusion, there's a strong non-syringomycin dependent and MAMP-independent immune response being induced by the pathogen. Due to the fact that both the pronase and lipase treatments failed to inhibit a strong ROS response in the plants, the molecule responsible for this reaction is likely not a protein nor a lipid. However, lipase, unlike pronase, is a singular enzyme and as such there are some lipids that it is unable to degrade. In the future, further experimentation will hopefully shed light onto what type of complex molecule is underlying this response in plants using chromatography to isolate the compound.

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REFERENCES

1. Thoms, David, et al. "Innate Immunity Can Distinguish Beneficial from Pathogenic Rhizosphere Microbiota." bioRxiv, Cold Spring Harbor Laboratory, 1 Jan. 2023, www.biorxiv.org/content/10.1101/2023.01.07.523123v1.full.