

# The sugarbeet phyllosphere harbors bacteria capable of inhibiting *Cercospora beticola*, the causal agent of *Cercospora* leaf spot

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Madison Christenson

USDA-ARS

NDSU Plant Pathology

Fargo, ND

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# Plant Microbiome Dynamics

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**Phyllosphere** - total above-ground portions of a plant and serves as a habitat for microorganisms

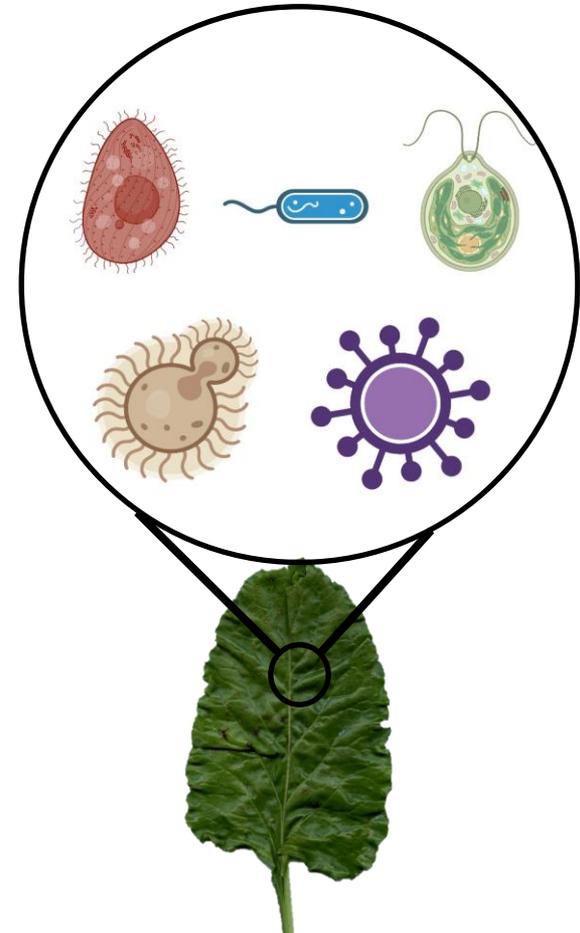
## What determines community structure?

### Abiotic factors

- \* Ephemeral environment
  - Daily fluctuations in temperature, UV exposure, water availability, plant life cycle

### Biotic factors

- \* Microbial residents
  - Bacteria, fungi, archaea, algae, viruses
  - Trophic mode

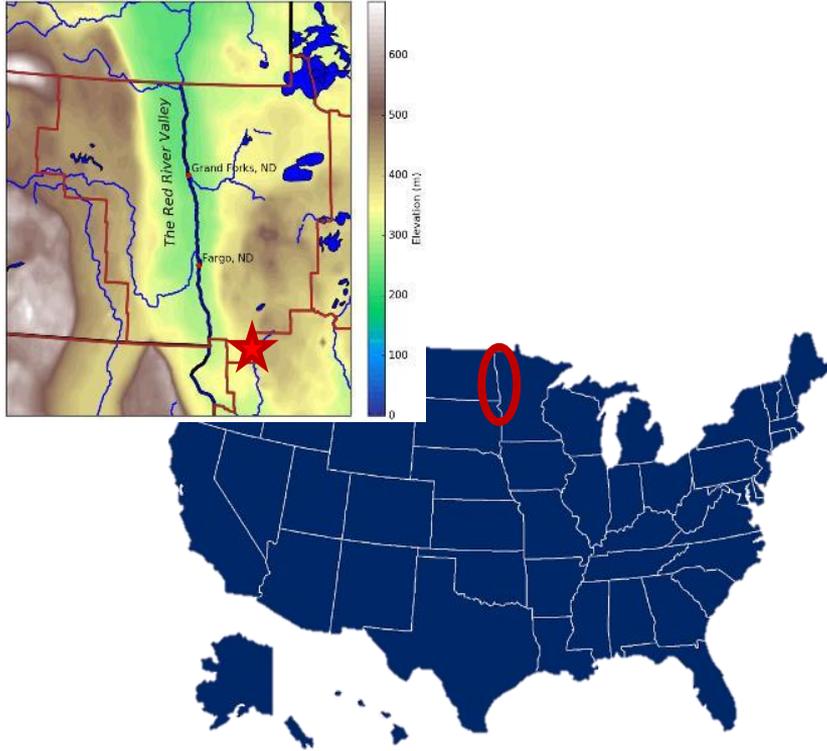


# Hypothesis:

The sugarbeet leaf microbiome harbors bacteria with the capability to ward off other microbes, such as *C. beticola*

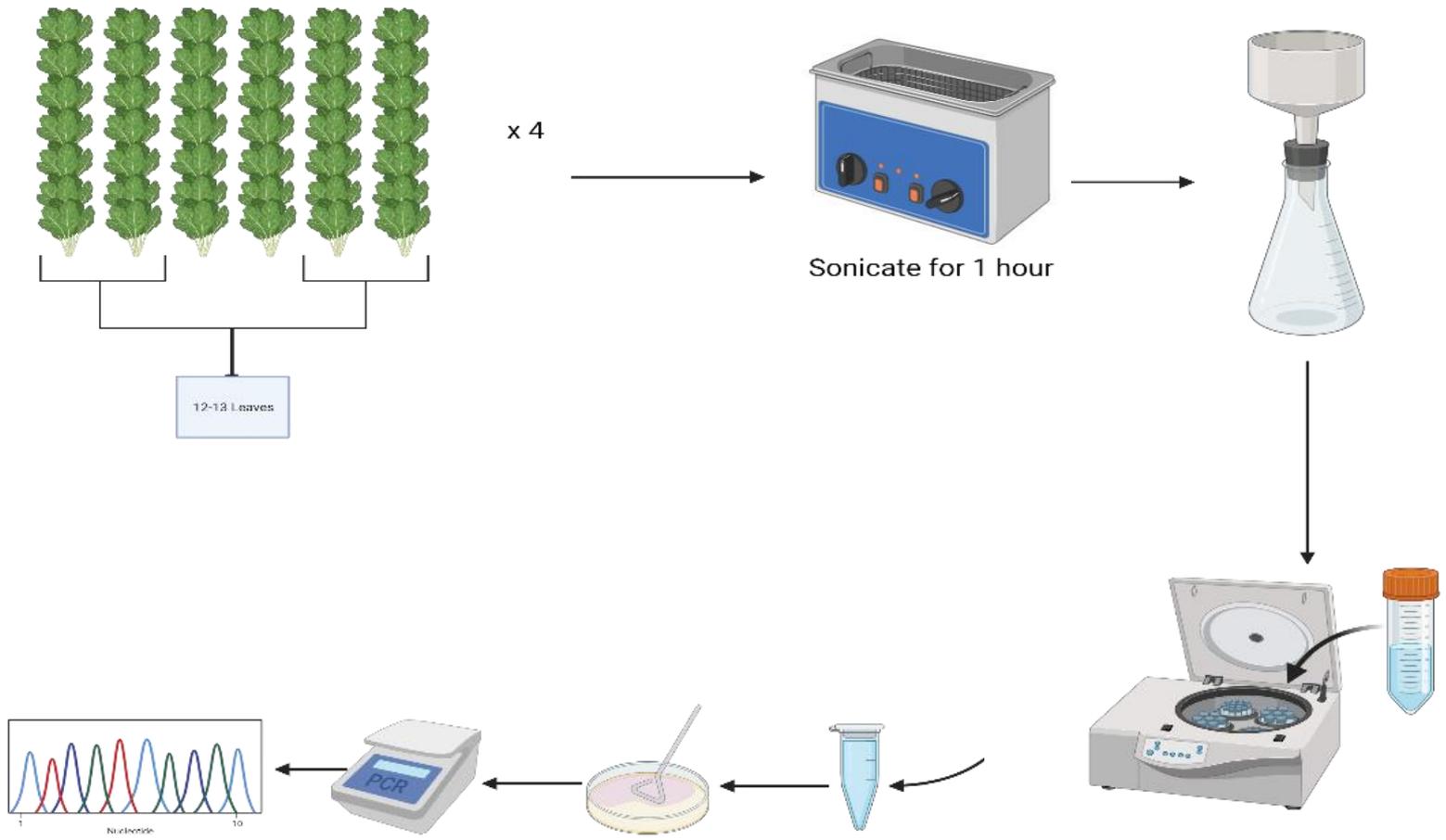
# Field Sampling

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- \* Disease nursery near Foxhome, MN
- \* Five sampling timepoints
- \* Plots allowed to acquire CLS
  - Susceptible and Resistant varieties to CLS
  - Sampled 50 leaves per timepoint
  - Recovered epiphytic bacteria

# Processing Samples

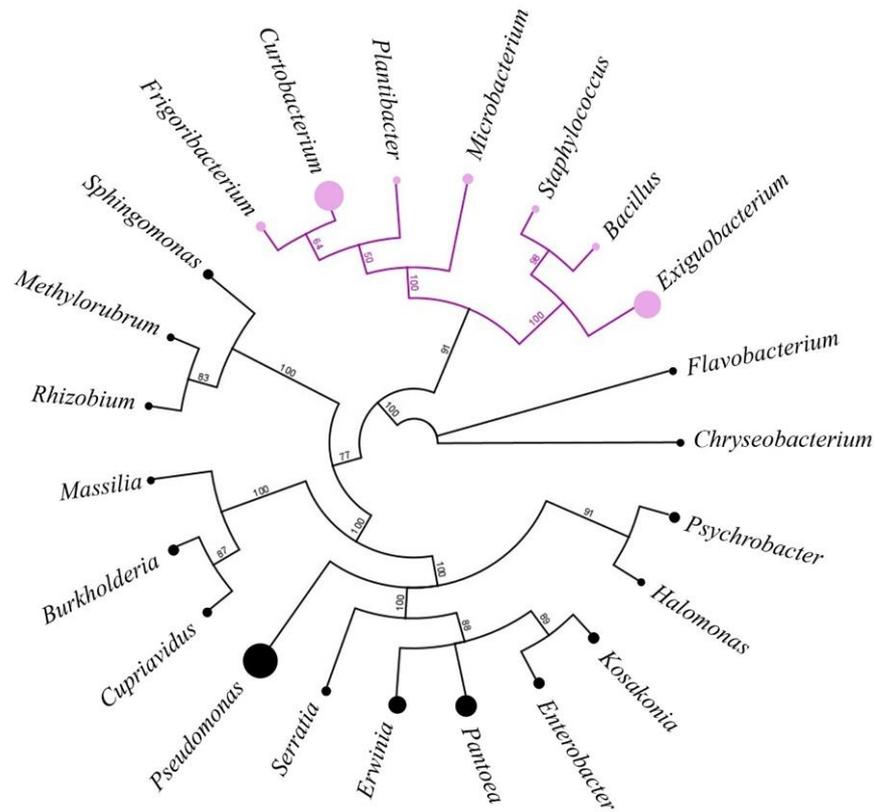


# Sampling Results

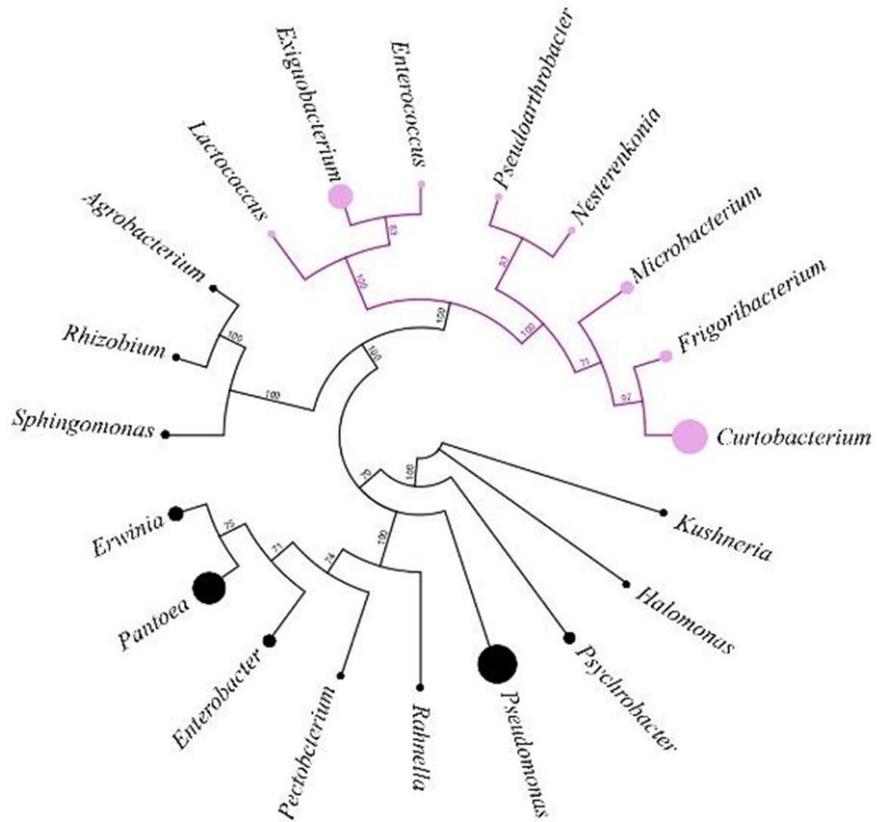
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# Resistant

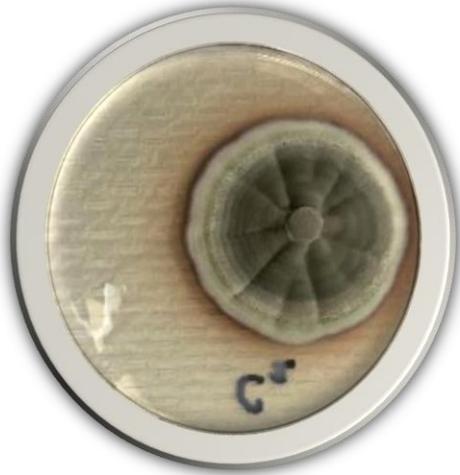


# Susceptible



# Screening for antagonistic properties

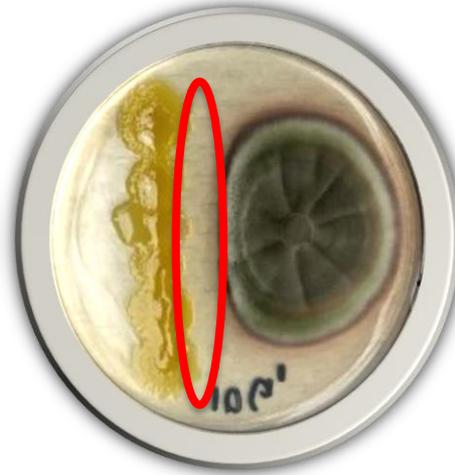
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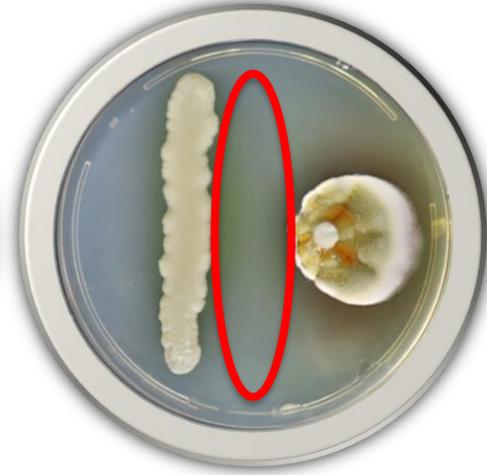
Control  
(no bacteria present)



Bacterial species that  
do not inhibit  
*Cercospora*



Bacterial species that  
partially inhibits  
*Cercospora*



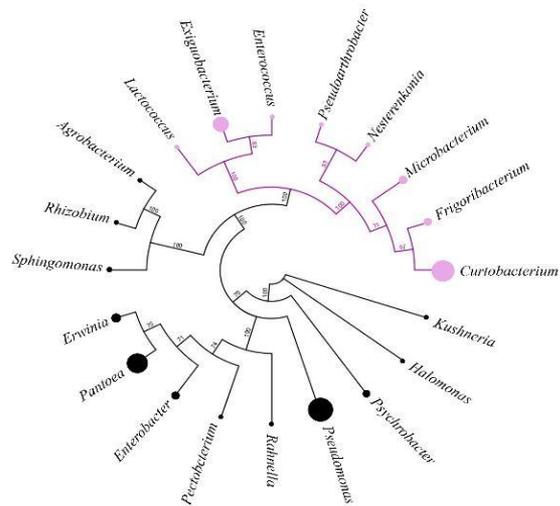
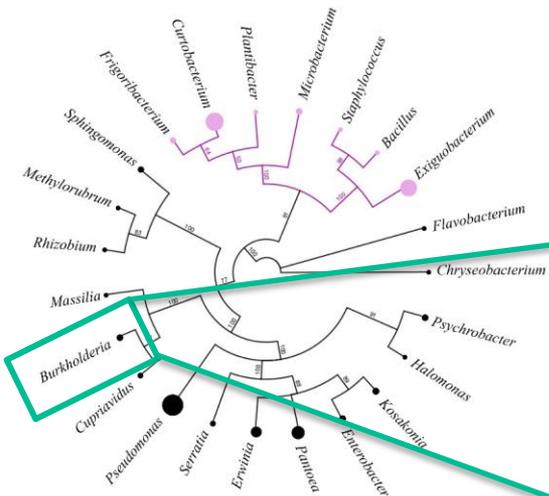
Bacterial species  
that significantly  
inhibits *Cercospora*

# What inhibits *C. beticola*?

Resistant  
Cultivar

All *Burkholderia* species recovered

- Only isolated at timepoint 3 from the resistant cultivar
- Gram negative bacteria
- Some species found to be antagonistic towards plant pathogens



Susceptible  
Cultivar





# ***Burkholderia* vs *C. beticola***

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*C. beticola* 154



*C. beticola* 11



*C. beticola* 6



*C. beticola* 116

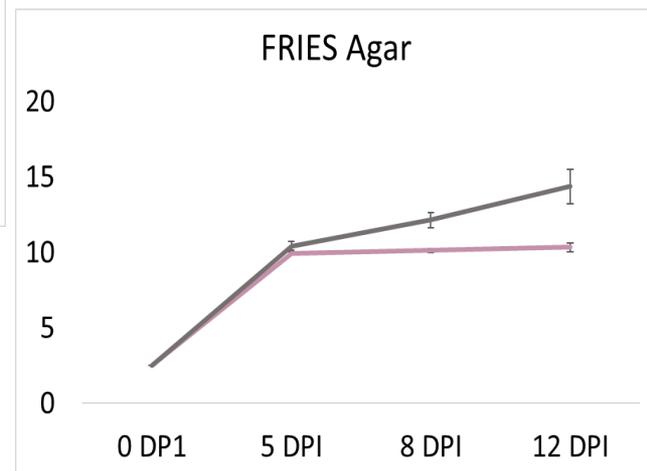
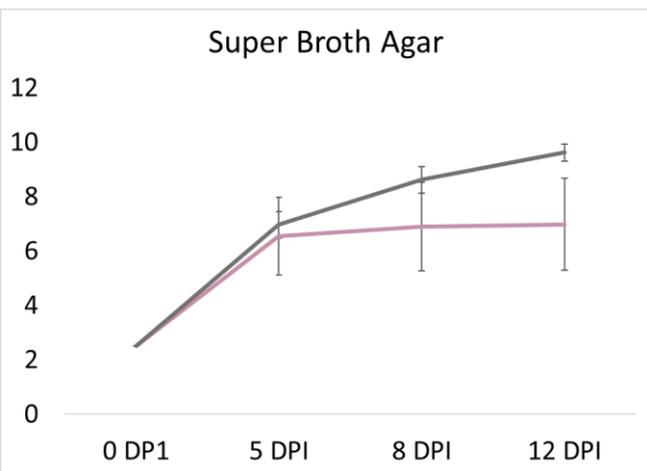
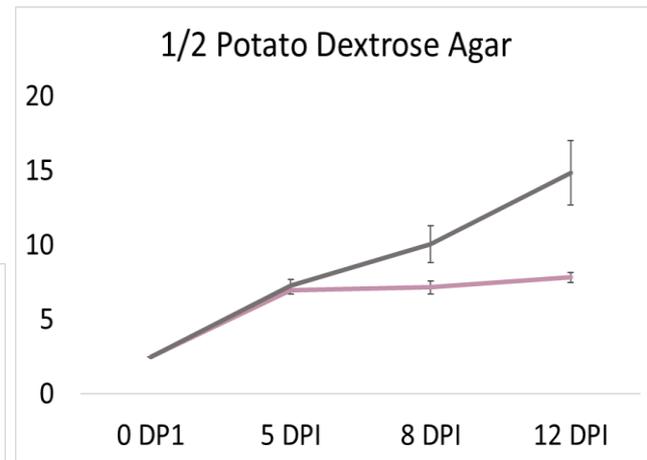
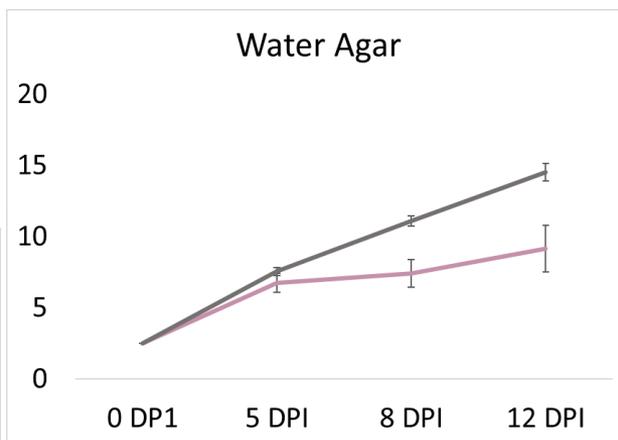
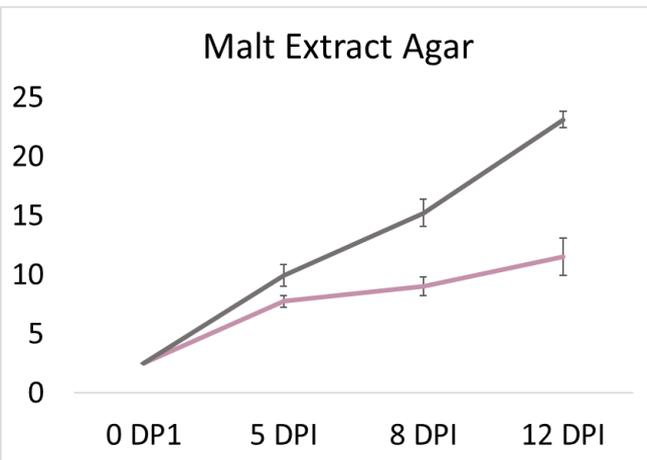


*C. beticola* 12



- *Burkholderia*  
inhibited multiple  
strains of *C. beticola*

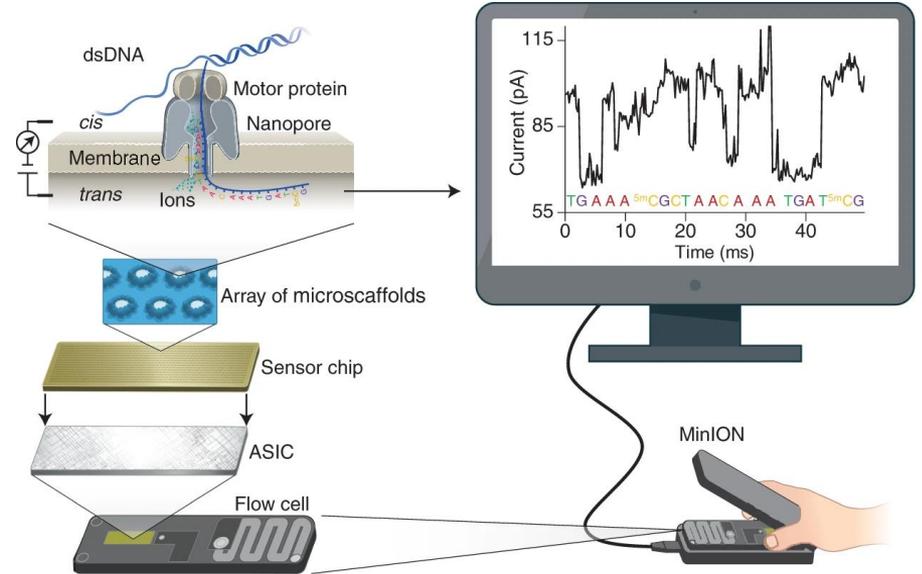
# *Burkholderia* vs *C. beticola* on different media



— *Burkholderia* vs *C. beticola*  
— *C. beticola*

# Sequencing of *Burkholderia*

- \* One *Burkholderia* isolate chosen
- \* Whole genome sequencing using Nanopore's MinION
  - o Size: 8.7 Mb



*Burkholderia  
contaminans*

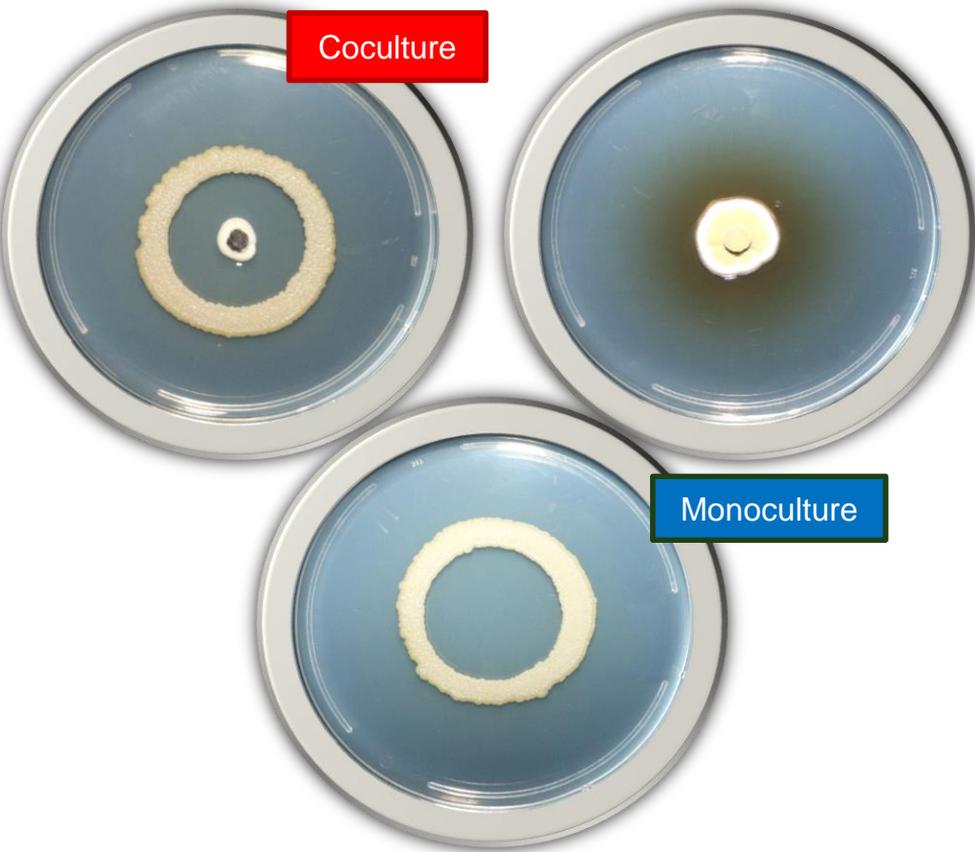
Transcriptomics

Volatile Assay

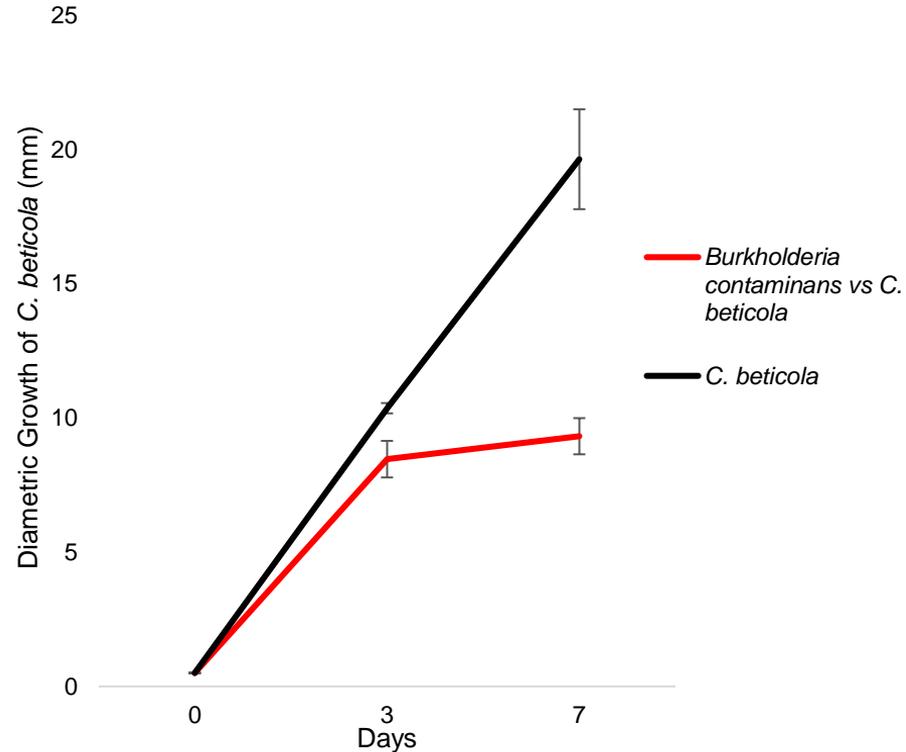
Fungal Stain

Metabolomics

# *Burkholderia contaminans* vs *C. beticola* 309-10



Extracted bacterial RNA at 7 DPI



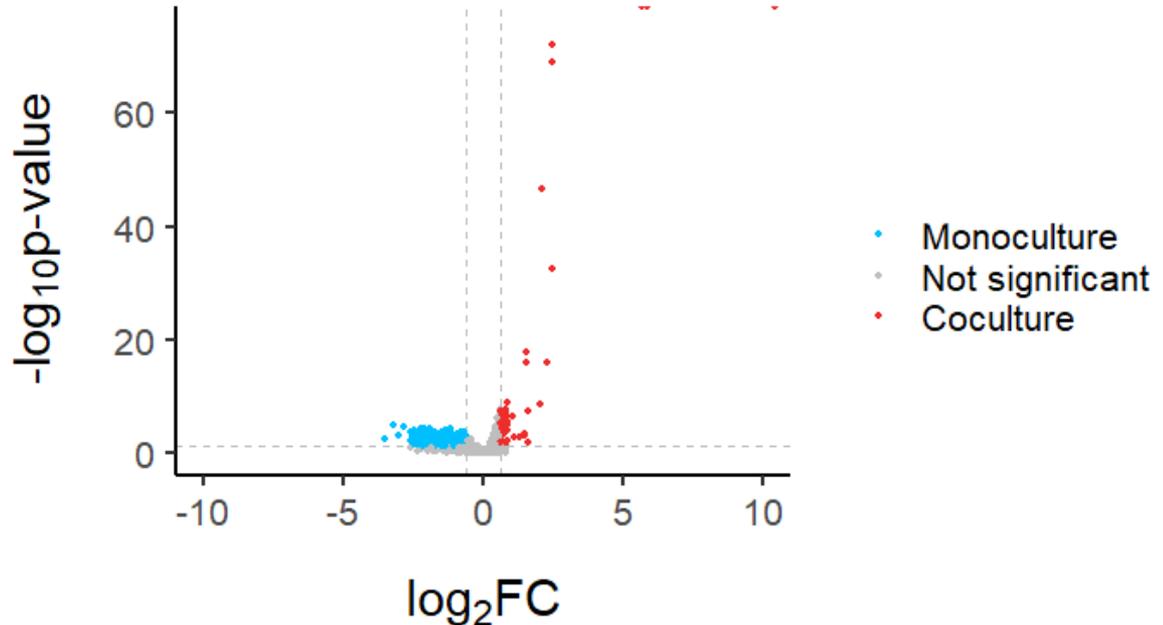
# Bacterial RNA-seq

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172 total differentially expressed genes with a p-value cutoff 0.05

- **115** genes are up-regulated in the coculture

Volcano plot of bacterial gene expression in presence/absence of *Cercospora beticola*



# Candidate Genes

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- \* **115** genes are up-regulated in the coculture
  - o Predicted product of the 10 most differentially expressed genes

Predicted Product
hypothetical protein
IS200/IS605 family transposase ISPa80
putative transporter
N-ethylmaleimide reductase
Biodegradative arginine decarboxylase
Putrescine transporter PotE
Inducible ornithine decarboxylase
Long-chain-alcohol dehydrogenase 1
hypothetical protein
hypothetical protein

*Burkholderia  
contaminans*

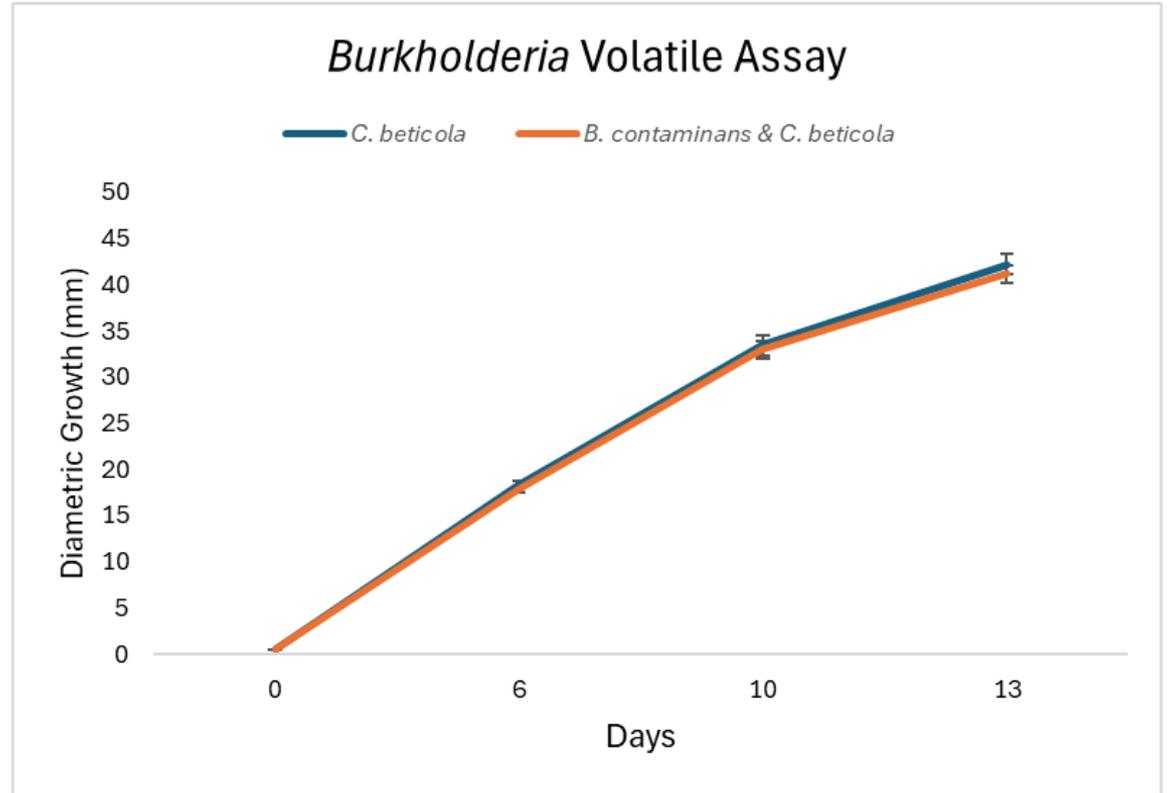
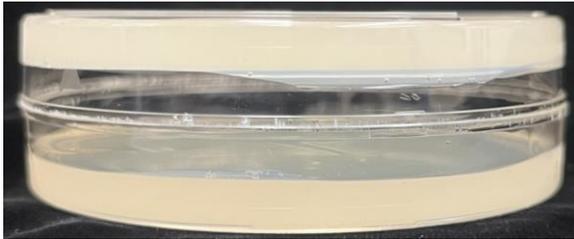
Transcriptomics

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Metabolomics

# Volatile Assay



*Burkholderia  
contaminans*

Transcriptomics

Volatile Assay

Fungal Stain

Metabolomics

# Fungal Stain

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- \* Set up antagonism assay of *Burkholderia contaminans* vs *C. beticola*
- \* Day 15 stained hyphae with Evans Blue or Neutral Red
  - o Hyphae that uptake the red are alive
  - o Hyphae that uptake the blue are dead
- \* Used microscope to observe

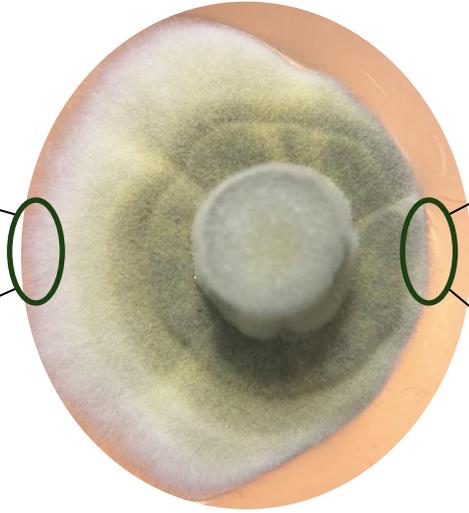
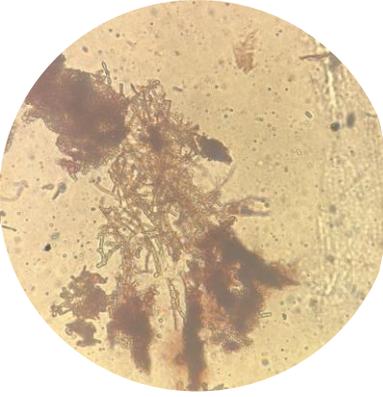
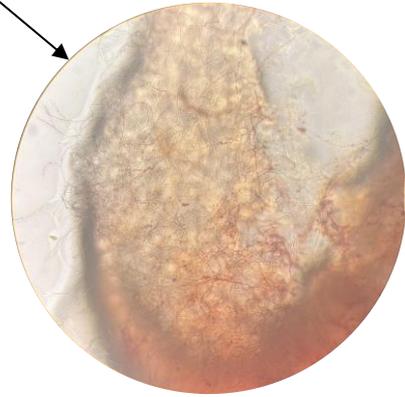
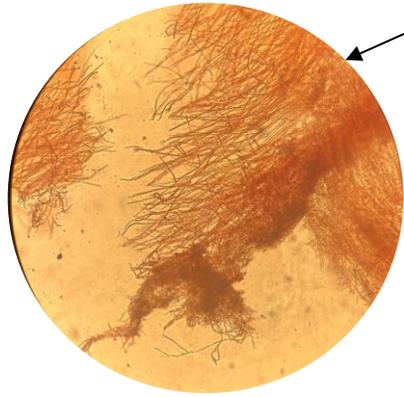
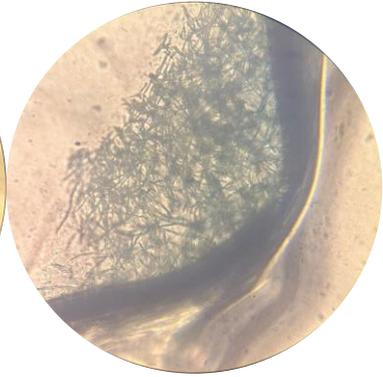
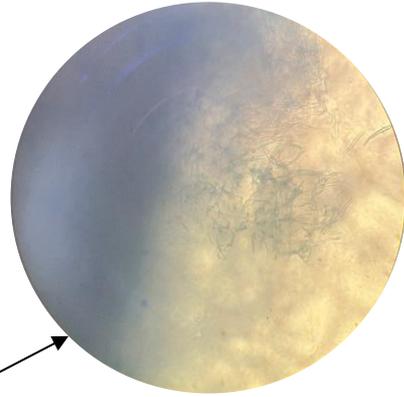
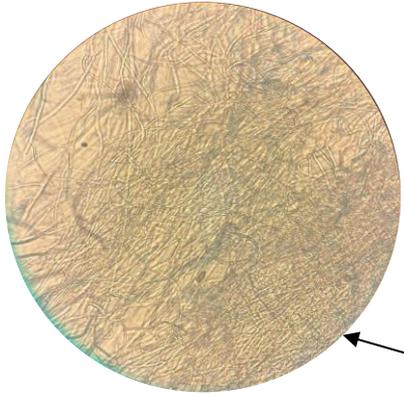


# Fungal Stain

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Blue= Dead

Red= Alive



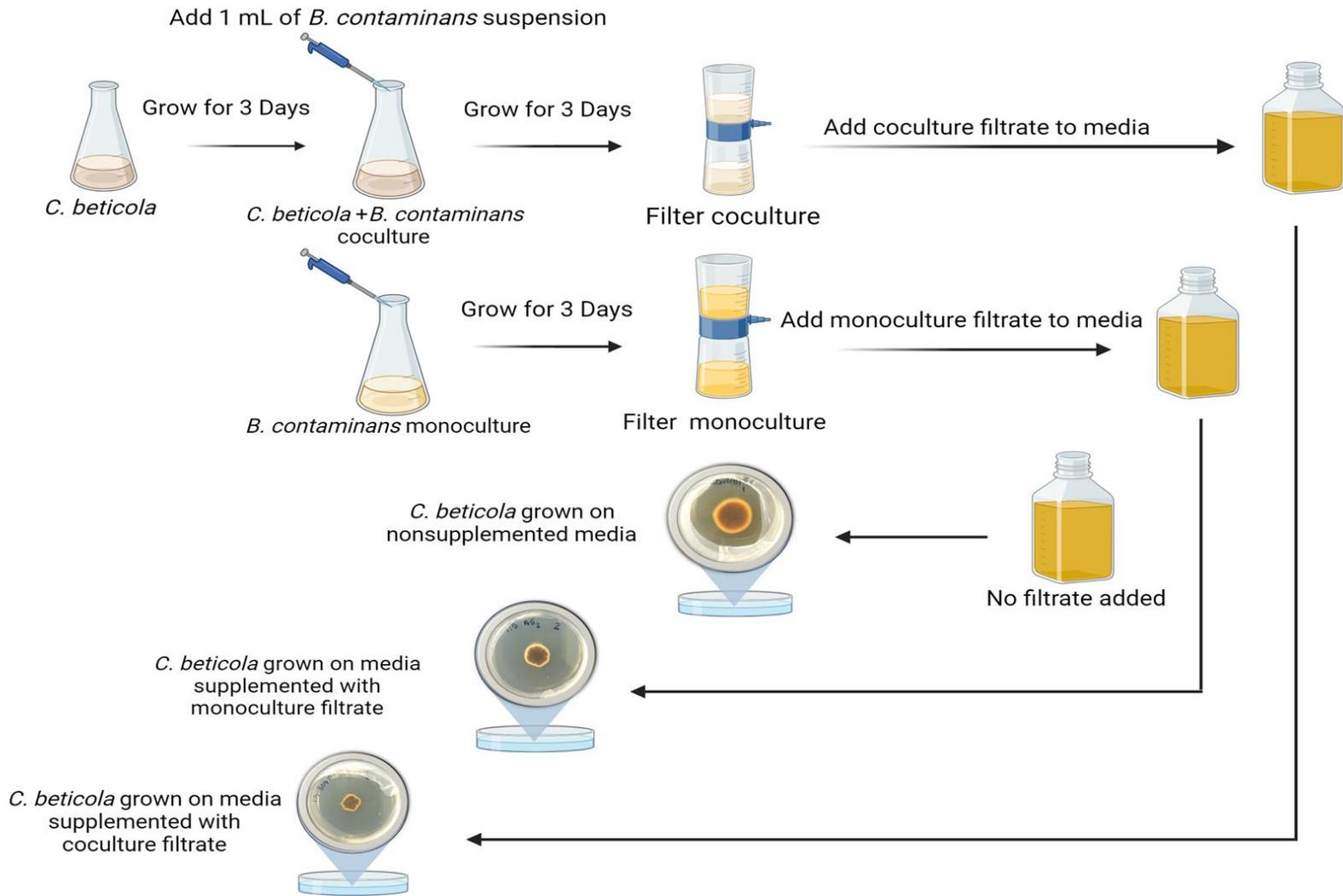
*Burkholderia  
contaminans*

Transcriptomics

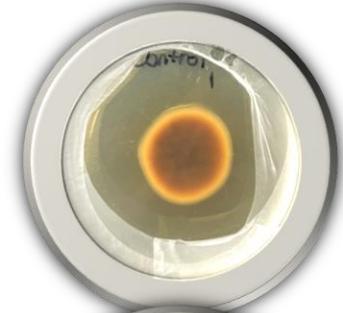
Volatile Assay

Fungal Stain

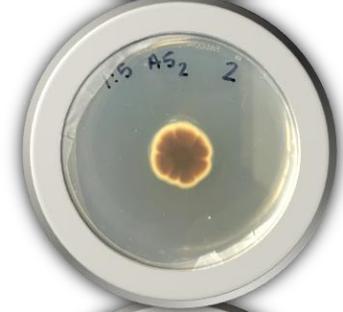
Metabolomics



# Poison Plate Assay



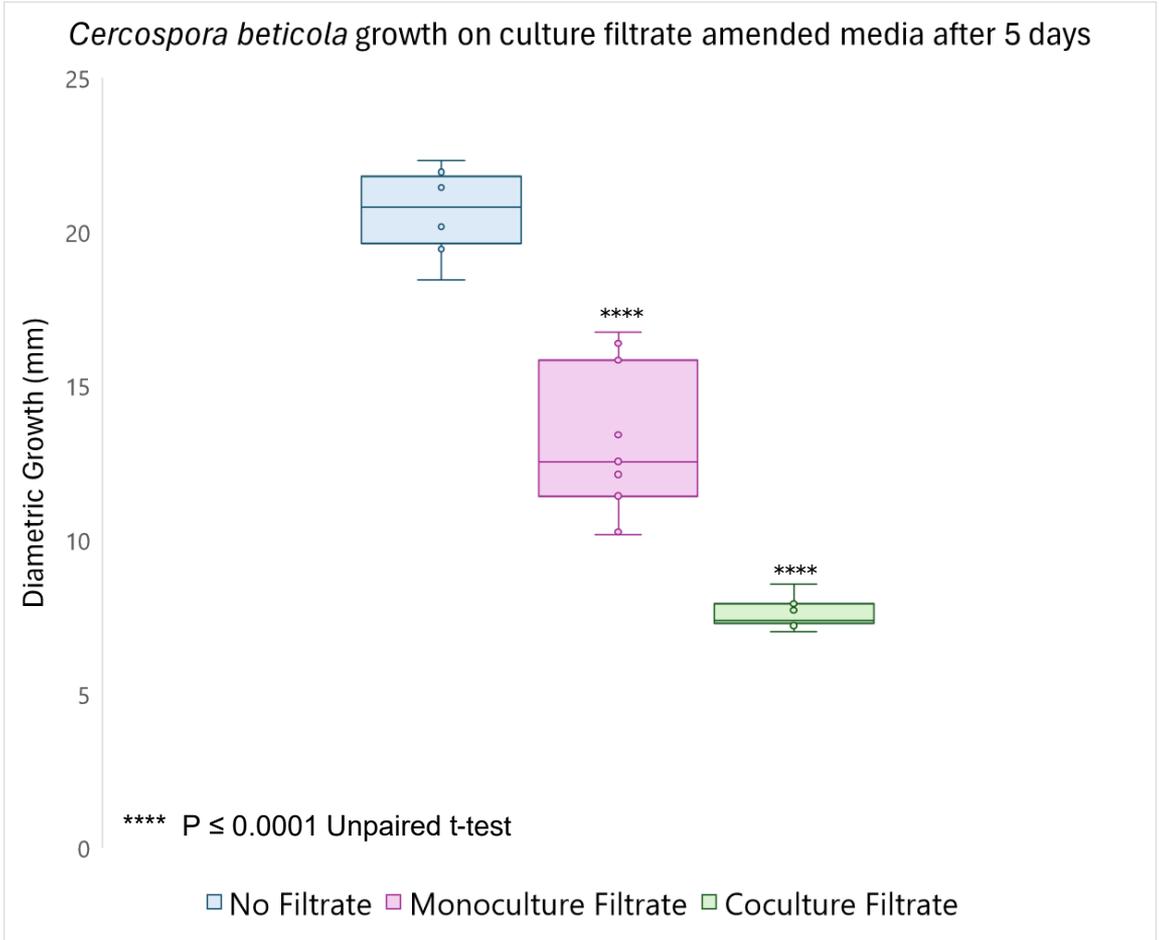
No Filtrate



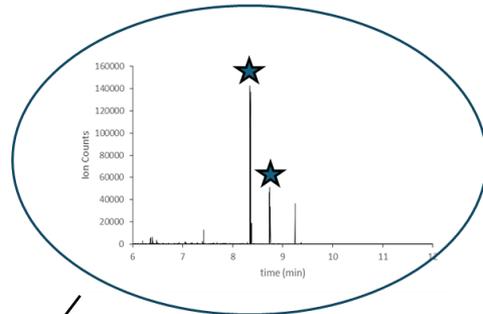
Monoculture  
Filtrate



Coculture  
Filtrate



# What molecule causes the inhibition?



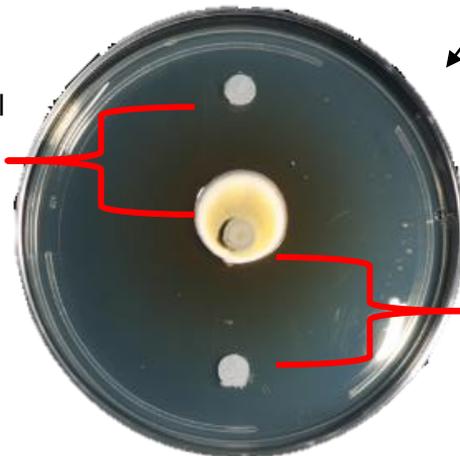
Coculture of *C. beticola*  
and *B. contaminans*

LCMS



Dr. Daniel Back  
Research Chemist

Discs embedded with methanol  
(control) do not inhibit  
*Cercospora* growth

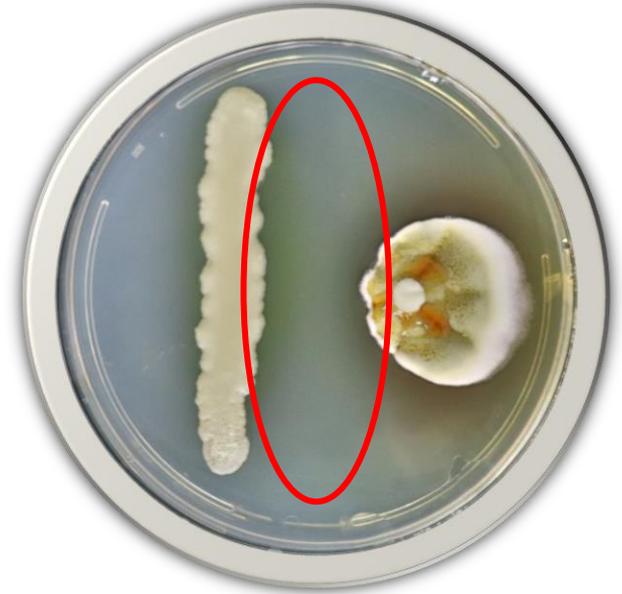


Discs embedded with methanol +  
identified metabolite inhibit *Cercospora*  
growth

# Current Work

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- \* Knock-out gene candidates in *B. contaminans*
  - Antagonism assays
- \* Test the viability of *C. beticola* after being challenged with *B. contaminans*
- \* Applying the identified metabolite on plants to test for CLS inhibition



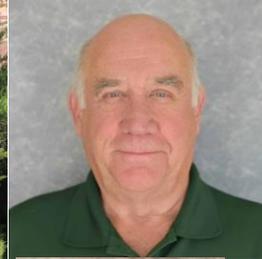
# Acknowledgements



Bolton Lab – USDA ARS



Dr. L. Rangel- The James Hutton Institute



Dr. G. Secor – NDSU



Dr. Nathan Wyatt – USDA ARS



A wide-angle photograph of a large agricultural field. The foreground and middle ground are filled with rows of lush green leafy plants, possibly a type of vegetable or cover crop. The plants are growing in dark soil. In the distance, a flat horizon line separates the field from a blue sky with scattered white clouds. A few small trees and a red tractor are visible on the horizon.

**Thank You!**

**Questions?**