Characterization of Type Three Effectors from *Pseudomonas syringae* pv. *tomato* DC3000

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Why should we care about plants or plant pathogens?

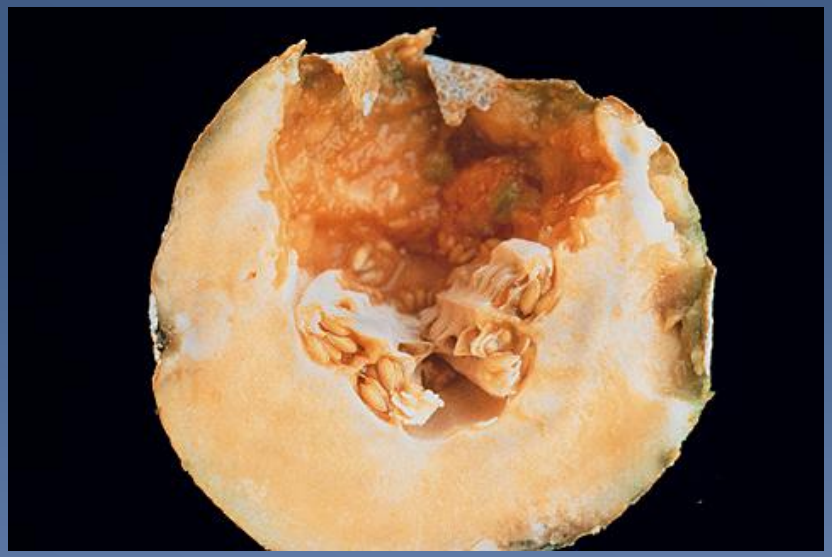
- Agriculture is essential for food production
- In the U.S. 10-20% of crops are lost to disease annually
- Billions of dollars each year
- Threat to food availability

(http://www.reeis.usda.gov/web/crisprojectpages/198484.html)

(http://www.gov.mb.ca/agriculture/crops/diseases/fac43s00.html)

Plants are susceptible to disease

Bacterial speck disease:
Pseudomonas syringae



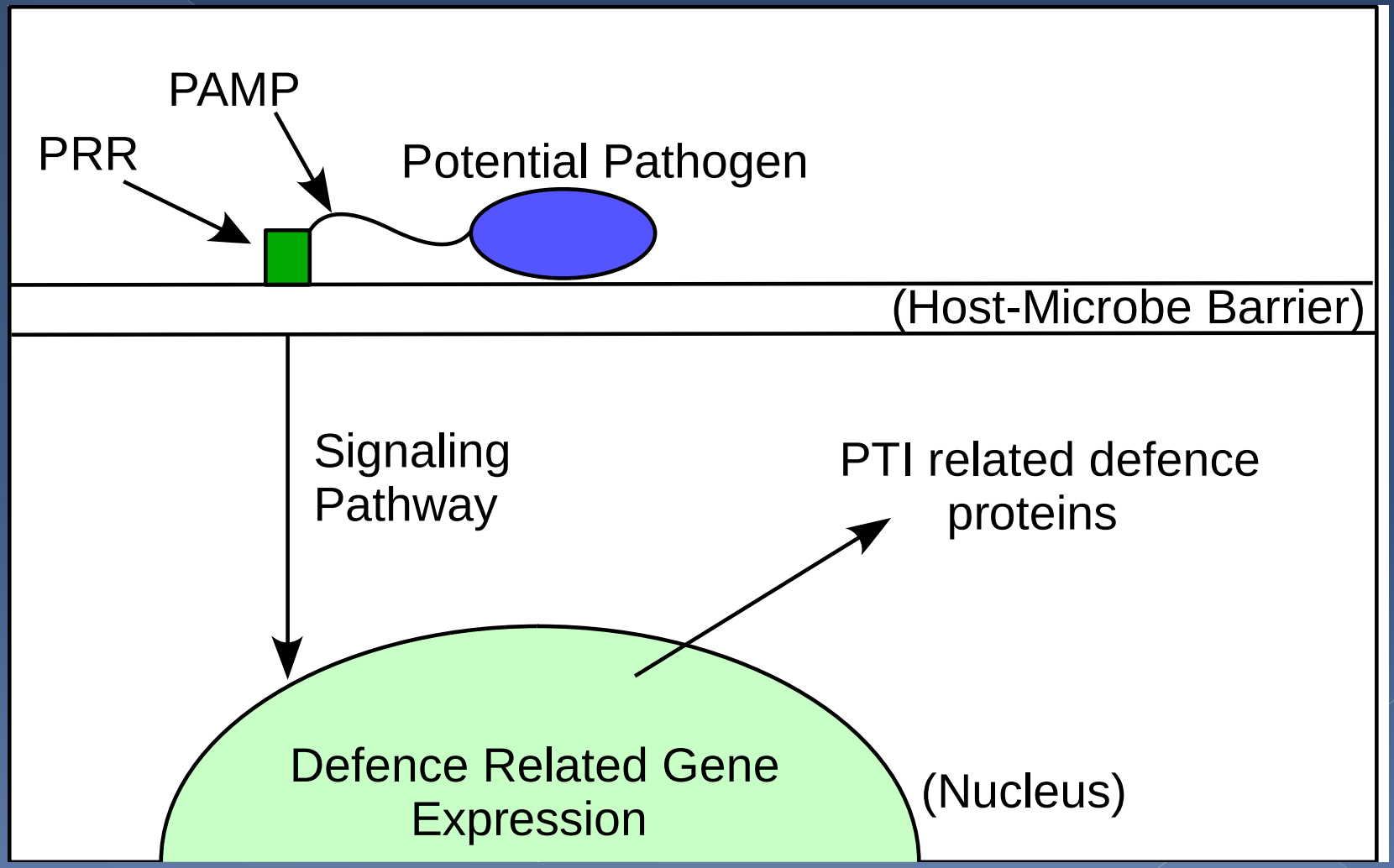
Bacterial soft rot:
Erwinia carotovora

Pictures courtesy of www.apsnet.org/education/IntroPlantPath

Plants have immune systems for protection against pathogens

- Two branches of immunity
- First branch: PAMP-Triggered Immunity (PTI)
- PAMP = Pathogen Associated Molecular Pattern
  - ex- Flagella protein: flg22
- Plant Pattern Recognition Receptors (PRRs) detect PAMPs
- Broad range detection

PAMP-Triggered Immunity

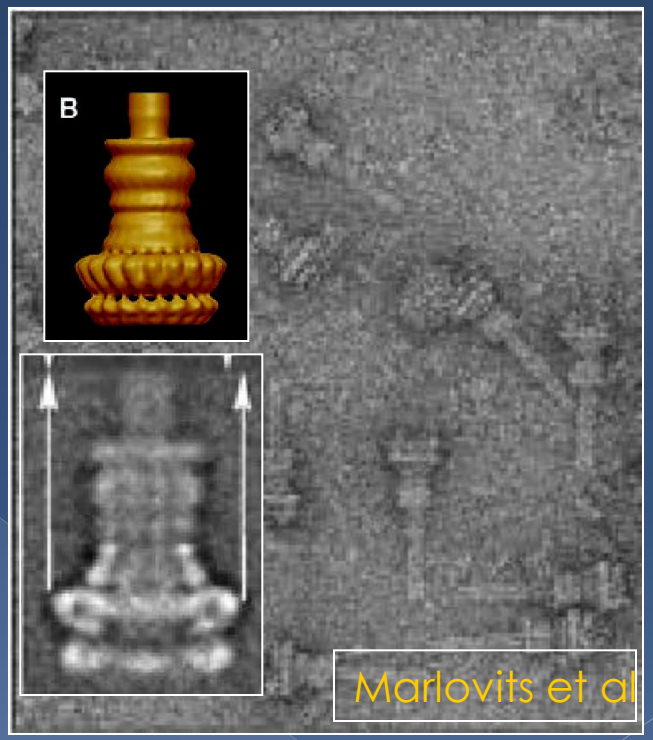


PTI-elicited defense

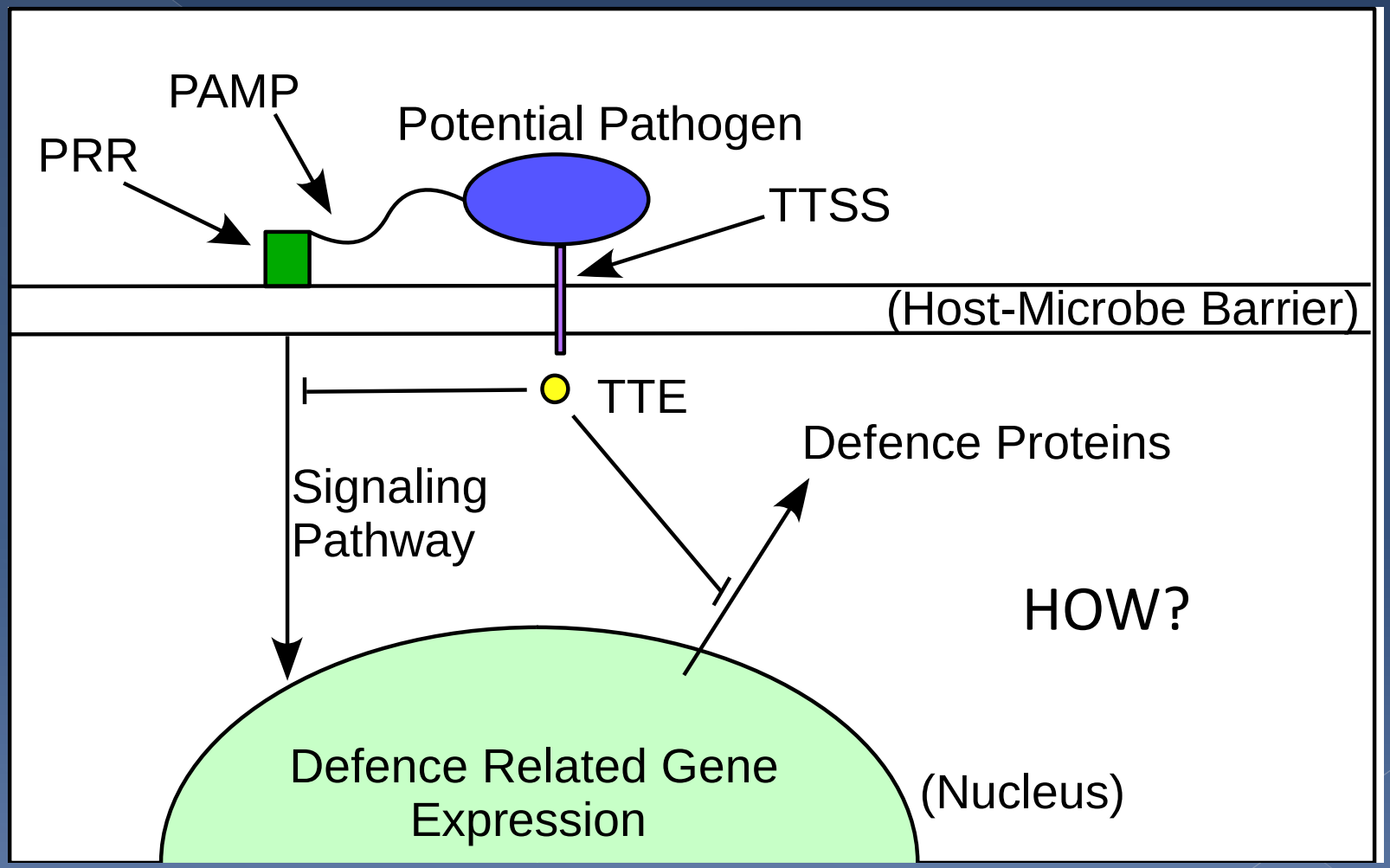
- Callose deposition in plant cell wall
- Goal: Increase physical barriers to help limit an infection
- Callose deposition can be used as a way to measure the response of PAMP-triggered immunity

How are bacteria able to infect a plant?

- Many Gram-negative bacteria use a type three secretion system (TTSS)
- Molecular syringes
- Injected proteins are known as type three effectors (TTE)



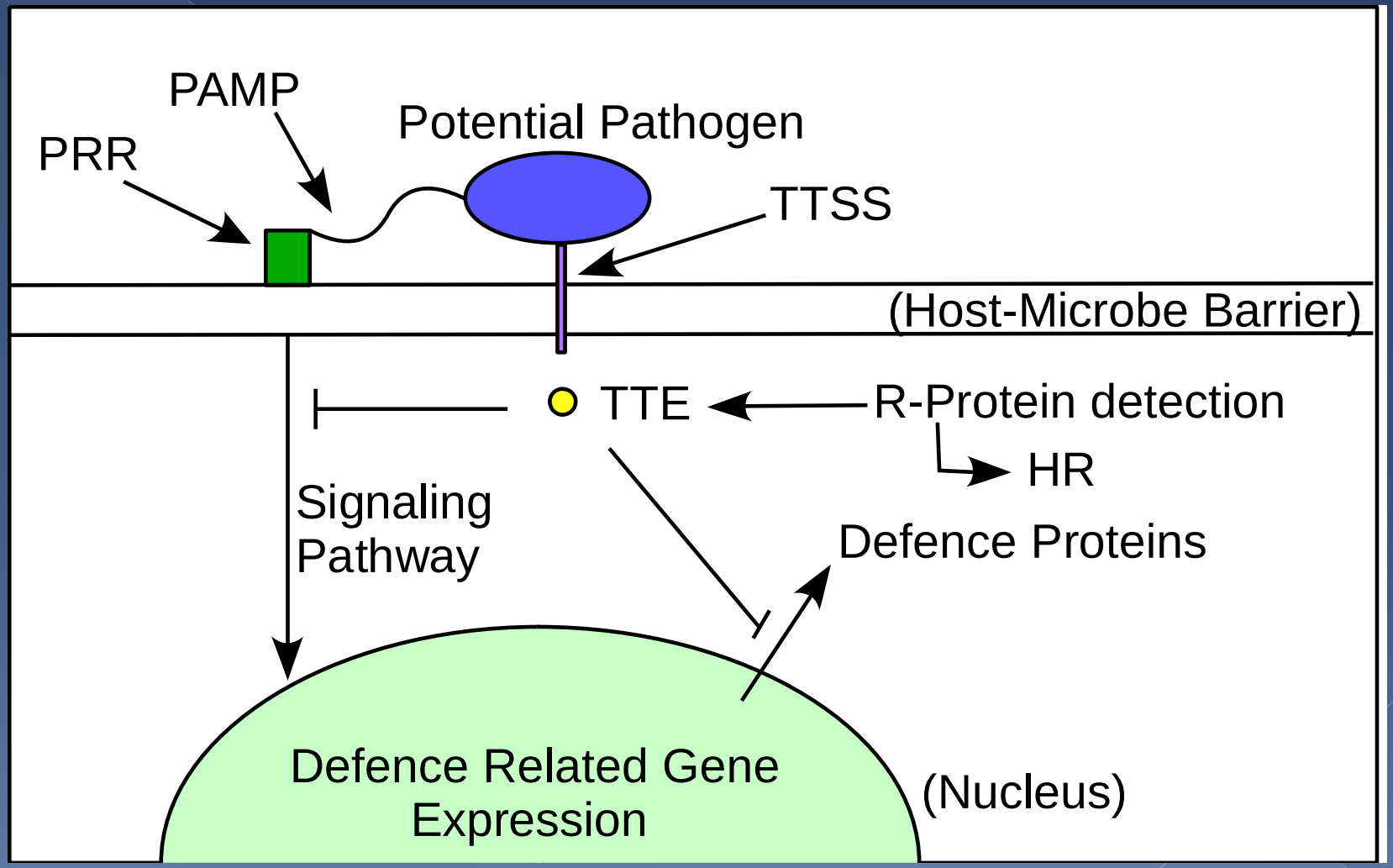
Type Three Effector Function



Second Branch of Plant Immunity

- Effector-Triggered Immunity (ETI)
- Plant R-proteins recognize TTEs
- R-proteins can only detect specific TTEs
- If R-proteins do not recognize any of the TTEs secreted by the pathogen, susceptibility occurs

Effector-Triggered Immunity



ETI Response

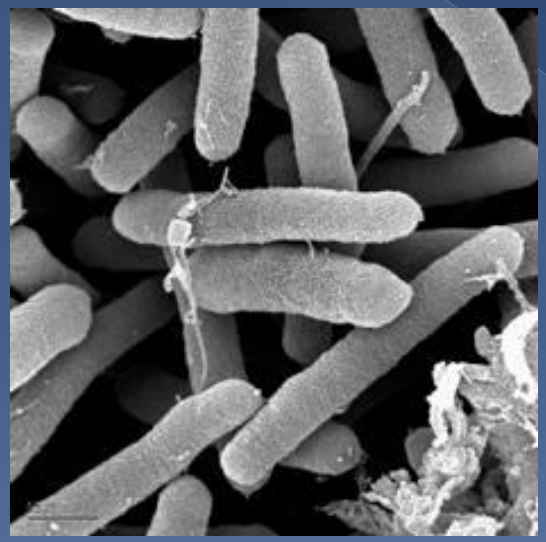
- Detection of effectors will cause a hypersensitive response (HR)
- Goal: Sacrifice cells to save organism
- HR can be used to measure ETI response

Review

PAMP-Triggered Immunity	Effector-Triggered Immunity
Broad range detection	More specific detection
Uses pattern recognition receptors (PRRs)	Uses R-proteins
Detects PAMPs on bacteria	Detects effectors inside the host cell
Read-out: Callose deposits	Read-out: Hypersensitive response (HR)
Less robust	More robust

The Research

- Characterizing individual type three effectors
- Pathogenic *Pseudomonas syringae* pv. *tomato* DC3000



http://wishart.biology.ualberta.ca/BacMap/includes/species/Pseudomonas_syringae.png
http://microbiology.msu.edu/97.html

Type Three Effectors of DC3000

- TTEs from DC3000 are not recognized by arabidopsis
- Sequencing information has made it possible to identify and clone the TTEs from DC3000
- Ultimate goal: What are the effectors doing in the plant?

T3Es	T3Es
HopE1	HopX1
SchN1-HopN1	SchM1-HopM1
HopC1	HopQ1-1
HopAM1-2	HopAA1-2
HopY1	HopAA1-1
HopAM1-1	SchA-HopA1
HopP1	ShcV1-HopV1
HopAF1	HopAB2
AvrPto1	HopR1
HopH1	HopAO1
HopK1	AvrE1
HopI1	HrpK-HopB1
HopG1	HopD1
SchO1-HopO1-HopT1	SchF2-HopF2-HopU1

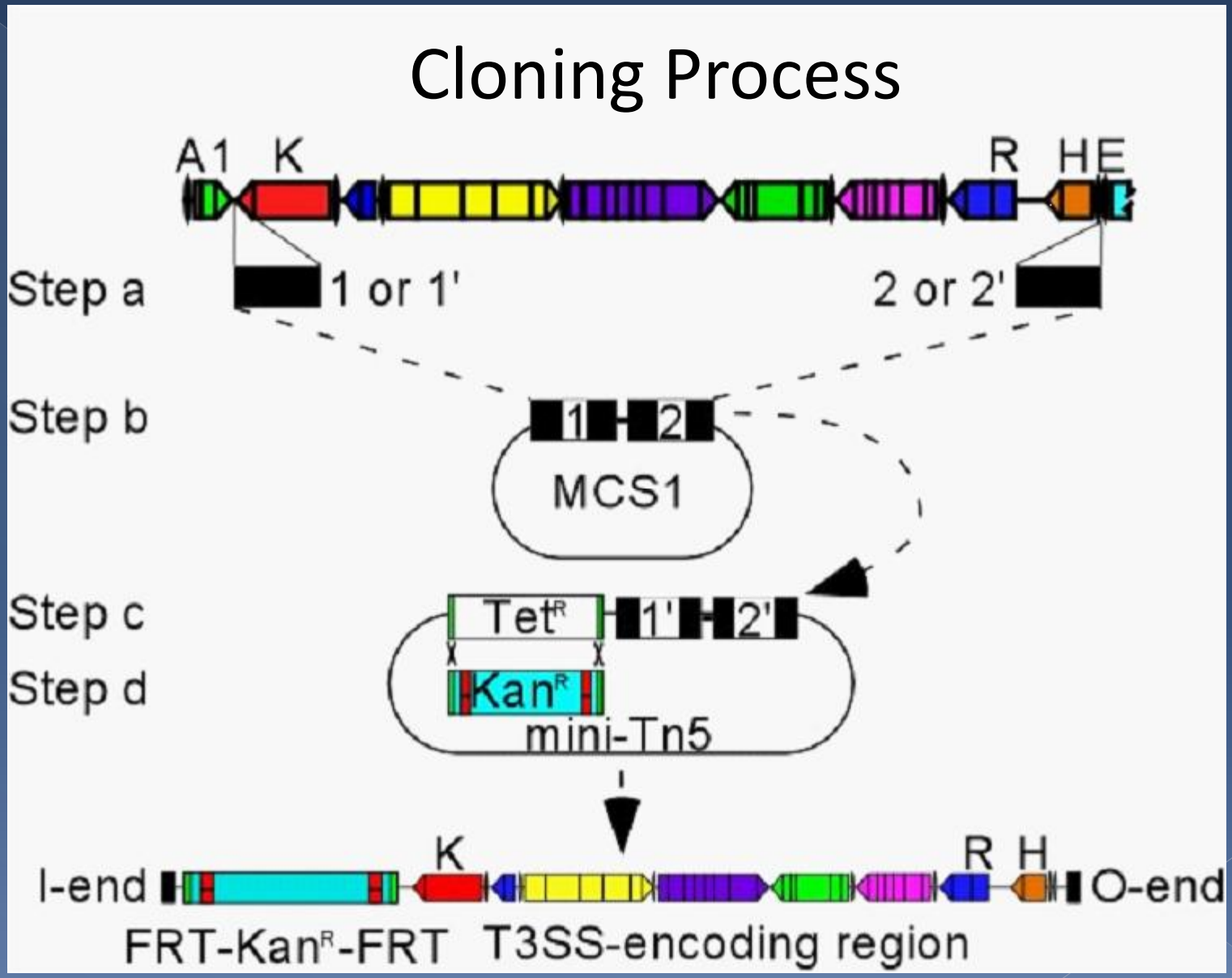
Challenge

- For accurate data, we want to study TTEs that are delivered into a host by bacteria
- Problem- DC3000 delivers all type three effectors at once
- Needed to design a single effector delivery system for individual TTE characterization

The Effector to Host Analyzer: EtHAn

- Approach:
  - Use recombineering to clone out TTSS encoding gene cluster from *P. syringae*
  - Integrate into a non-host associated bacteria (Pf0-1)
- Only contains genes necessary to build a functional TTSS

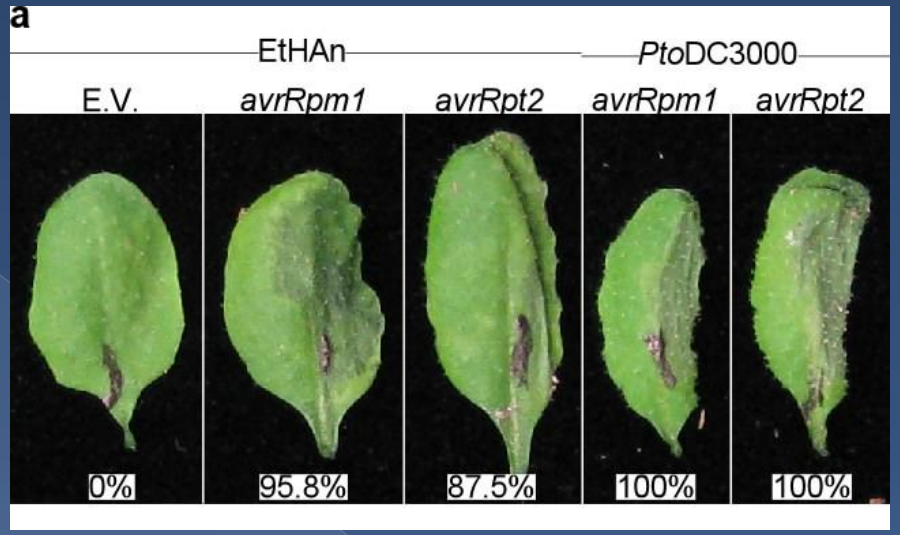
Cloning Process



EtHAn Validation

HR Assays

- First test: carrying an effector on a plasmid
- Gateway::Δ79AvrRpt2
- Confirm delivery



Growth Curve

- EtHAn does not grow in arabidopsis or produce a phenotype

Questions

1. Are individual TTEs able to significantly suppress PTI?
2. Do individual TTEs cause a phenotype in Arabidopsis?
3. Where do proteins go once secreted into the plant?
4. What proteins do TTEs interact with?

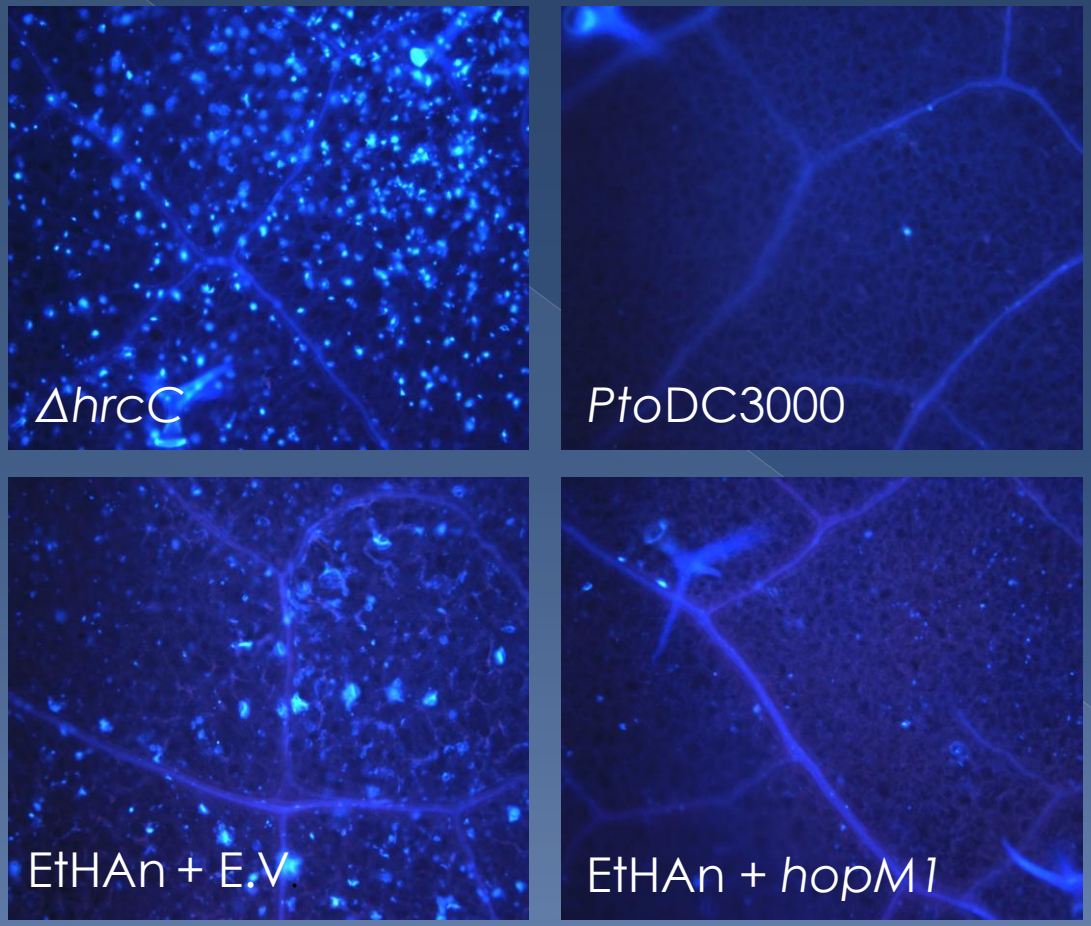
Individual Effector Characterization

- Clone all of the delivered effector genes out of DC3000
- Use Gateway cloning system to put all effectors into several different vectors
- Equals over 60 different clones per strain

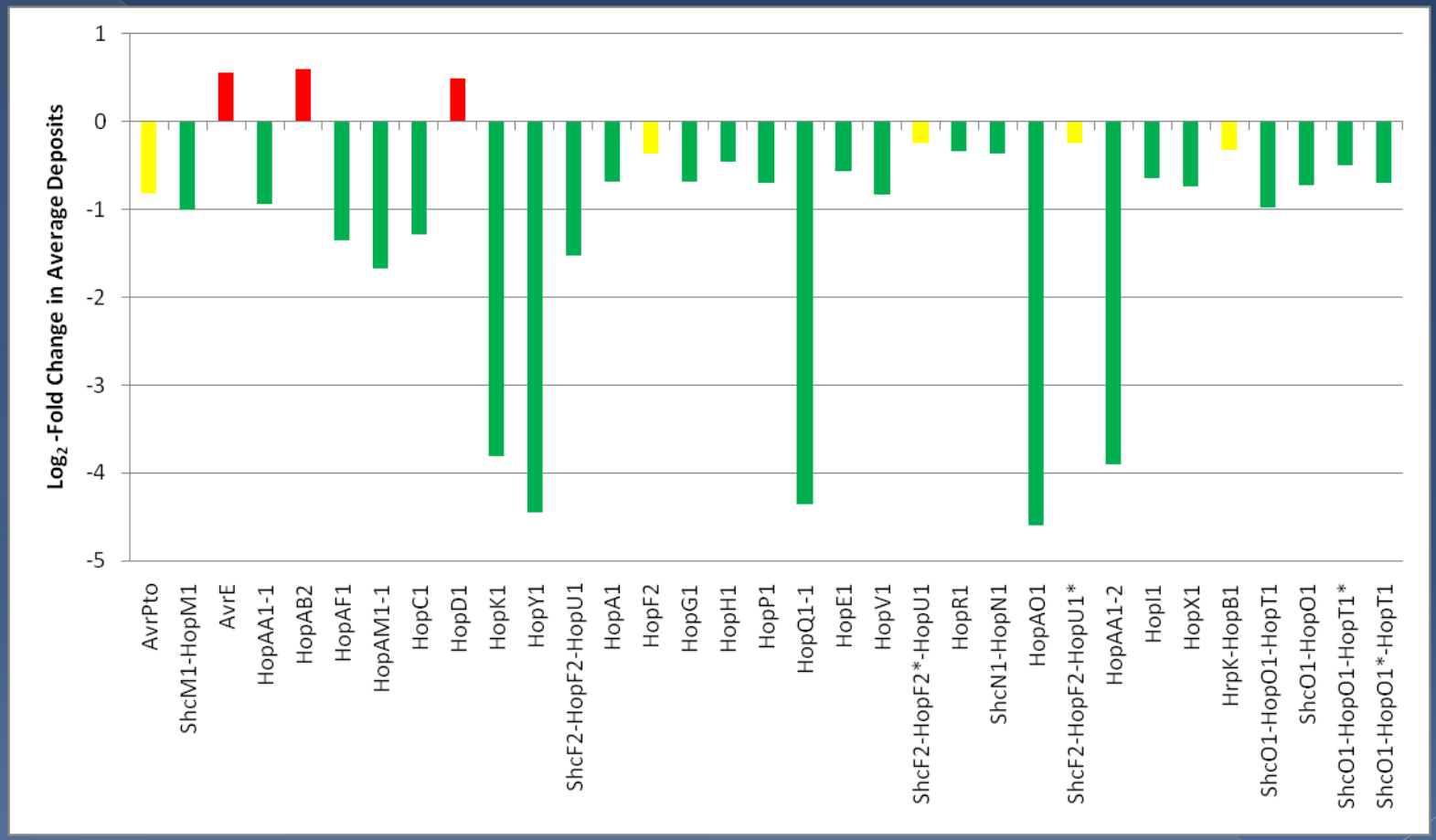
Vector	**Purpose**
Gateway::Δ79AvrRpt2	HR testing
Gateway::HA	Callose deposits, phenotyping
Gateway yeast-two hybrid	Effector-protein interactions
Gateway::YFP binary	Effector localization

Callose Assay

- Can individual effectors suppress PTI response?



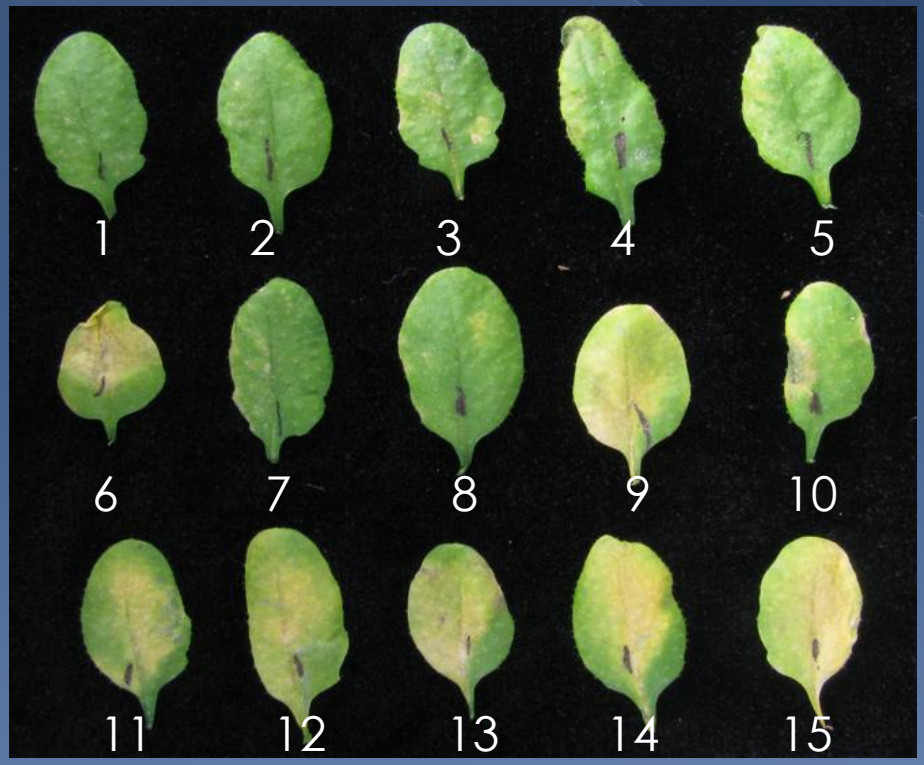
Callose Data



Green Bars: P-Value <0.01
Yellow Bars: P-Value <0.05

Phenotyping

- Do individual effectors cause a phenotype in arabidopsis?



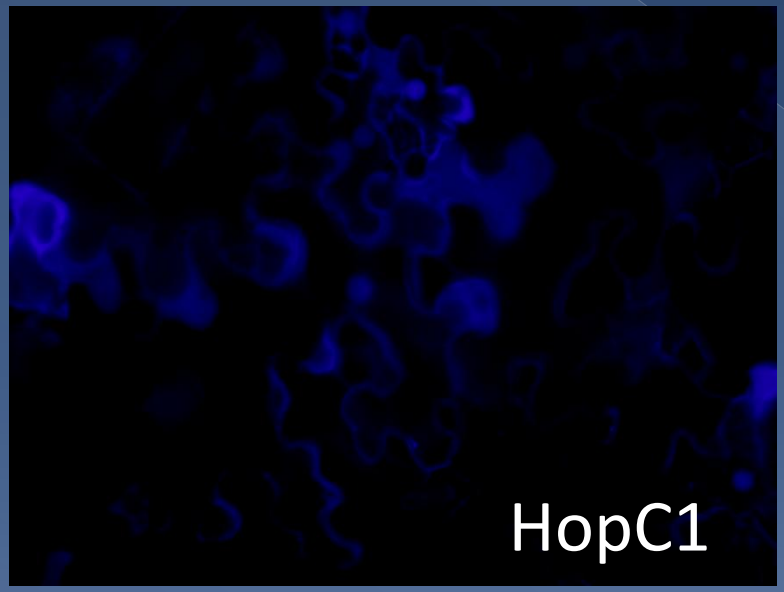
Leaf #	Effector	Leaves responding
1	Pf0-1	5/18
2	EtHAn	8/18
3	ShcM1-HopM1	14/18
4	HopE1	12/18
5	HopD1	7/18
6	ShcF2-HopF2	10/18
7	HopAM1-2	6/18
8	HopX1	8/18
9	ShcF2-HopU1	13/18
10	HopC1	10/18
11	ShcF2-HopF2-HopU1	13/18
12	HopK1	12/18
13	HopP1	15/18
14	HopAA1-1	14/18
15	ShcA-HopA1	15/18

Defining a “pathogen”

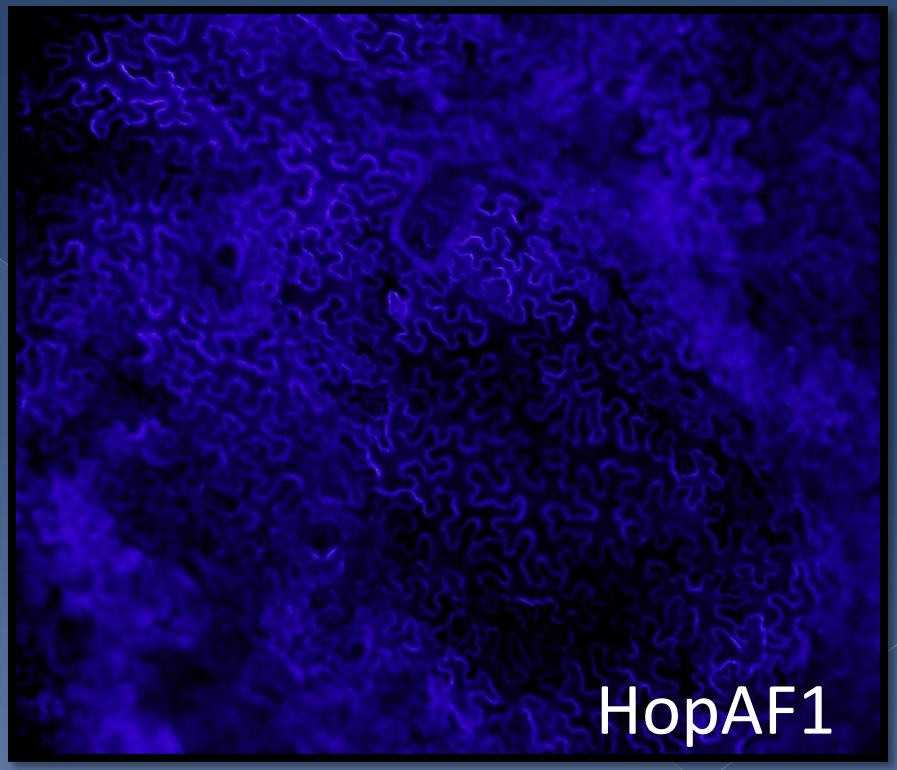
- Does disease mean a pathogen?
- Tested effectors with phenotype – no growth

Preliminary localization Data

- In collaboration with Brad Day's lab at Michigan State University



Nucleus/ PM?



Plasma Membrane

Conclusions

- We have developed a new approach for delivering individual TTEs into host cells
- EtHAn can be used to characterize TTE
- Most TTEs are capable of blocking host PTI defense
- Some individual TTEs allow EtHAn to cause disease-like symptoms
- TTEs are able to localize to specific areas in the plant cell

Future Directions

- Use the yeast-two hybrid clones to determine protein interactions with TTEs
- Define “phytopathogen”
- Observe phenotypes from TTEs in immuno-compromised arabidopsis

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