

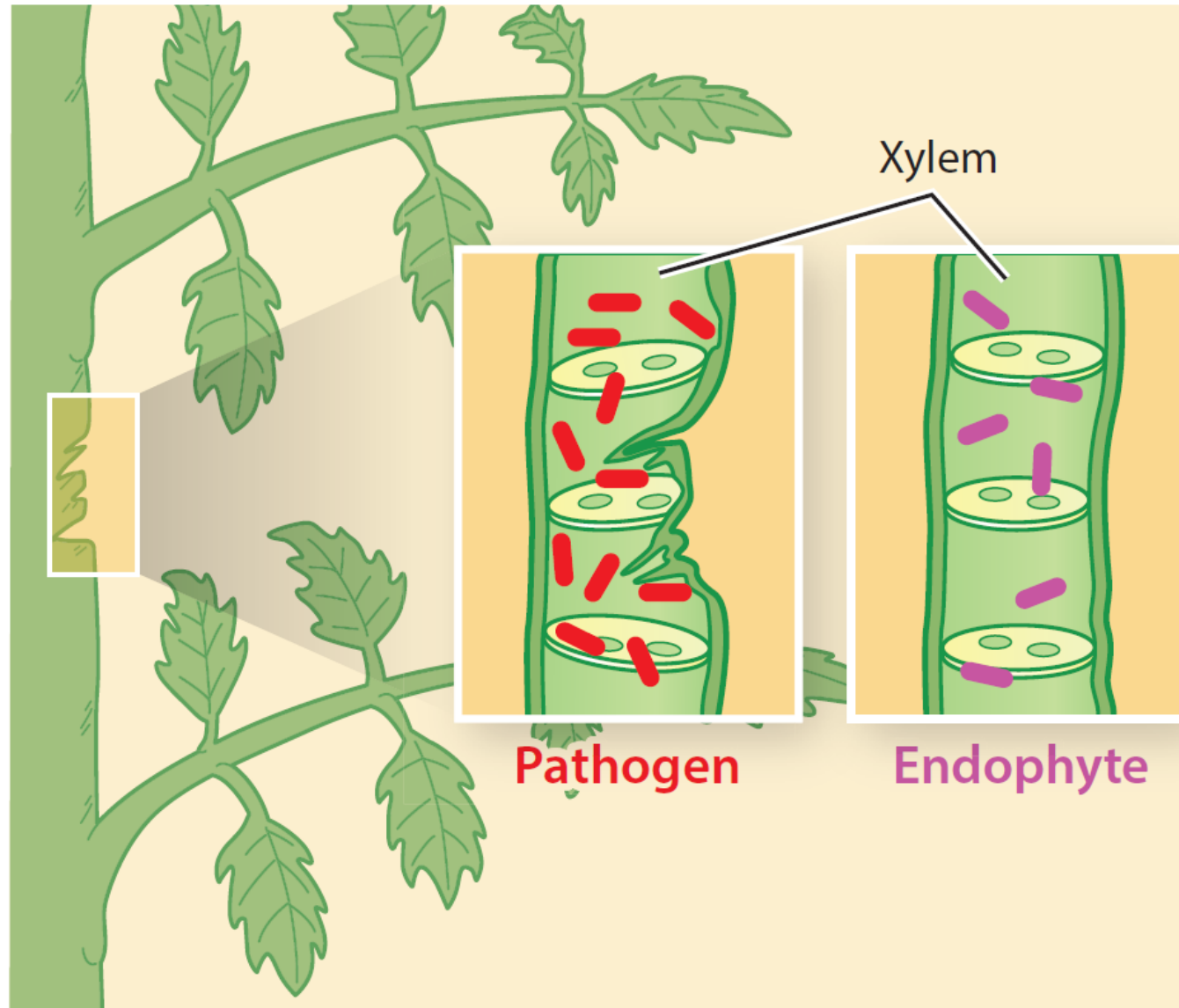


Bacterial canker of tomato

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Clavibacter



- **Host:**
monocots,
dicots
- **Disease:**
cankers, ring
rot, wilts, and
blights
- **Key features:**
xylem-
colonizing,
seedborne

Bacterial canker of tomato caused by *C. michiganensis*

Leaf yellowing/necrosis



Necrotic lesions



Stem cankers

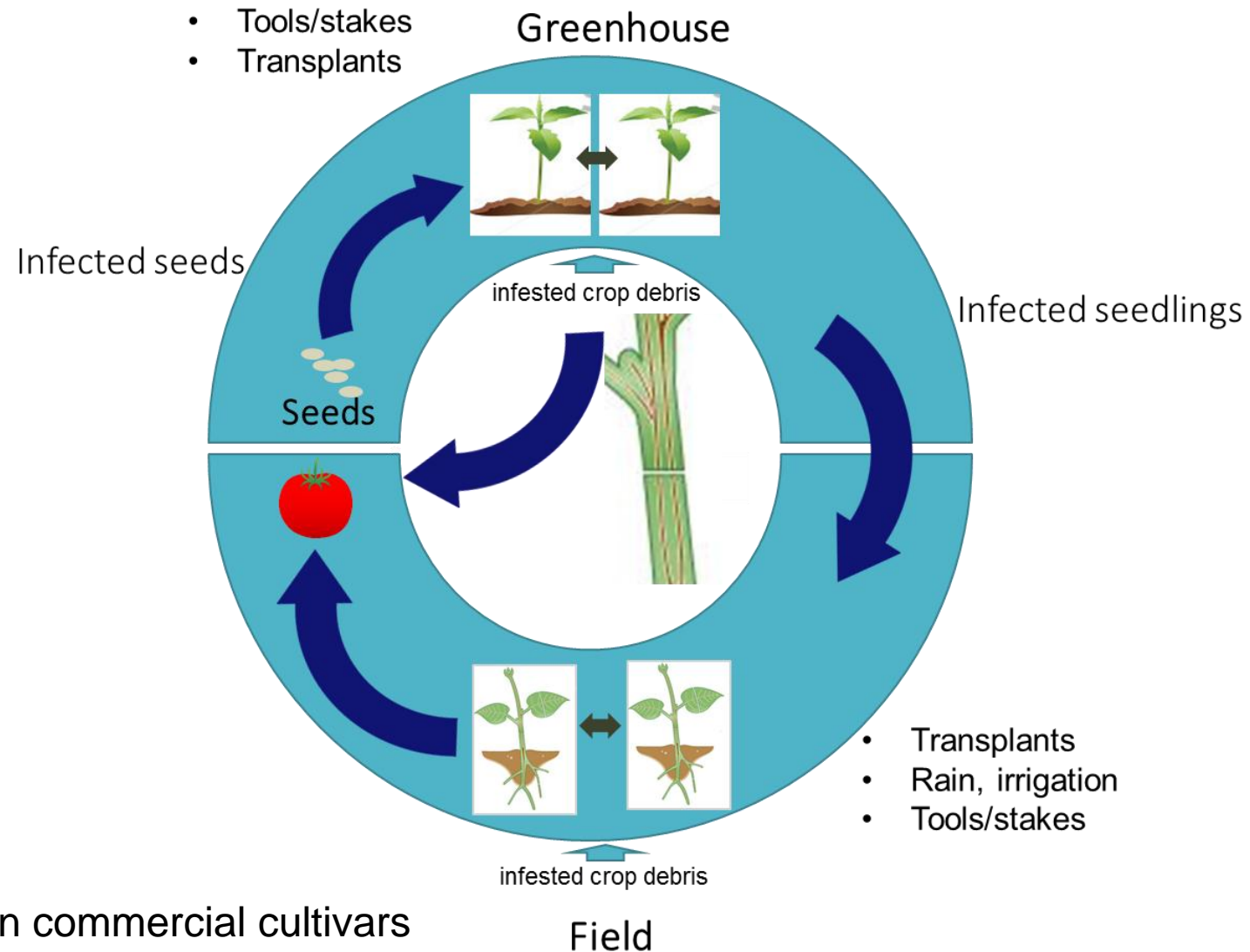


Bird's-eye spots



- Continuing issues with bacterial canker disease pressure in California fields
- *Clavibacter-Fusarium* co-infection

Clavibacter michiganensis transmission

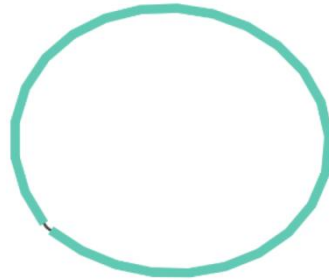
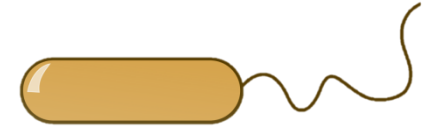


- Genetic resistance lacking in commercial cultivars
- Seed borne and mechanically transmitted
- Control strategy: clean seed & good horticultural practices

Central Questions

- ▶ How diverse is the *Clavibacter* genus?
- ▶ What regulates pathogenicity on tomato and other crops?
- ▶ What *Clavibacter* species are frequently associated with tomato?
- ▶ Can we identify genetic resistance to *Clavibacter*?
- ▶ Can genomic comparisons guide development of more accurate diagnostic tools for *Clavibacter michiganensis* detection?

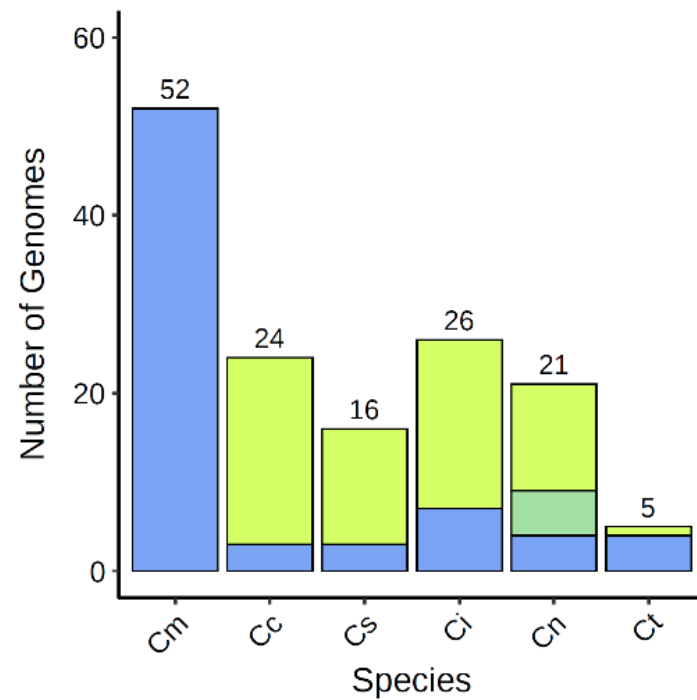
Oxford Nanopore Long Read Sequencing enables complete high quality genomes when paired with Illumina



Strengths:

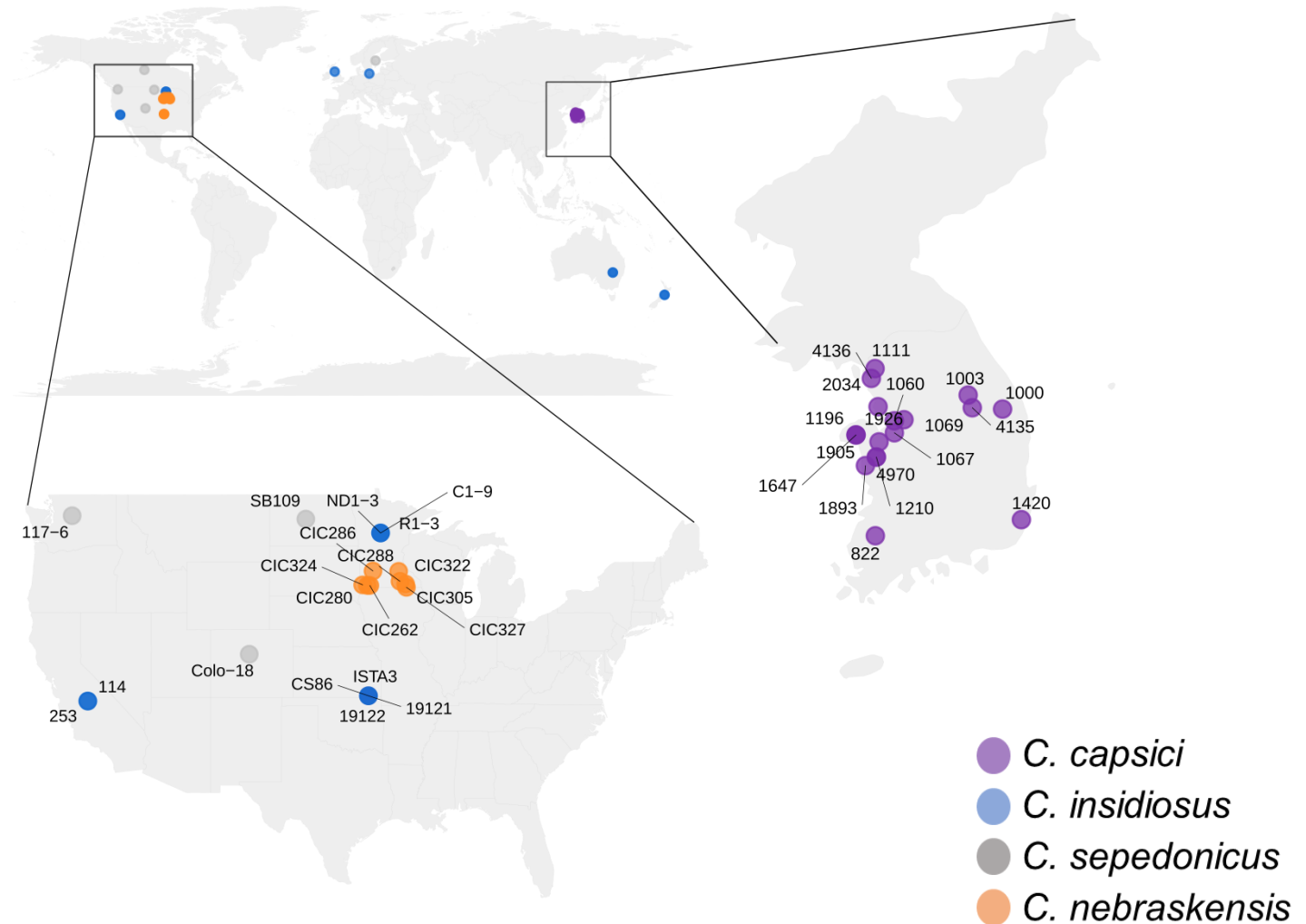
- Complete genome w/ low errors
- Reasonable costs per genome (\$80-150)
- No GC biases

Generating a high-quality *Clavibacter* genomic data set



Legend for the bar chart:

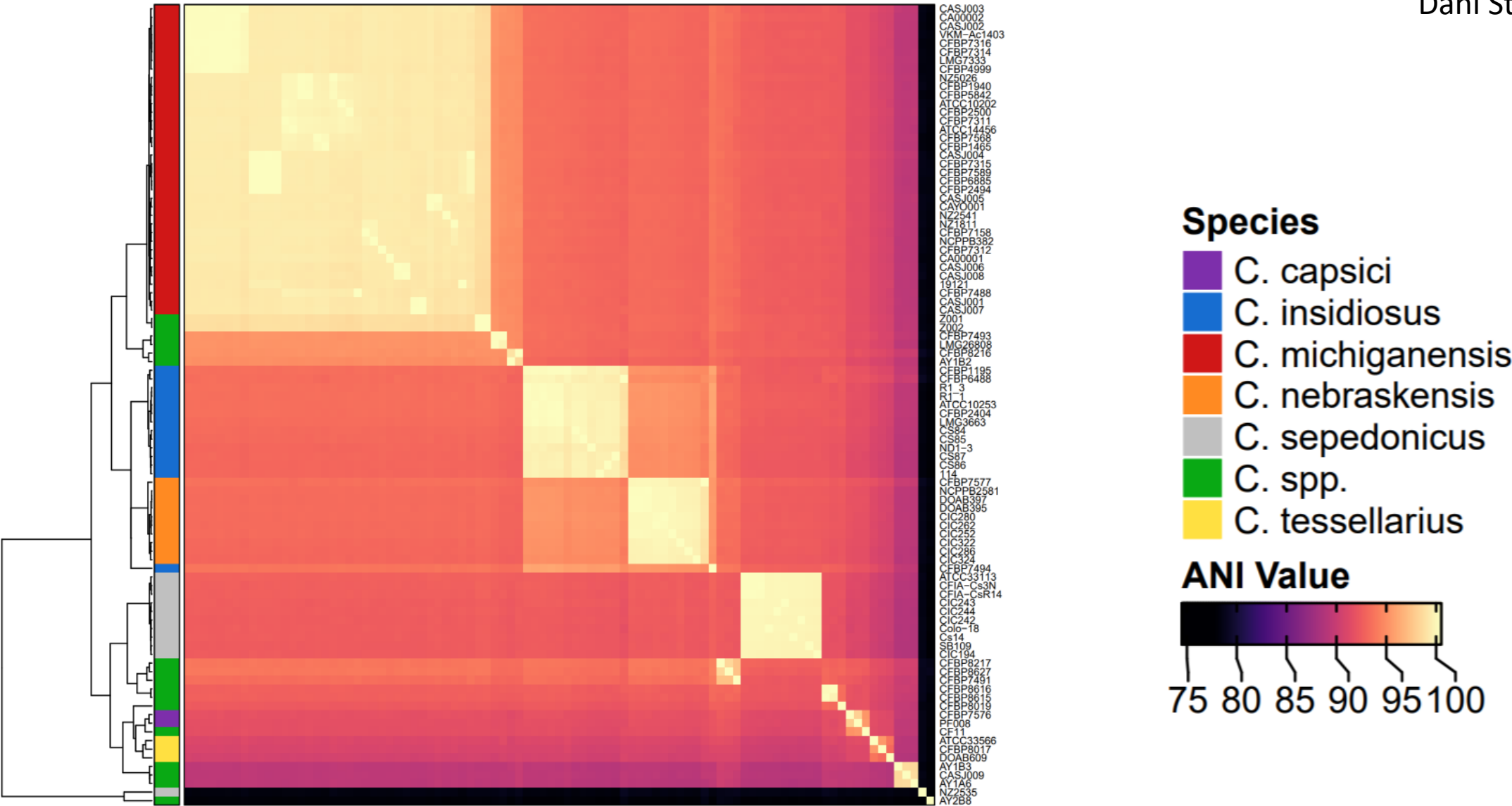
- Already in NCBI (Blue)
- Isolates to be Sequenced (Yellow)
- Sequences to be Shared (Green)



Clavibacter isolates are diverse and comprise > 6 species



Dani Stevens



Clavibacter endophytes common in tomato



CASJ002
(Pathogen)

CASJ009
(Endophyte)

CFBP7494
(Endophyte)

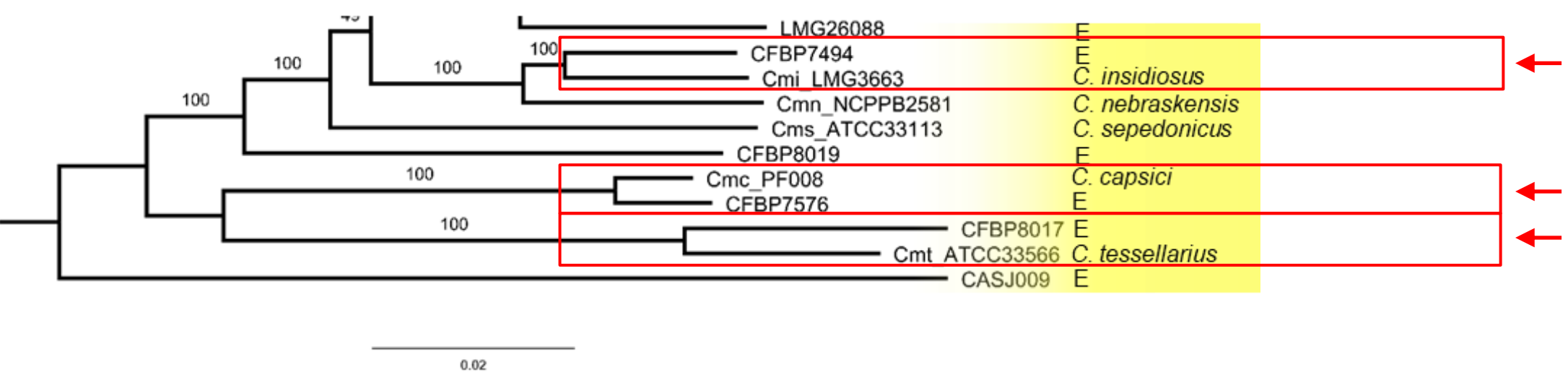


CFBP7576
(Endophyte)

CFBP8017
(Endophyte)

CFBP8019
(Endophyte)

Endophytes cluster with *Clavibacter* infecting other crops



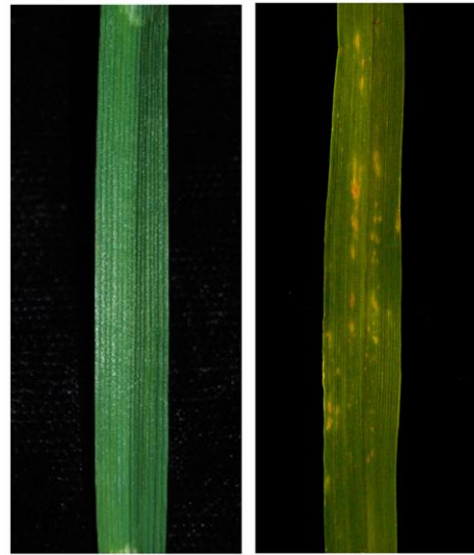
Tomato endophytes are pathogenic on other hosts



CASJ009

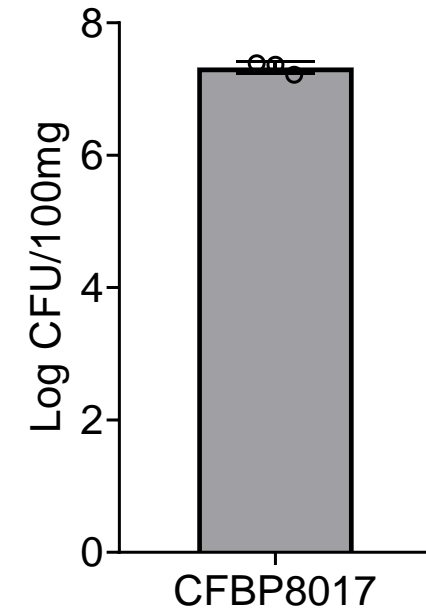
CFBP8017

Wheat cv. 'Bobwhite'



CASJ009

CFBP8017



- Different *Clavibacter* species can co-exist on a single tomato host.
- Mixed infection influences pathogen detection.

Specific *C. michiganensis* detection is challenging

1. Presence of other *Clavibacter* species in tomato plants
2. Genetic variation within pathogenic *C. michiganensis*

Detection historically based on:

1. Semi-selective media followed by pathogenicity tests
2. Serological
3. Molecular methods based on PCR of a single target



-

Weak +

+

ImmunoStrips

High rate of false positives

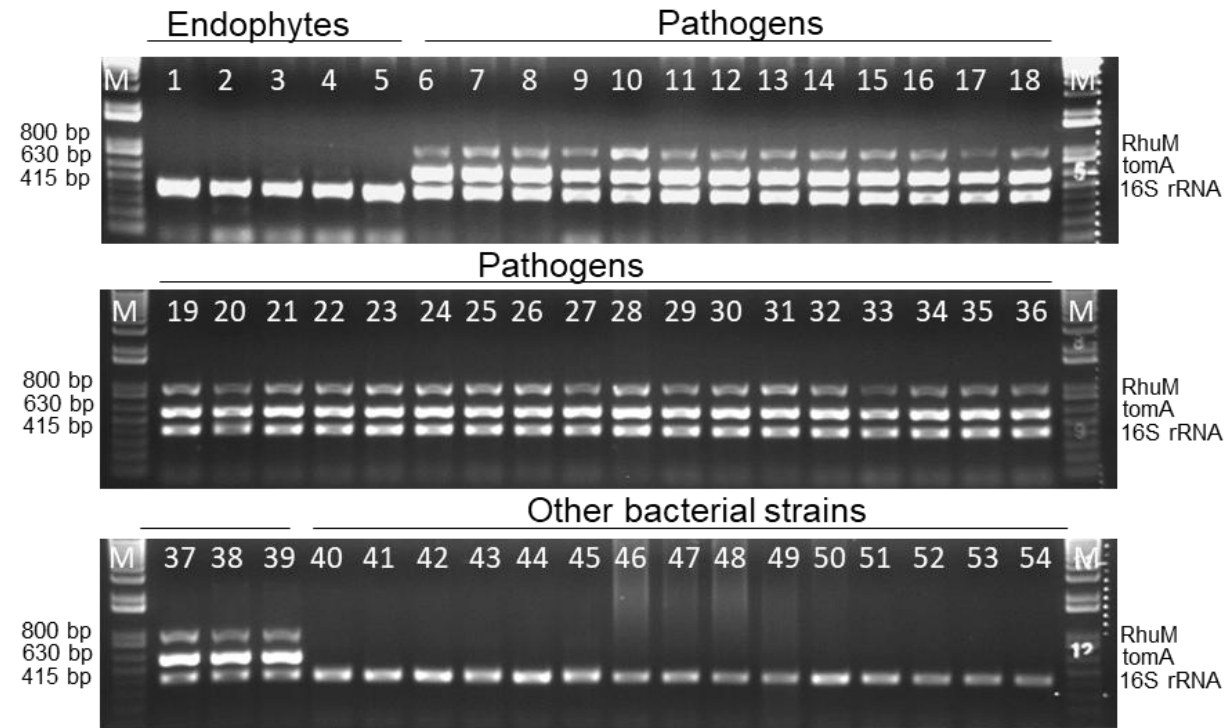
Cross-reacts with other *Clavibacter* species

Cmm detection via multiplex PCR



Shree Thapa

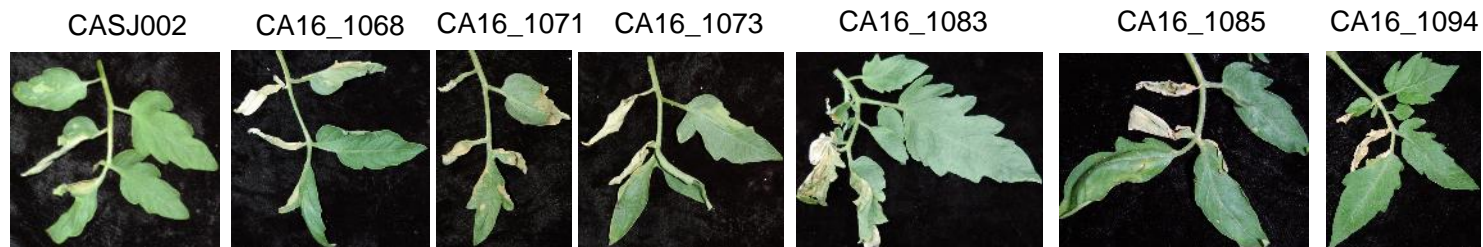
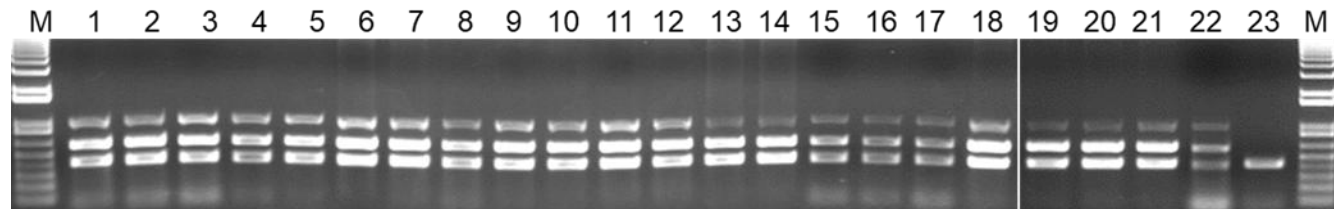
Bacteria	# strains	tomA	RhuM 16S rRNA	
<i>C. michiganensis</i>	82	+	+	+
Endophytic <i>Clavibacter</i>	6	-	-	+
<i>C. sepedonicus</i>	11	-	-	+
Other strains	20	-	-	+



Detection in field samples (2016-2019)



Shree Thapa



Summary

- ✓ *Clavibacter* is a diverse genus comprised of pathogens and endophytes
- ✓ Diverse *Clavibacter* isolates are associated with tomato and not all can cause disease.
- ✓ *Clavibacter* & *C. michiganensis* diversity has impacted pathogen detection
 - ✓ Clean seed = clean plants
 - ✓ Developed multiplex PCR platform to specifically detect *Clavibacter michiganensis*

Acknowledgements

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Clavibacter

G+ bacteria

Associated with many plant species

Pathogens = narrow host range

