Exploring the Diversity of *Peronospora belbahrii* effector proteins

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Abstract

Effectors are small, secreted proteins used by a pathogen to infect a plant host. In this experiment, we investigated the presence of computationally-predicted effector proteins from the downy mildew *Peronospora belbahrii*, which infects basil plants. In recent years there have been reports of *P. belbahrii* infecting previously resistant basil cultivars, suggesting that the pathogen may be changing to colonize these new hosts. Because changes in virulence are likely tied to changes in the effector proteins which make this process possible, we began our investigation with first choosing two predicted effector proteins, REL1 and REL2, and testing for their presence across all of our collected isolates.

Background

Effectors: Small secreted proteins (generally <150 aa) needed for host colonization.

Why do we care?

- Basil is the most cultivated herb worldwide and downy mildew causes significant crop loss every year
- Understanding the mechanism of infection can speed development of resistant basil cultivars
- Due to the small size of these proteins, nucleotide polymorphisms which change even single amino acids may be significant

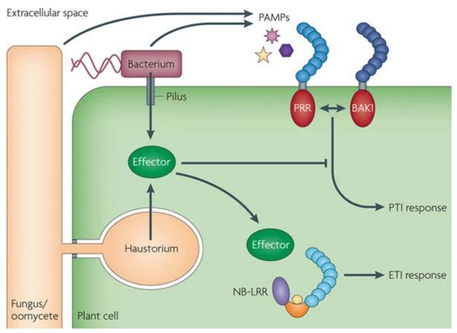

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Figure 4. A broad overview of plant immunity. Most oomycete pathogens, such as *P. belbahrii*, use small effector proteins to manipulate the immune response of their plant host. The pathogen is able to penetrate the cell wall of the hosts cells, and then secrete effector proteins which remain outside the cell membrane (apoplastic) or are taken into the host cell (cytoplasmic). These proteins may actively suppress the plants immune system, preventing a PTI response, or may induce effector-triggered immunity (ETI), depending on the pathogens mechanism of colonization, and the presence of resistance-conferring genes in the host. Image from: https://www.nature.com/articles/nrg2812

METHODS

Using a list of computationally-predicted effectors and the published *P. belbahrii* genome, primers were designed for eight different predicted effector proteins. Then, the presence of these effectors was successfully confirmed in a cultivated pathogen isolate using polymerase chain reaction (PCR).

Two effectors, REL1 and REL2, were chosen due to their amplification across all of our isolates, significant difference in size and structure, and a potential insertion event observed in the REL2 sequence.

RESULTS

1. Isolate Collection & Information

- Obligate pathogen, *P. belbahrii* isolates used in this study were collected directly by the research group, or shipped from the New England/New York area from 2018-2020
- Basil cultivars were grown in the UMass research greenhouse, inoculated with *P. belbahrii* sporangia
- One week after inoculation, infected basil plants were subjected to high humidity to force sporulation of downy mildew, which were then collected and stored at -80C

Figure 1. *Peronospora belbahrii* (basil downy mildew) sporulating on the underside of an infected *Ocimum basilicum* (sweet basil) leaf. Image from: https://apsjournals.apsnet.org/doi/10.1094/PHYTO-02-15-0032-FI

2. Validation of effectors identified through meta-transcriptomics

- All eight effectors successfully amplified in isolate KAMA.20

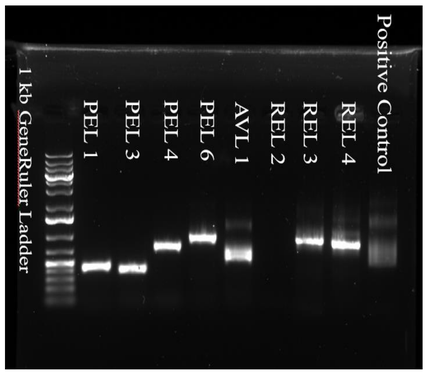

Figure 2. Visualization of effector coding sequence PCR products after gel electrophoresis using 1% agarose. REL2 was later successfully amplified in the same isolate, but not pictured. All amplified effector coding regions matched the anticipated size according to the 1 kb GeneRuler Ladder.

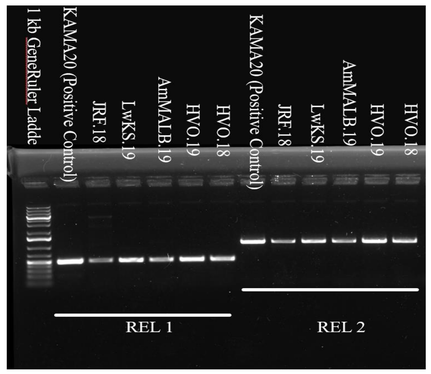

Figure 3. Visualization of PCR products of REL1 and REL2 coding sequences from six distinct *P. belbahrii* isolates after gel electrophoresis using 1% agarose. The labels above each lane indicate the isolate in which the effector gene was amplified.

3. Detection of two effectors among six isolates

- Six distinct isolates collected from New England and New York, from 2018 through 2020
- REL1 & REL2 successfully amplified using the same primer set in all six isolates
- Small size differences in REL1 observed using gel electrophoresis

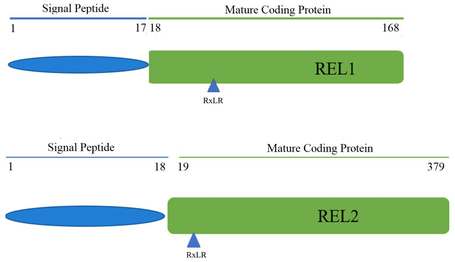

Figure 5. Gene maps of REL1 and REL2 effector proteins. The blue oval represents the signal peptide, which is cleaved during the excretion process. The green rectangle indicates the coding region of each effector. The approximate location of the RxLR motif is indicated by the blue triangle. The length of each component is indicated in amino acids.

Figure 6. Structural predictions for REL1 and REL2 coding sequences, generated using the RaptorX web server, hosted by the Xu Group at the University of Chicago. The color of the residues corresponds to their chemical properties: blue = acidic, magenta = basic, and white = unknown. Prediction software available at: http://raptorx.uchicago.edu/documentation/

4. Sequence divergence among REL1 and REL2

- Multiple SNPs observed in preliminary sequencing results for REL1 & REL2
- Identified need for more high-fidelity sequencing of effectors across collected isolates

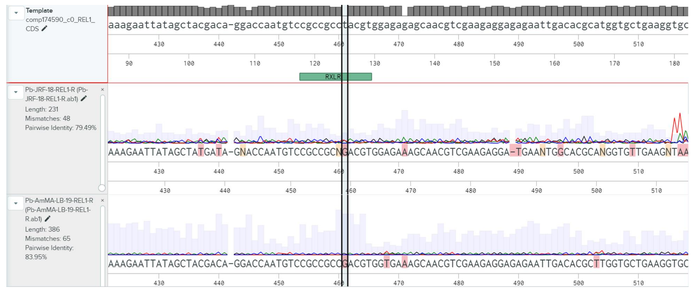

Figure 7. Multiple sequence alignment of the coding sequence of effector REL1 from the published *Peronospora belbahrii* genome available from Michigan State University on NCBI. The RxLR motif is highlighted in green, and a single nucleotide polymorphism (SNP) of interest is highlighted at position 461 on all three sequences. The change from a CTA codon to CGA is a nonsynonymous mutation. Investigation into the structural impact of these SNPs across our collected isolates is still ongoing. Multiple sequence alignment was completed using Benching available at: https://benchling.com

CONCLUSION/ DISCUSSION

- Ability to amplify eight effectors in a cultivated isolate, using primers designed from the reference genome, suggests conservation across population
- Presence of REL1 & REl2 across all collected isolates indicate an important role in the colonization process
- Observed SNPs and size differences across REL1 and REL2 must be further investigated to determine effect on overall protein structure

FUTURE DIRECTIONS

- Develop a more diverse collection of *P. belbahrii* isolates to observe if effectors are highly conserved among a wider population
- Refine the sequencing of all effectors across the currently collected isolates to better understand present nucleotide polymorphisms and their structural impacts
- Whole genome sequencing of *P. belbahrii* isolates to understand emerging speciation among the pathogen population and more precisely compare differences in effector coding sequences.

References

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