The investigation of plant-microbe interactions associated with noroviruses and leafy greens

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Pre-Harvest Contamination of Leafy Greens

Brandl, 2006
Do plants recognize norovirus as a plant pathogen?
Questions and Objectives:

1. Does norovirus elicit an immune response in leafy greens and, if so, which pathway is used?
2. How are noroviruses recognized (MAMPs; PAMPs)? What is the receptor (i.e. sialic acid receptors)?
3. Do plant hormones associated with plant defenses, such as salicylic acid, affect the persistence of internalized norovirus?
4. How do noroviruses interact with the epiphytic microbiota within the phyllosphere of plants?
5. Can we utilize biocontrol agents to reduce contamination and persistence of norovirus on plants?
Summary of Methods

- **Plant immune response studies:**
  - All plants were grown in either sterile magenta boxes (*A. thaliana*) or within a controlled growth chamber (Romaine lettuce).
  - For immune response studies, whole plants (*A. thaliana*) or leaves (Romaine lettuce) were pooled into one sample and frozen in liquid nitrogen prior to total plant RNA extraction and semi-quantitative PCR.
  - Gene expression was quantified using markers for specific plant immune response pathways.

- **Salicylic acid persistence studies:**
  - Salicylic acid was dissolved in HBSS and persistence studies were performed in 15 ml tubes at room temperature for 20 min.
  - SA was neutralized with FBS and frozen at -20 C until total viral RNA extraction was performed prior to qPCR.
Effects of MNV on the immune response of plant model *Arabidopsis thaliana*

Average Fold Change in Expression from Control

- PR-1
- PDF1.2
- Vsp2a

Hours Post Inoculation

0  2  4  6  8  10  12

0  2  4  6  8  10  12
Effects of human norovirus GI.6 on the immune response of Romaine lettuce
Effects of plant signaling hormone salicylic acid on the persistence of norovirus surrogates

* Samples were neutralized with FBS 20 min post-inoculation. Infectivity assays have not yet been performed.
Conclusions

• Immune response studies in *A. thaliana* as well as lettuce indicate plants do recognize noroviruses through potential MAMPs.

• ISR and SAR may be induced.

• MNV and TV were shown to persist at levels comparable to controls in up to 500 mM (pH 2.4) of salicylic acid for 20 min indicating the ability of internalized norovirus to survive in plants.
Future Research

• Immune response of *A. thaliana* to MNV, TV and HuNoV as well as “knockout” plant lines.

• Immune response of Romaine lettuce to surrogates MNV and TV. Wound and non-wound inoculations.

• Persistence of noroviruses in salicylic acid for up to 24 h (including infectivity assays).

• Interactions of noroviruses in biofilms associated with the natural microbiome of leafy greens (ie. *Pseudomonas*).

• How the application of PGPR (UD1022) effect the persistence and internalization of norovirus on leafy greens through plant immune response.
Thank you, NoroCORE!