*Green*STEM's microbe technology is an efficient, effective, and affordable way to clean up fuel and chemical spills

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Petroleum Hydrocarbons in the Environment

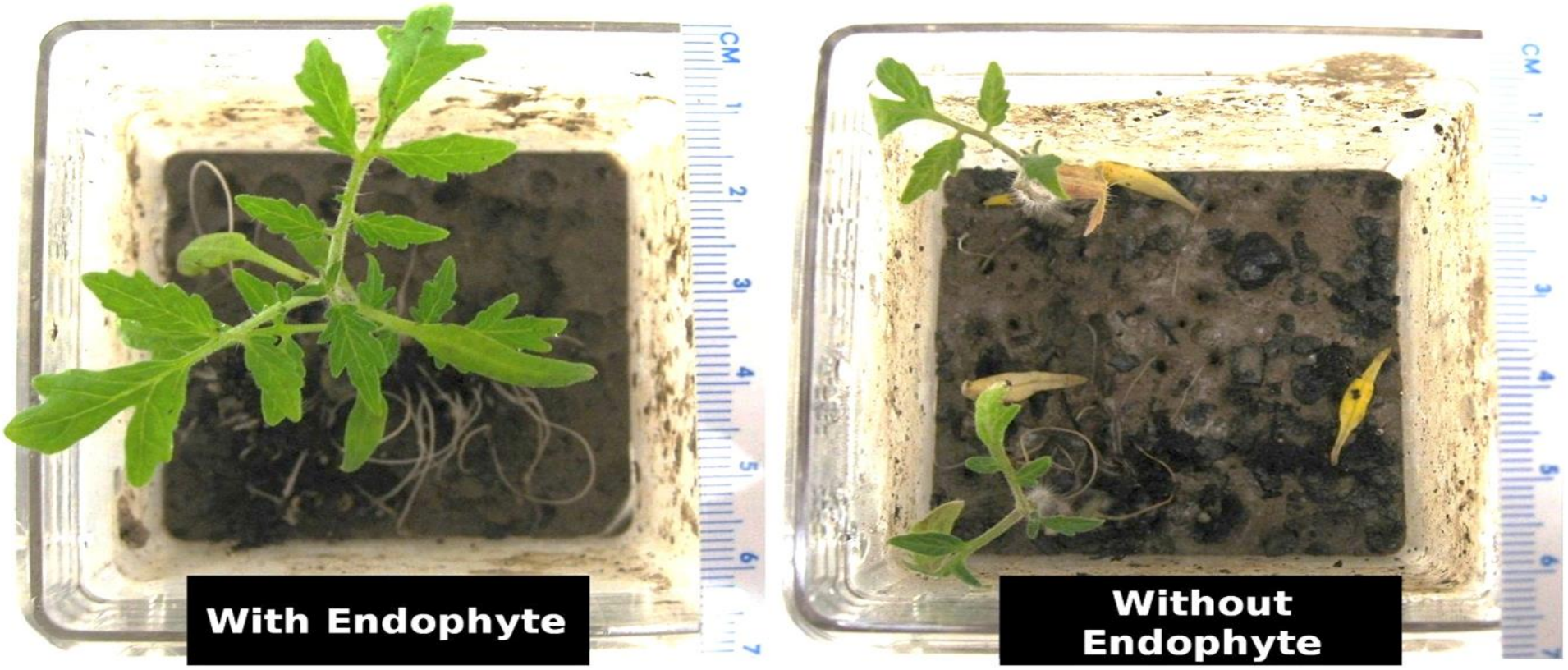
Collateral damage from the quest for hydrocarbons In the Athabasca (Alberta) tar sands

Remediation of Petroleum Hydrocarbons

- **Bioremediation technology:** is a promising technology

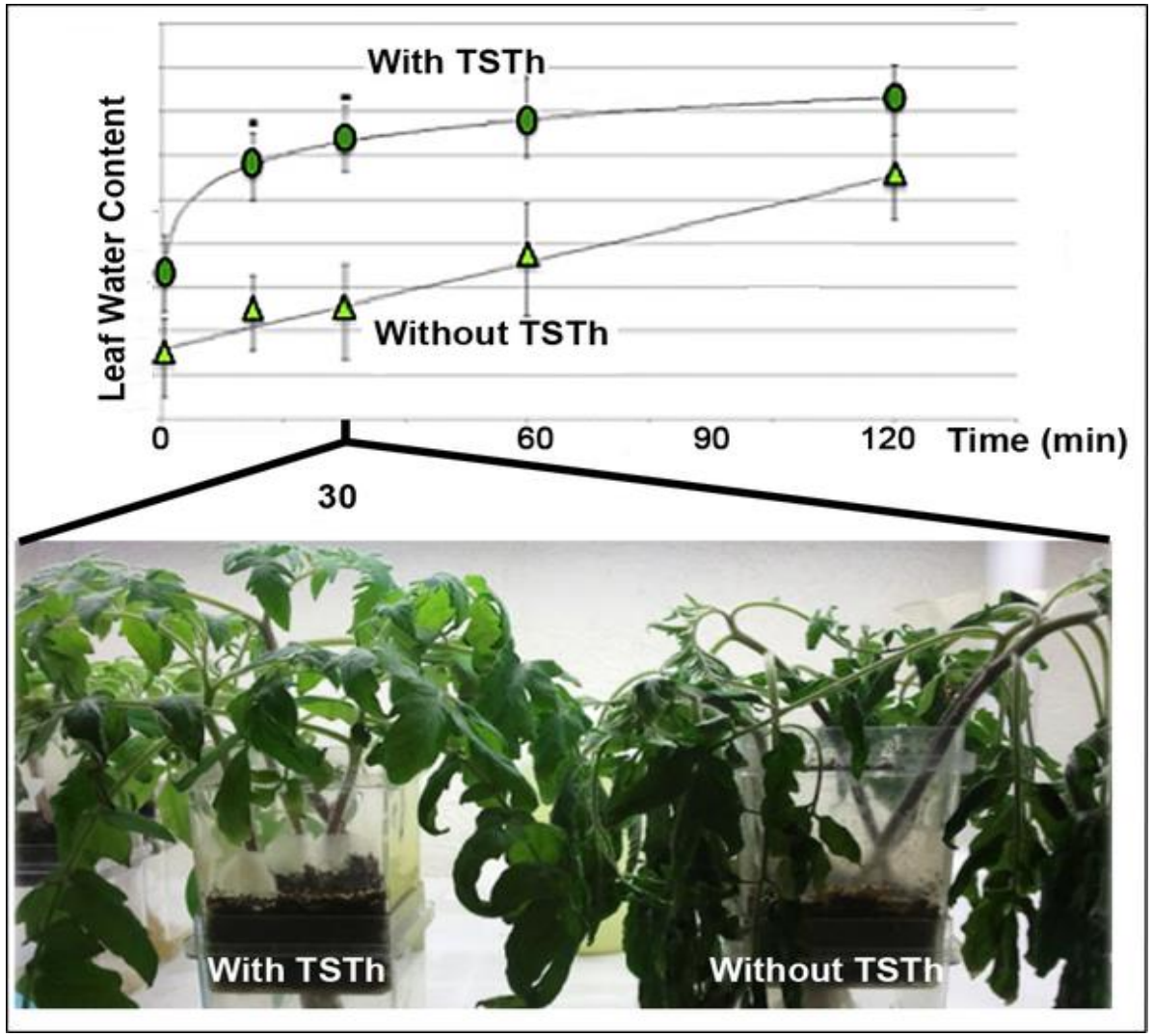
Previous work:TSTh20-1 was isolated from oil sand tailings

Improved Growth on Coarse Tailings



TSTh20-1, no fertilizer

Improved Drought Recovery with TSTh20-1



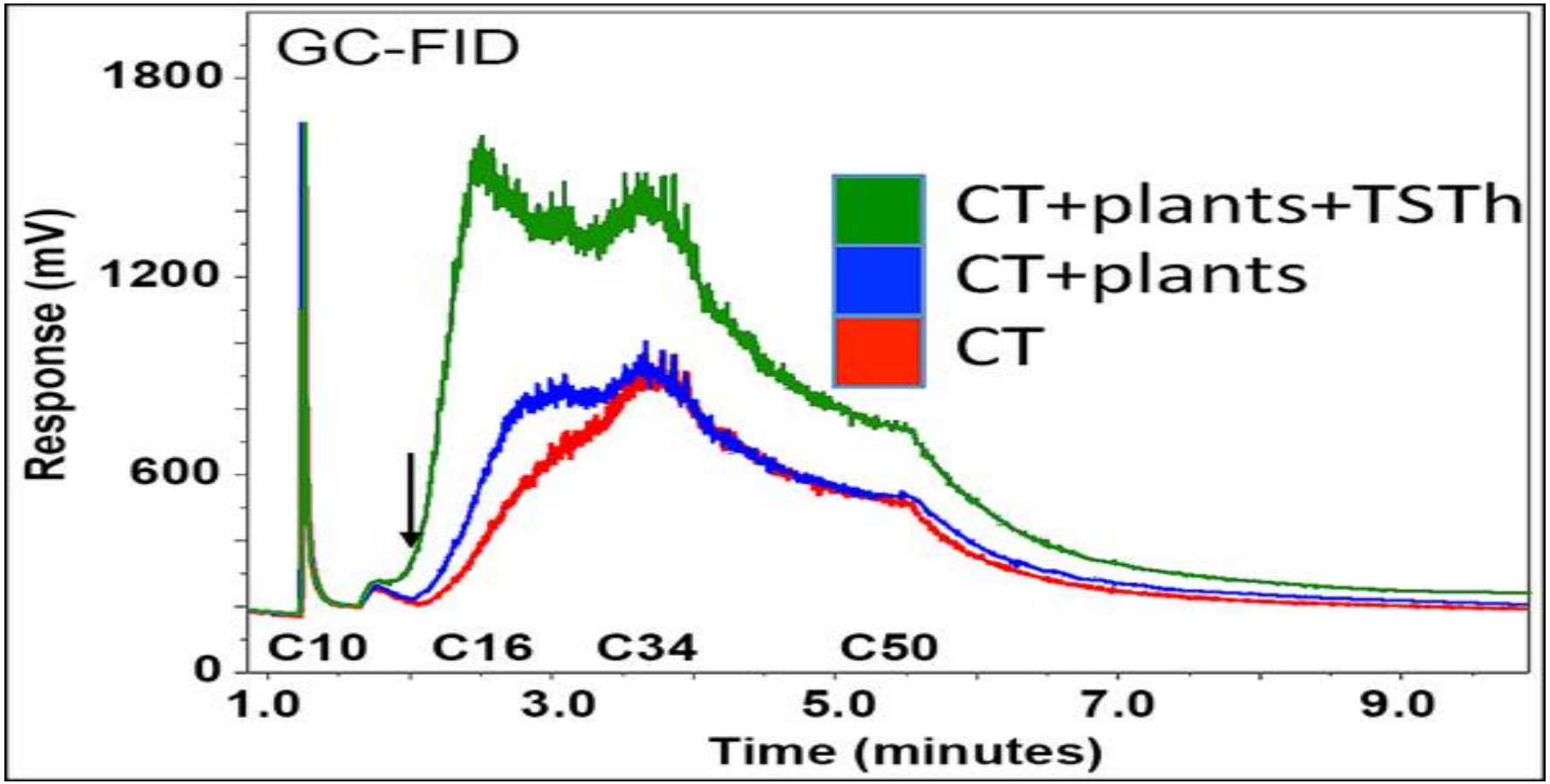
- Six week old tomatoes were deprived of water for 2d, then allowed to imbibe.
- Those inoculated with TSTh20-1 recovered exponentially; those without endophyte took longer.
- Picture shows relative recovery at 30 min.

Improved Germination with TSTh20-1

TSTh20-1 increases seed germination rate and % germination on potting mix as well as coarse tailings.

CT + NPK 20:20:20 CT + TSTh20-1 Potting mix + NPK

Hydrocarbon Profiles altered by TSTh20-1



- Presence of growing plants, particularly with TSTh20-1, had the unexpected effect of increasing the assayable levels of lower molecular weight PAHs.
- The assay is 'blind' to PAHs above C50, so we interpret this as evidence of their degradation

Our goal: Contribute to revegetation of extreme environments using microorganisms already growing in those environments

In May 2018


GreenSTEM Technology Corp
We use Science to Restore, Preserve and Protect the
Health and Beauty of our Land

Two locations

- Saskatoon, SK –Main office and primary lab location

- Fort St. John, BC –Satellite location for small experiments and product production

Research For Hire → established at *Green*STEM lab

Identify a Challenge

Identify Potential Solutions

Develop a Lab Testing Budget

Determine the Most Effective Solution Through Lab Trials

Scale-up and Test on a Real Site

Use Proven Technology at Other Sites

Collaboration re Specific Solutions

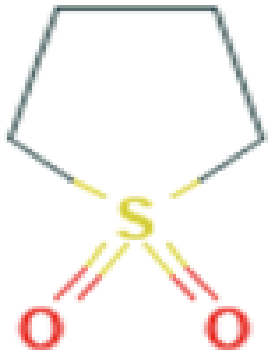
Shared Positive Publicity

Early Access to New Technologies

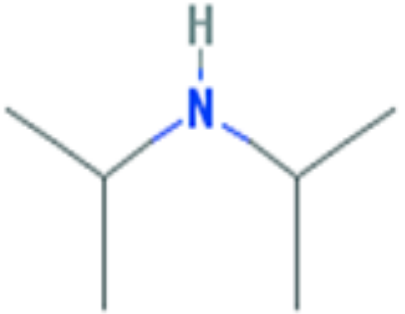
Contamination case study: Sulfolane and DIPA

- Former sour gas processing facility in southern AB
- Sulfolane and Diisopropylamine (DIPA) impacts to groundwater over 3 ha (100 m x 300 m)
- Approaches that were tested were unable to remove either contaminant effectively
- **GreenSTEM** started working with the client to identify a path forward and designing lab trials to identify and test other possible solutions

Analysis: Sulfolane and DIPA degraders



Sulfolane



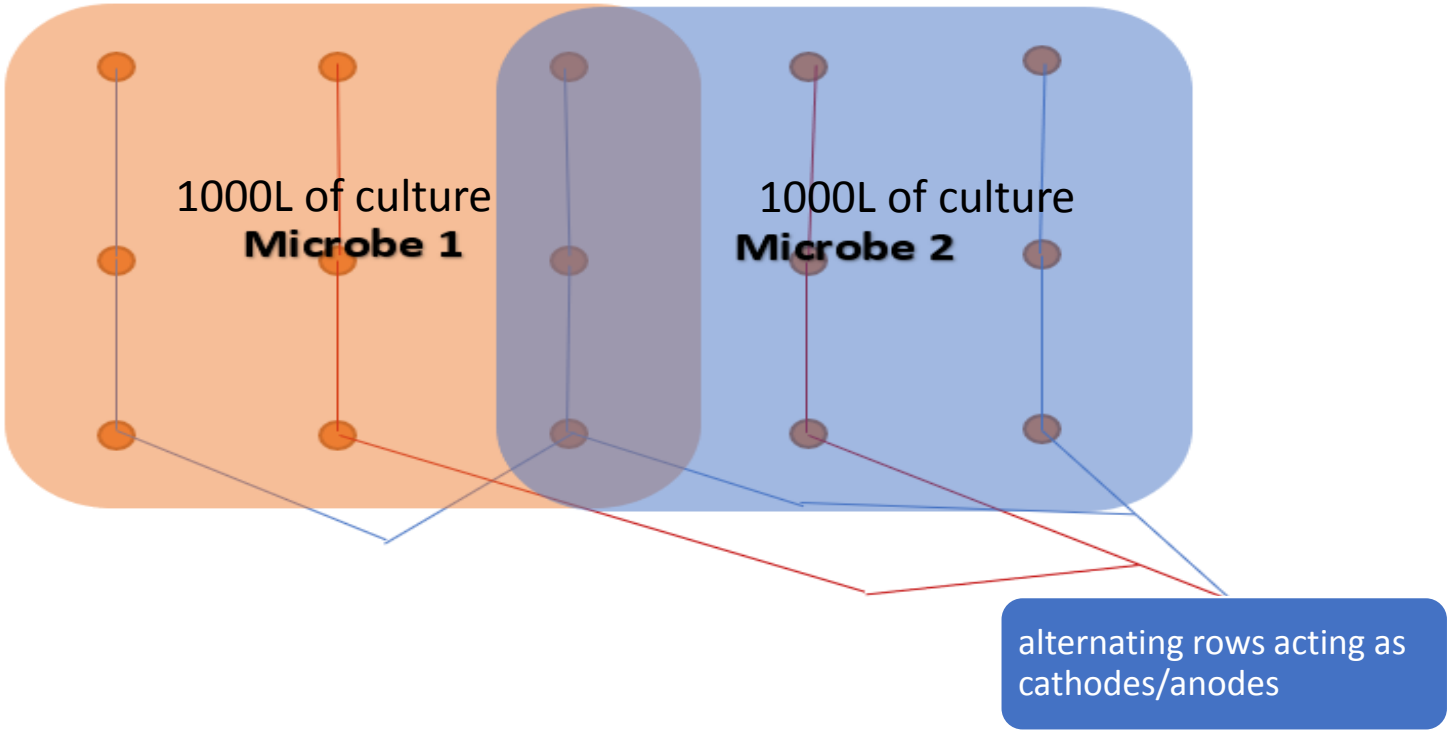
Diisopropylamine

Table 1. Analytical Results Of Desktop Trial After 2 Weeks Of Water Treatment

Parameter	Pre-Treatment	Microbe 1	Microbe 2
Sulfolane	16.1 mg/L	<0.002 mg/L	10.8 mg/L
DIPA	2150 mg/L	18.2 mg/L	15.3 mg/L

Table 2. Analytical Results of the Field Trial After 6 Days of Water Treatment

Parameter	Applicable Standard	Pre-Treatment 1	Pre-Treatment 2	Microbe 1 6 Days	Microbe 2 6 Days	Combined 6 Days
Sulfolane	0.09 mg/L	17 mg/L	18.2 mg/L	4.3 mg/L	6.6 mg/L	4.45 mg/L
DIPA	1.6 mg/L	25.2 mg/L	49.8 mg/L	10.2 mg/L	9.37 mg/L	4.22 mg/L



*Green*STEM Take-home Messages

- Microbe-based methods are affordable, effective, fast
- Can be used for bio-remediating a wide-variety of chemical and petrochemical pollutants
- We are developing new strains that are selected for unusual contaminants → xenobiotics (synthesized), natural gas condensate, glycol
- Also, new strains selected for customer-specific problems
- ***Strength through diversity → multiple methods from allied companies work Better and Faster***

Acknowledgements

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https://www.greenstem.ca/

www.usask.ca/biology/kaminskyj

Questions?

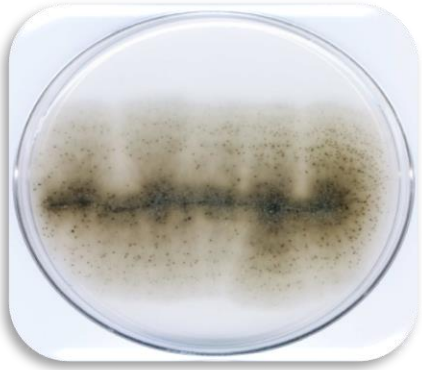
Methodology

Plant collected from saline site

Surface sterilized and cut into pieces

Plated on potato dextrose agar

Single strain isolated



Grown for spore harvest

Spores harvested suspended in water

Charcoal tested for good fungal growth

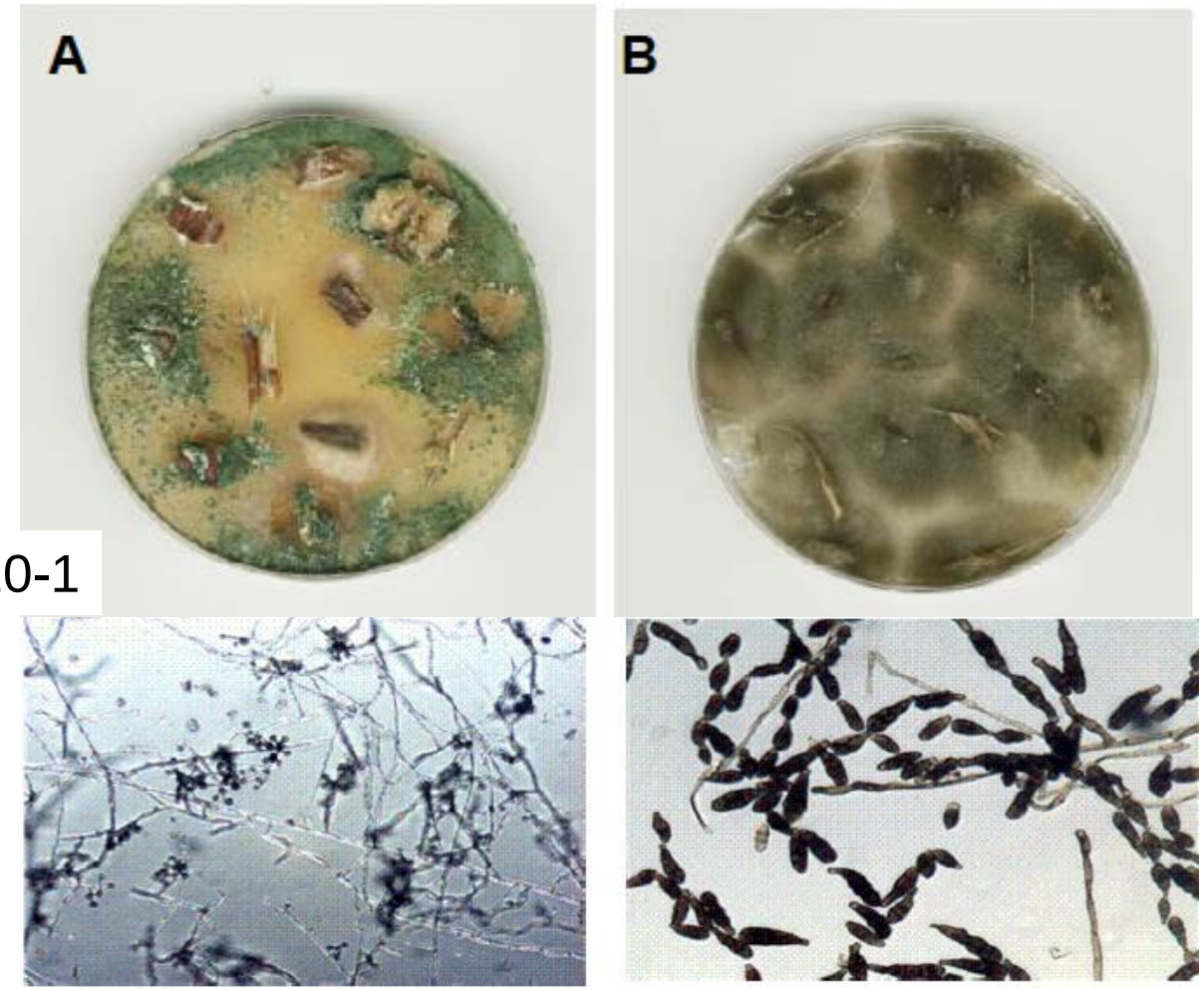
Spore suspension applied to charcoal granules

Colonized plant grown under variety of conditions according to experiment design

Charcoal granules placed with germination seeds

Isolation of tailing sands endophytes from pioneer plants

TSTh20-1



Endophyte function → does colonization lead to tolerance?

Confirm colonization → re-isolate

Determine identity → morphology and molecular genetics

Option for outgrowth from plant vs outgrowth from charcoal