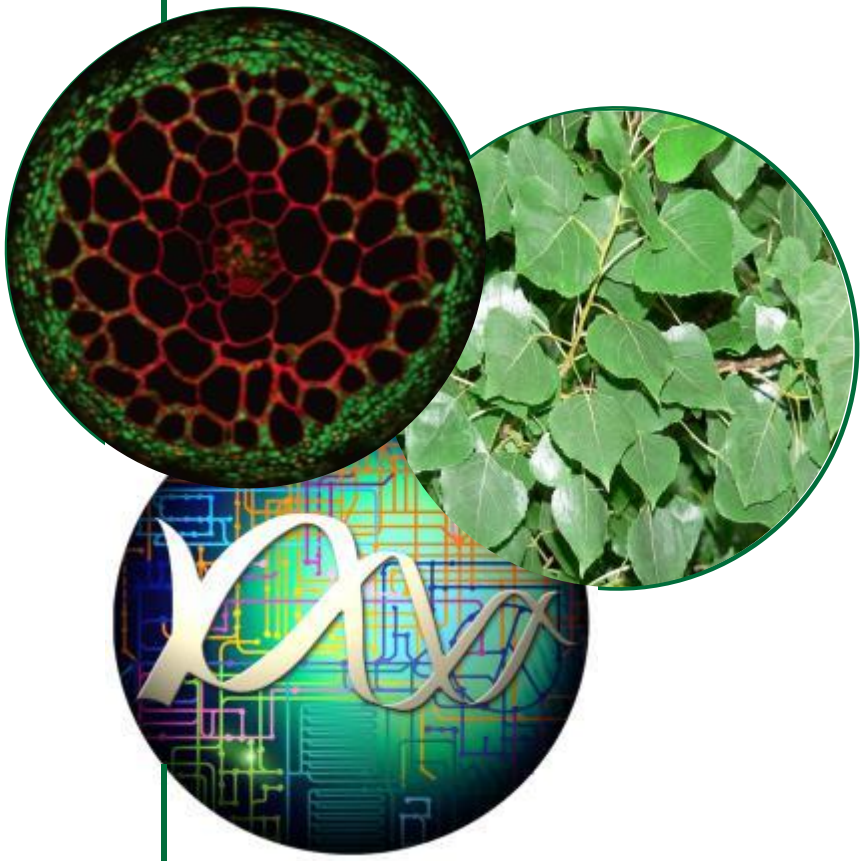
# *Fungal diversity within the Populus rhizosphere and endosphere*

**Gerald A.Tuskan**
Oak Ridge National Laboratory

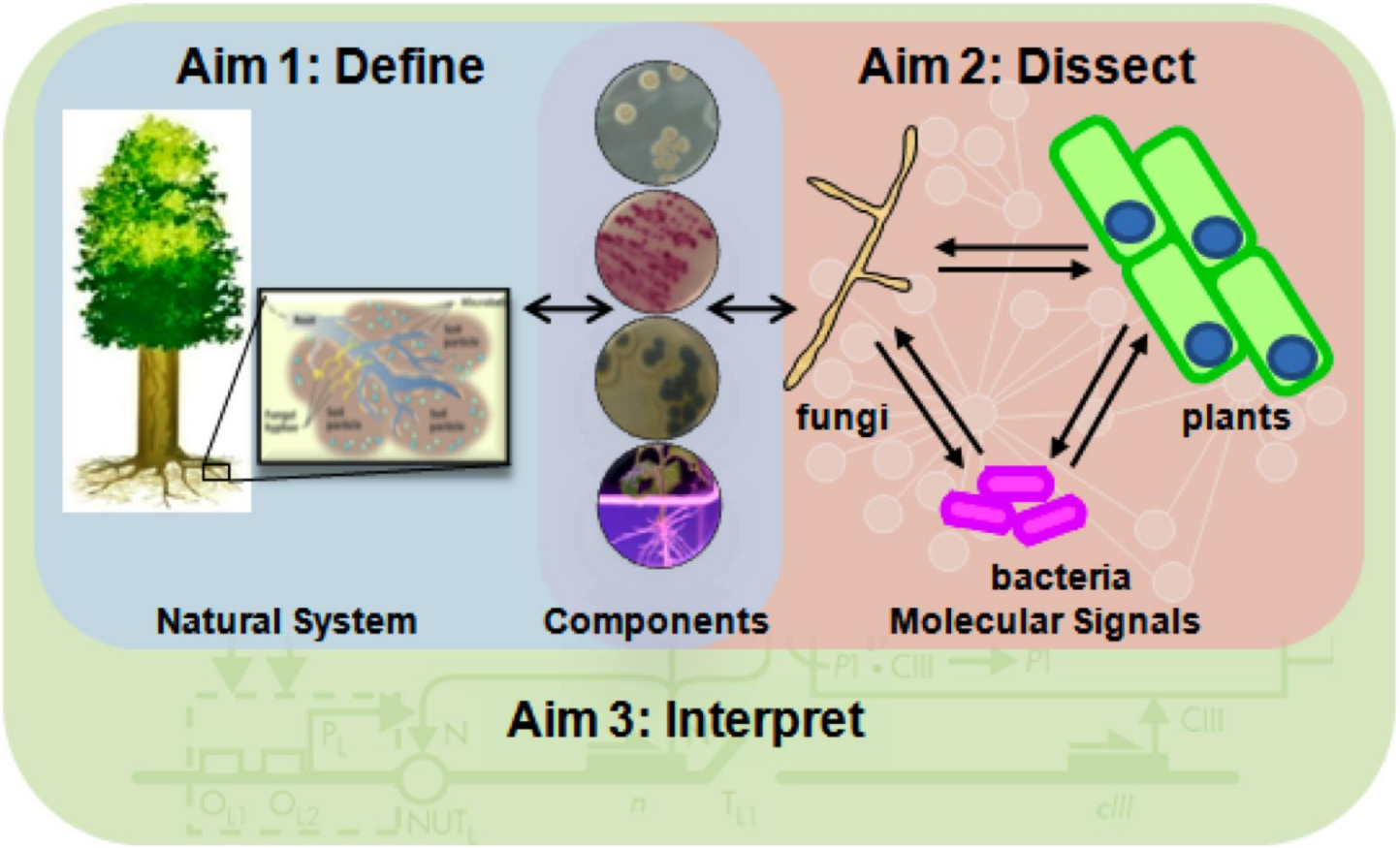
Jessy Labbe
Nora Dunkirk
Pascale Frey-Klett
Aurelie Deveau
Dale Pelletier
Gregory Bonito
Rytas Vilgalys



U.S. DEPARTMENT OF ENERGY

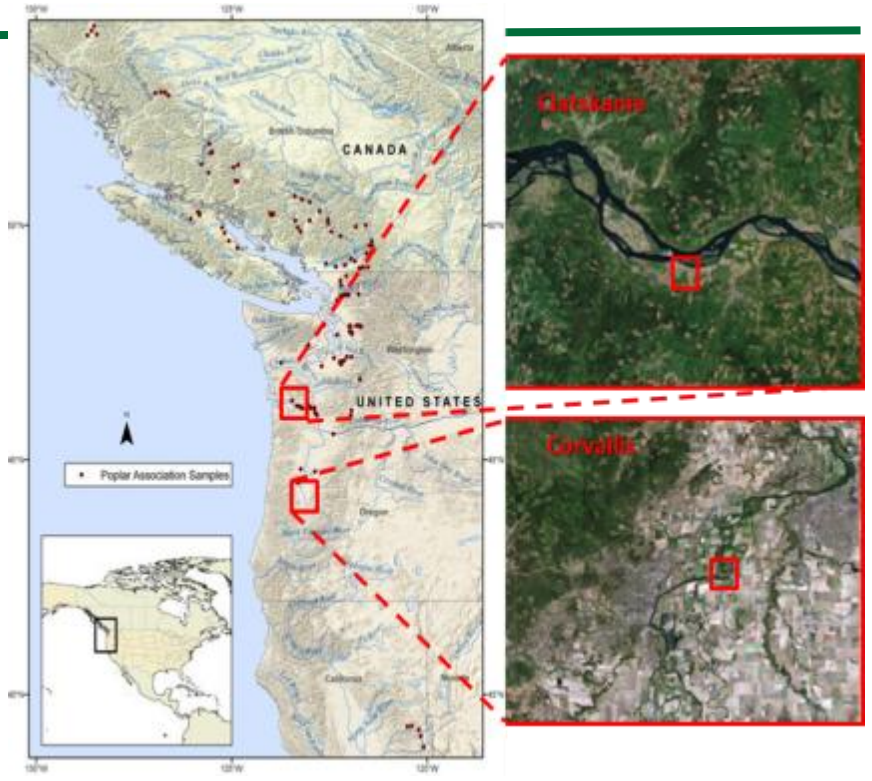
OAK RIDGE NATIONAL LABORATORY
MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY

# Defining, dissecting and interpreting the plant-microbe interface



# Next steps in defining the *Populus* microbiome

- Common garden studies
  - Partitioning of genotype by environment interactions through host resequencing and community metagenomics (JGI Collaboration)
- Understanding how members of the community function in concert
  - Metagenomic and metaproteomic measurements
  - Isolation of missing phyla
  - Model community studies




Beginning studies on well characterized common-garden populations that will allow examination of more detailed genomic-level information in a environmental setting

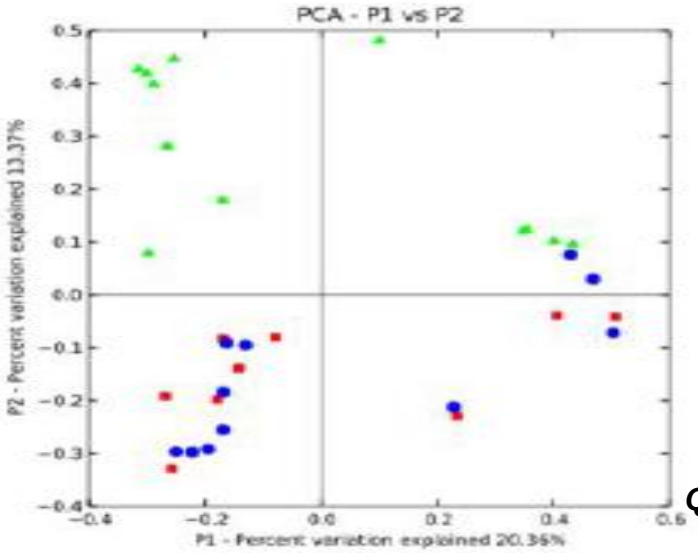
# Ectomycorrhiza Bioassay Experiment

- Allows experimental assessment of what portion of the available arbuscular (AM) and ectomycorrhizal (ECM) population, is capable of forming relationships with *Populus* roots of different genotypes (e.g., soil inoculum potential x genotype effects)
- Serves as source for ECM isolation & cultivation efforts in Task 1.3.1
- 5 genotypes of *P. deltoides*, 1 *P. dxt hybrid,* oak/pine & *Tuber* positive controls, are being used to access 5 different soils in the first experiment

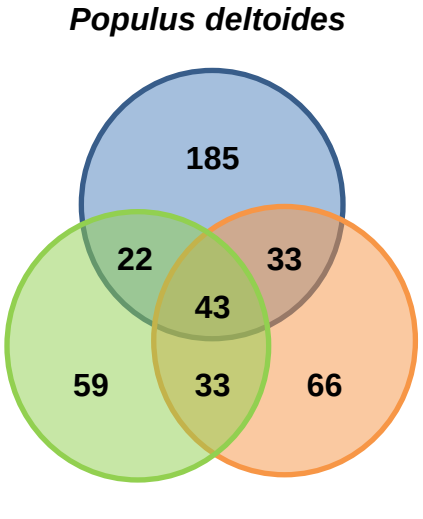
**J. Labbe, G. Bonito & R. Vilgalys**

# Trap-Plant Experiment 1

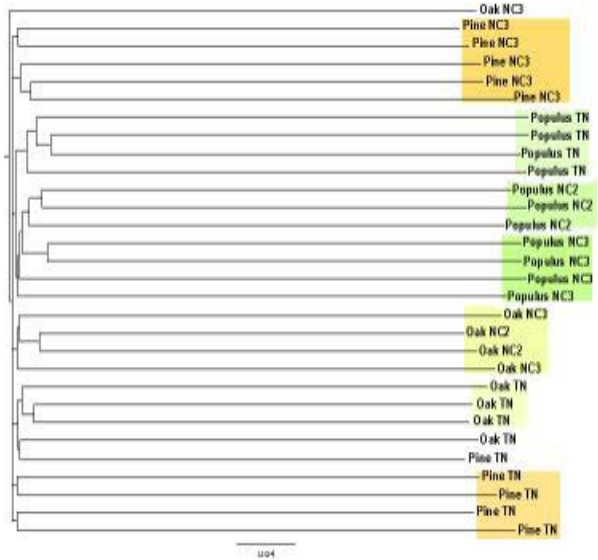
**Main finding: *Populus deltoides* hosts distinct endosphere fungal and bacterial communities from *Quercus alba* and *Pinus taeda***




**Fungal communities on three hosts**




**Fungal species (OTUs) shared between 3 hosts**



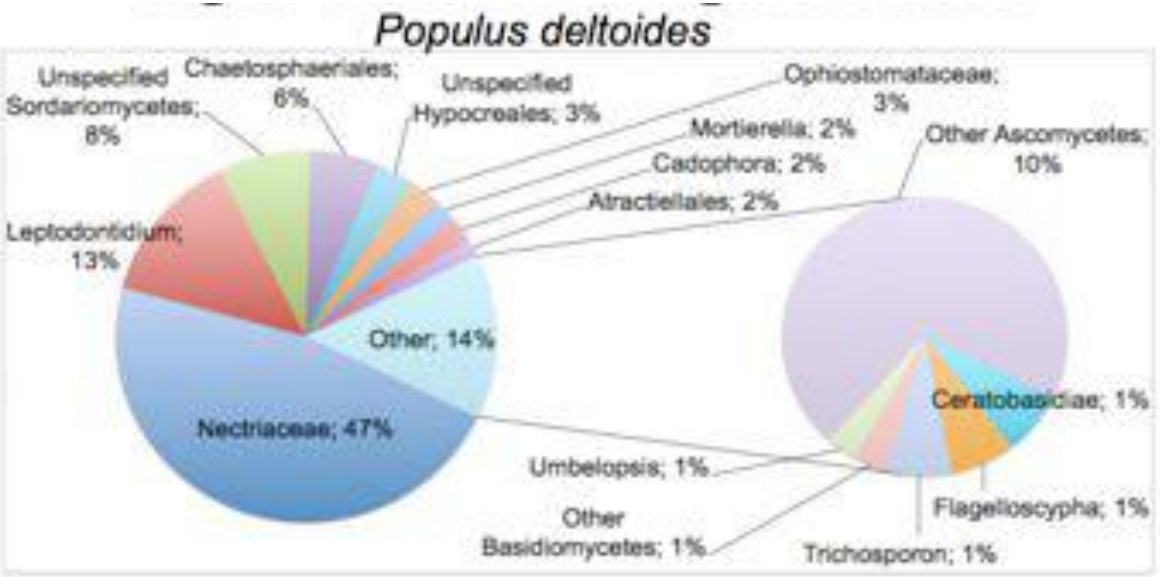
**Unifrac distances of bacterial communities based on 10248 16S sequences**

**Bonito et al. *In prep***

# PMI Fungal Culture Collection

**>1800 isolates**
**ITS DNA barcoded**
**Selected Taxa for**:

- Koch's postulates
- Characterization
- Genomics
- Transcriptomic Experiments




Ranked Abundance

| *P. Deltoides* | Shared | *P. trichocarpa* |
|---|---|---|
| Chaetosphaeriaceae | Cylindrocarpon | Ilyonectria radicicola |
| Ophiostomataceae | Leptodontium | Phomopsis |
| Atractiellales | Ilyonectria | Corticiaceae |
| Denodrosporium | Neonectria | Hypocrea |
| Thozetella | Fusarium | Paecilomyces |
| Trichosporon | Exophiala | Chaetomiaceae |
| Aspergillus | Mortierella | Cryptosporiopsis |
| Phoma | Lecythophora | Bionectria |
| Chaetosphaeria | Hymenoscyphus | Phialophora |
| Diaporthales | Mortierella | Aureobasiidum |

# Koch's Postulates

1) Causal organism must be associated with disease
2) Causal organism must be isolated from an infected plant & grown in culture
3) When a healthy susceptible host is inoculated with the pathogen from pure culture, symptoms of the original disease must develop
4) The same organism must be re-isolated from plant

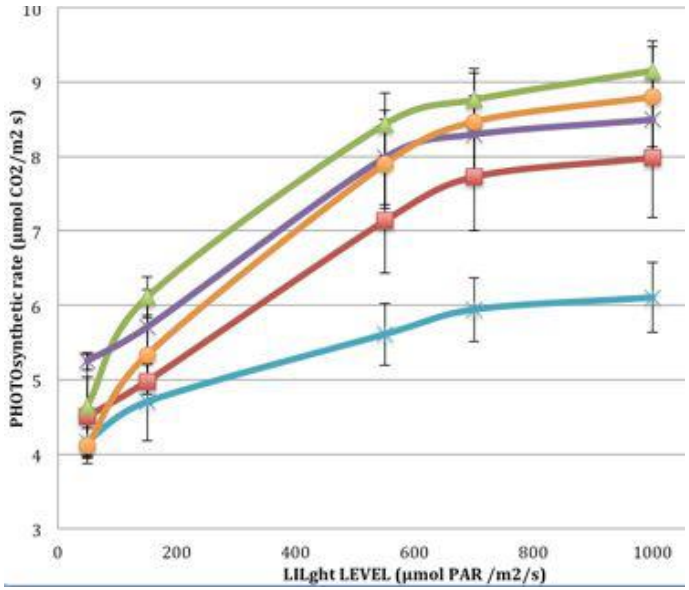
**Grow fungus on millet**

**Inoculate plants with colonized millet**

**Re-isolated fungus, characterize fungal and plant growth and health**

# Effects of fungal endophytes on plant growth & photosynthesis

## Mean photosynthesis of oaks inoculated with root endophytes



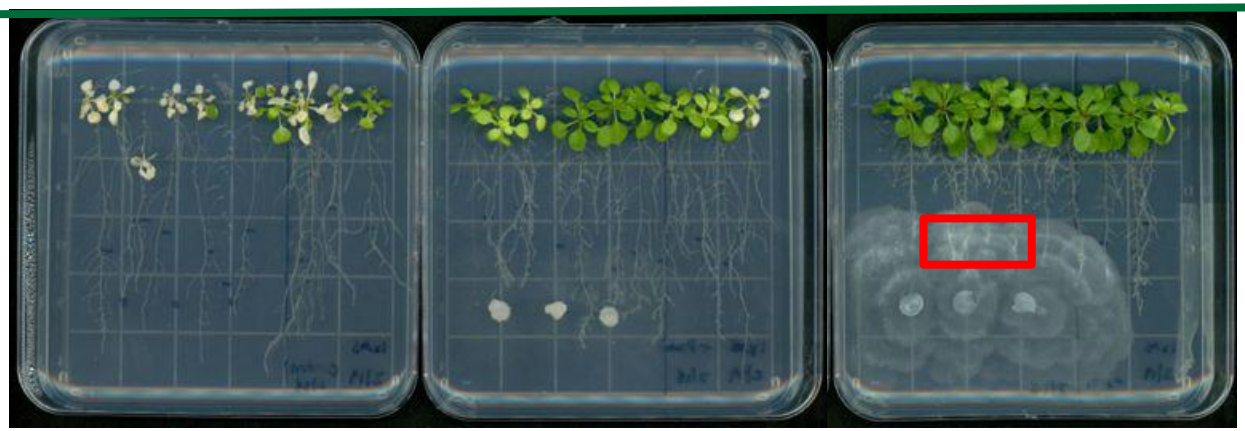

***Atractiella* sp. PMI95**
***Piriformaspora indica***
***Atractiella* sp. PMI242**
***Mortierella elongata* PMI93**

**Control uninoculated**

## *Atractiella* affects leaf area of red oak

**Control uninoculated PMI95 PMI242 PMI152**

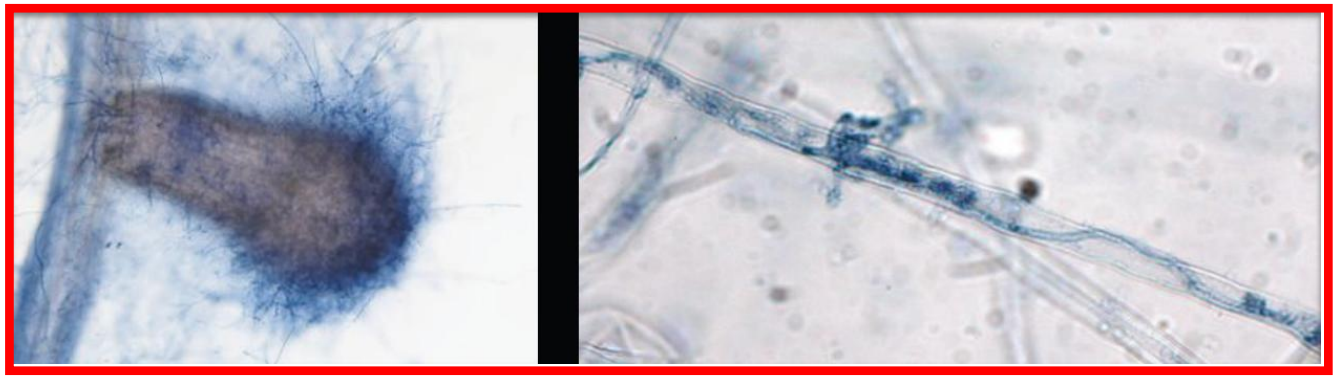
# Effect of fungal colonization on heat shock tolerance



Control
(no fungus)

+ *P. indica*

+ *Mortierella elongata* 77

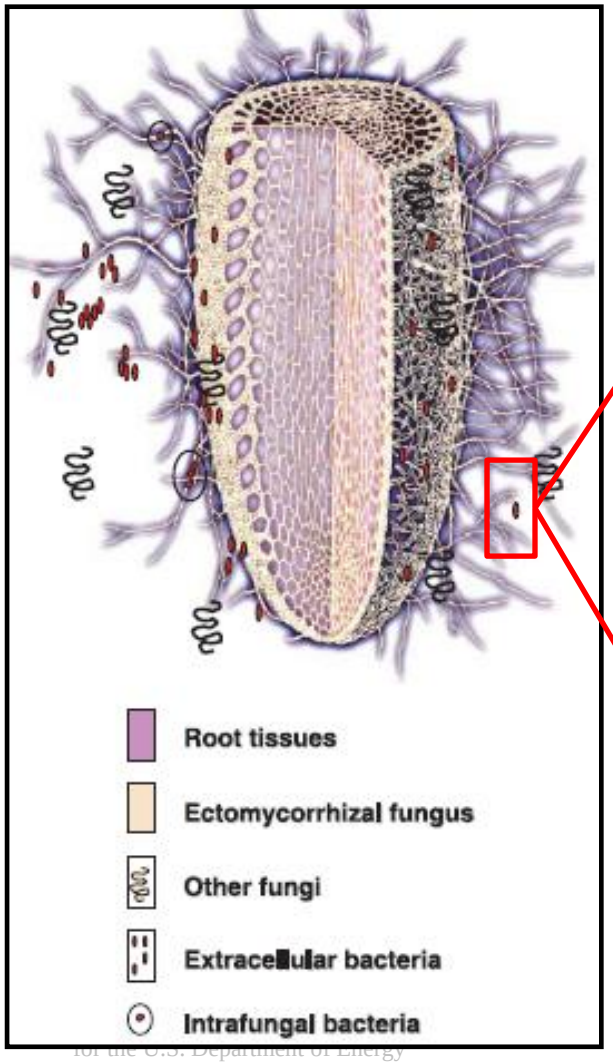


*Mortierella elongata* colonizing *Arabidopsis* roots

**Jeremy Lipkowitz (Duke Univ.)**

# Experimental characterization of Mycorrhizal Helper Bacteria (MHB)

Goal: identification of the molecular determinants of the helper effect



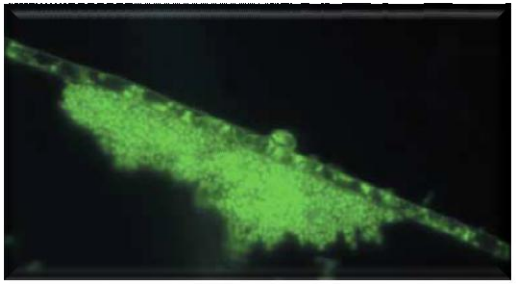

Frey-Klett et al 2005


Electron microscopic image

Mycorrhizal fungi are surrounded by complex microbial communities, which modulate the mycorrhizal symbiosis.

'Mycorrhiza helper bacteria' (MHB) assist mycorrhiza formation,

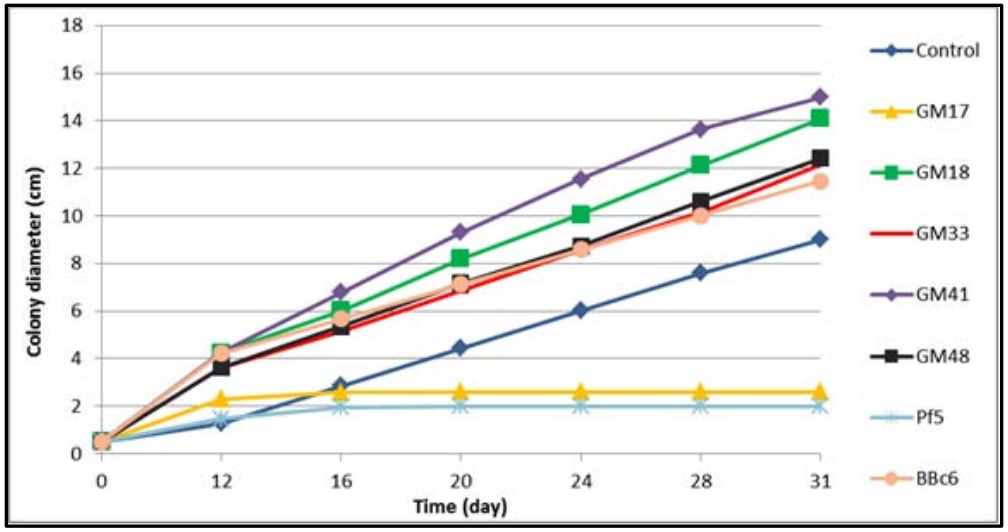
and interact positively with the functioning of the symbiosis.

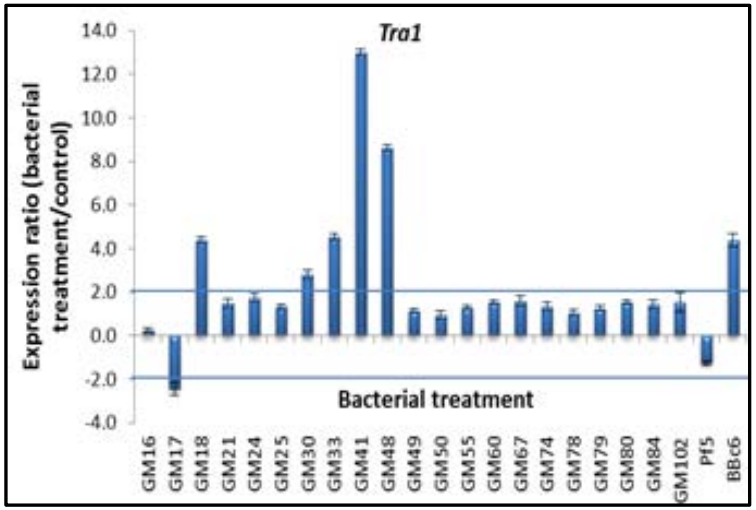


Epifluorescence microscopy of biofilm on fungal hyphae

# Summary of MHB Characterization

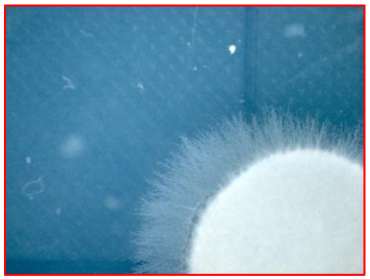
## MHB strains of the *Laccaria-Populus* interaction among the native *Populus deltoides* microbial communities



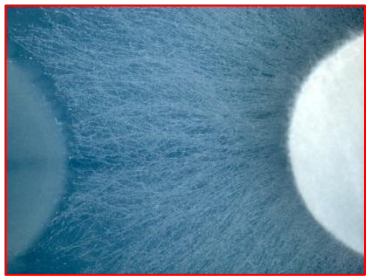




*Microscopic observation of L. bicolor mycelium (10X)* 12 DPI

BBc6 effect +



GM17 effect -



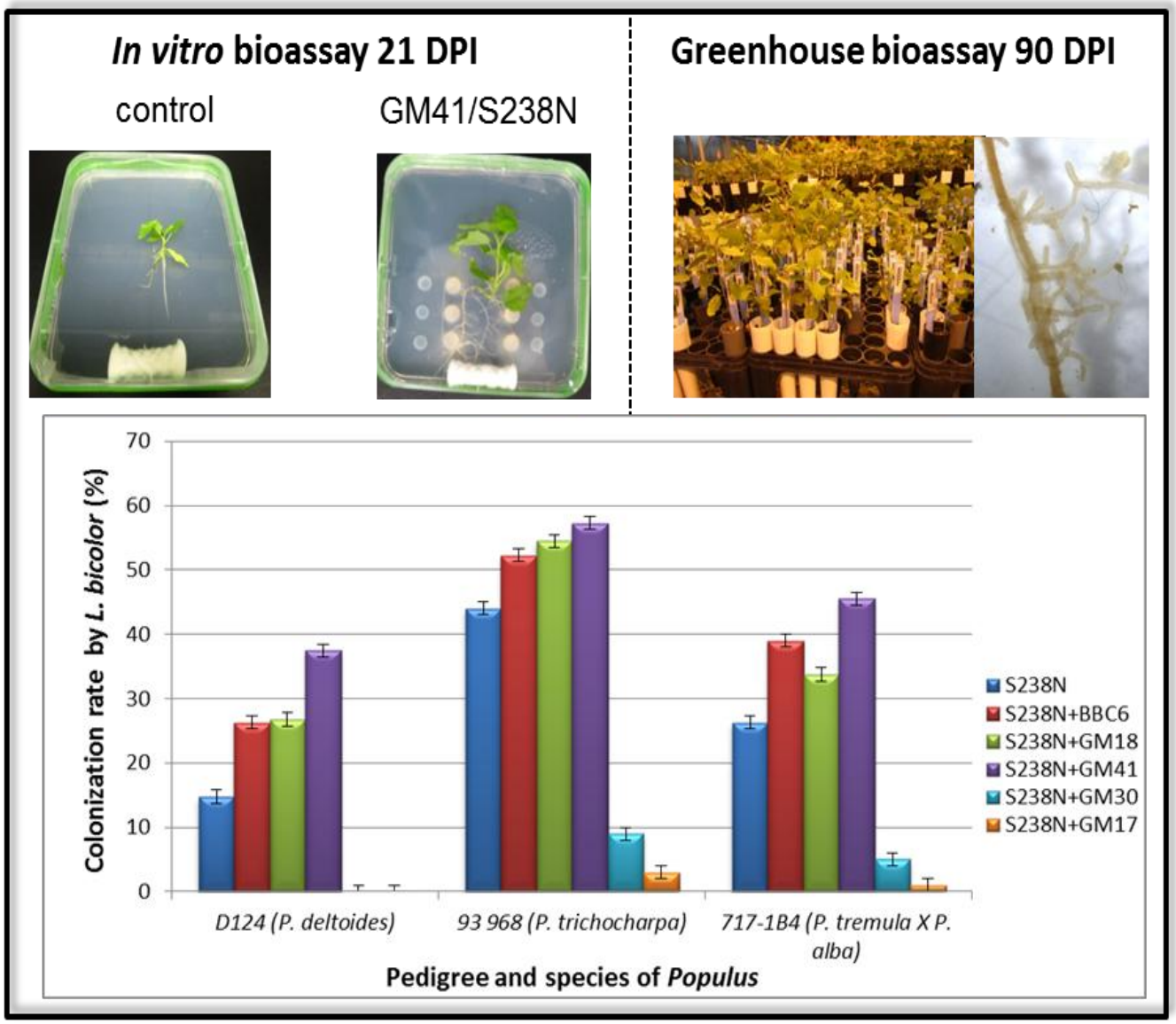
GM41 effect ++

We looked at the bacterial effect on *Laccaria* growth and mycelium morphology (branching and density)

and

the expression level of seven target genes in *L. bicolor* shown as regulated by the MHB strain BBc6R8

# Summary of MHB Characterization - con't



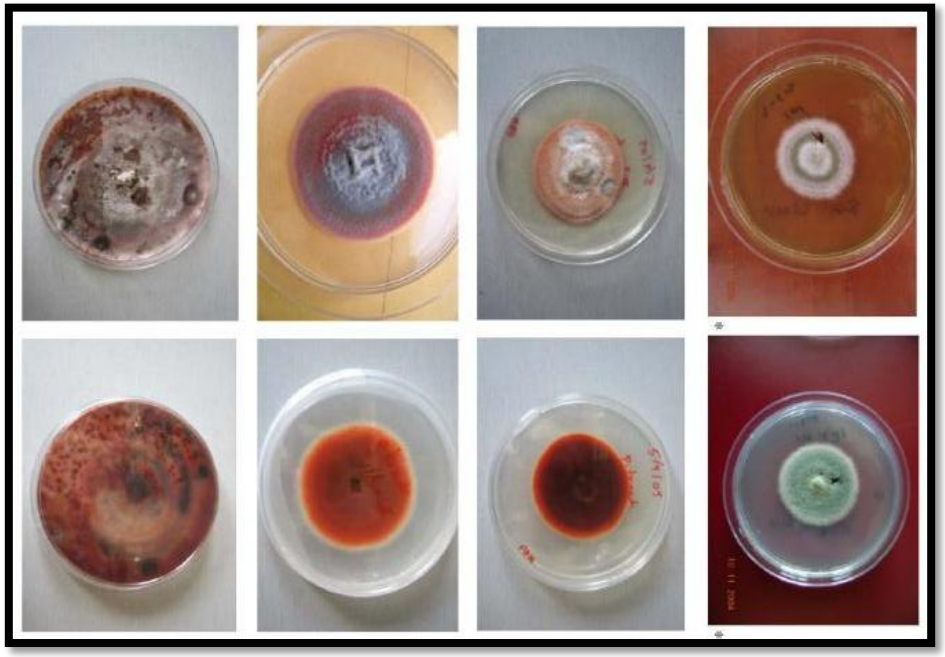

**We assessed the effect on plant of some interesting combinations by *in vitro* assays**

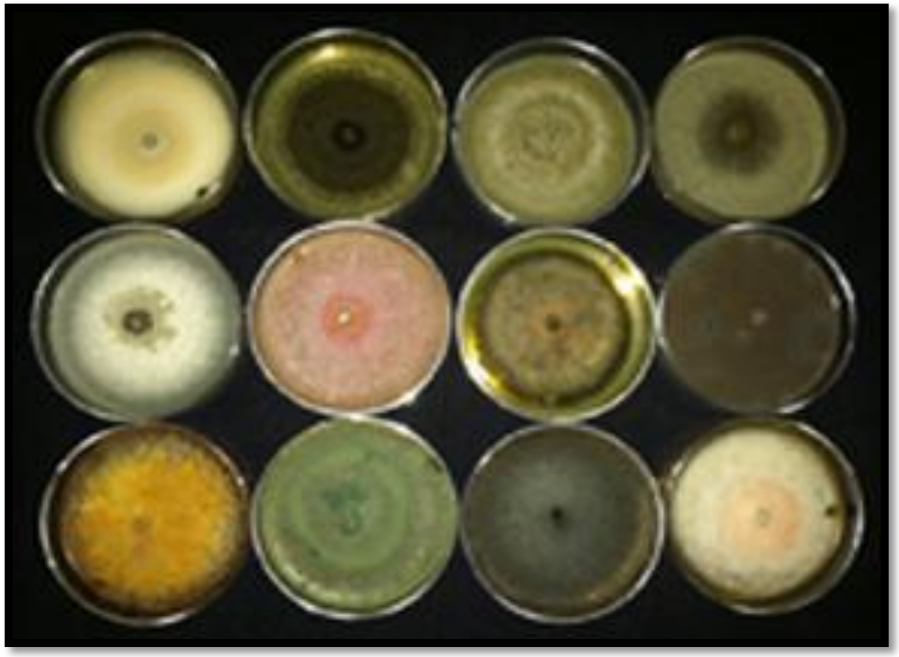
***As well, the ectomycorrhizal formation in greenhouse assays across 3 Populus species***

**4 MHB strains showed high helper effects of which one significantly improving *P. deltoides* colonization by *Laccaria***

# Evaluating the characterized MHB strains effect on other Fungi isolates

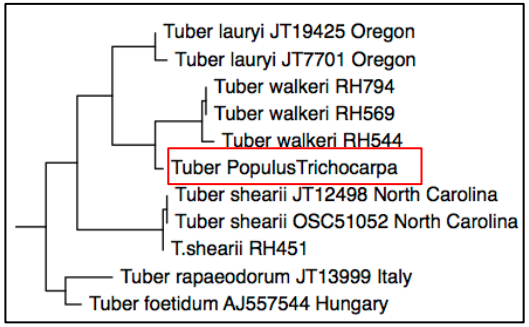
(experimental evaluation done on 20 fungal strains)

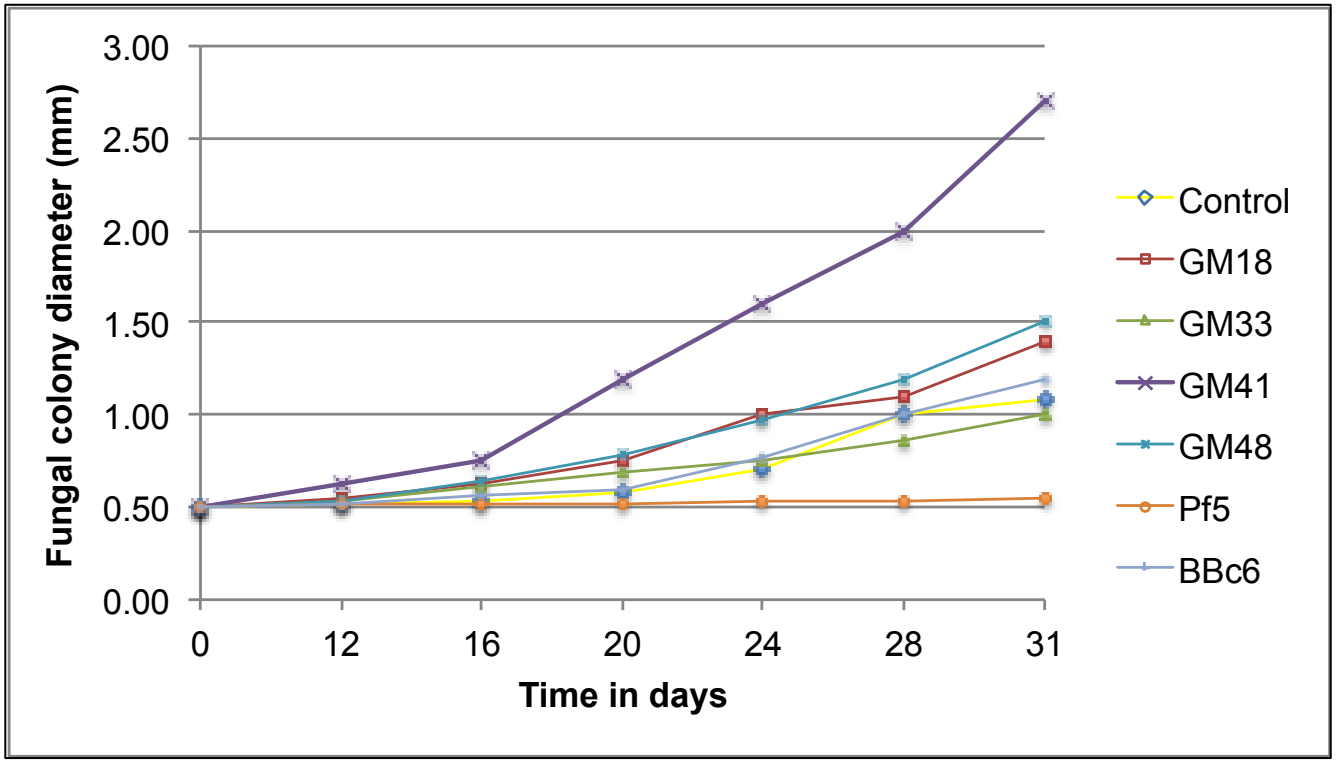




Look at the fungal growth and the branching density over a month of culture

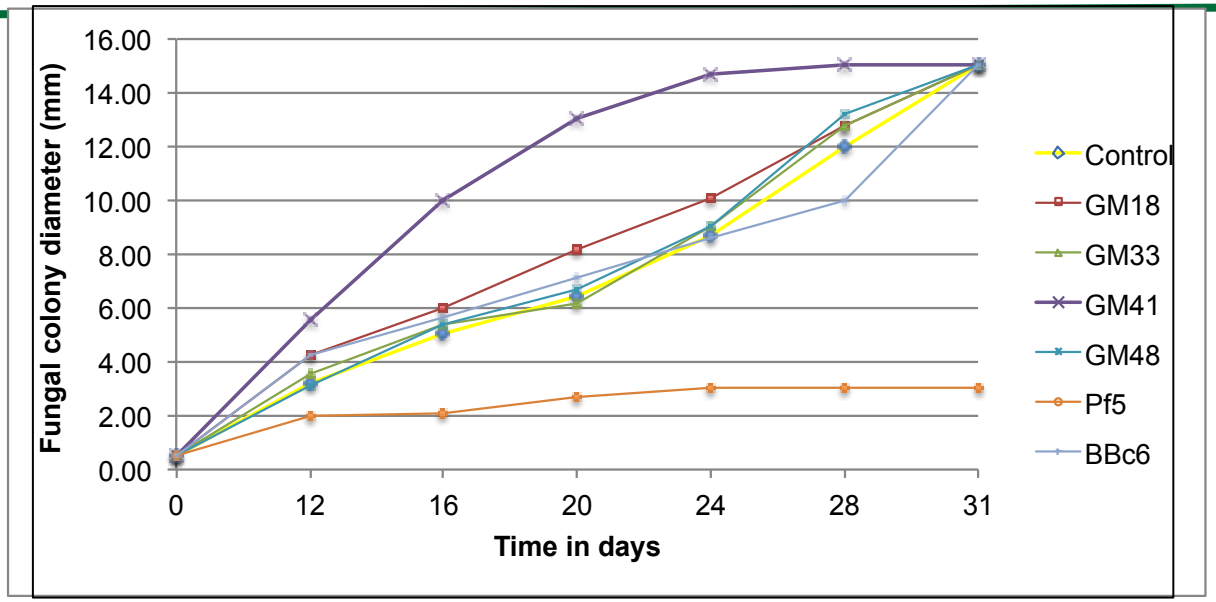
# Focus on the new isolated species of Tuber (not named yet)

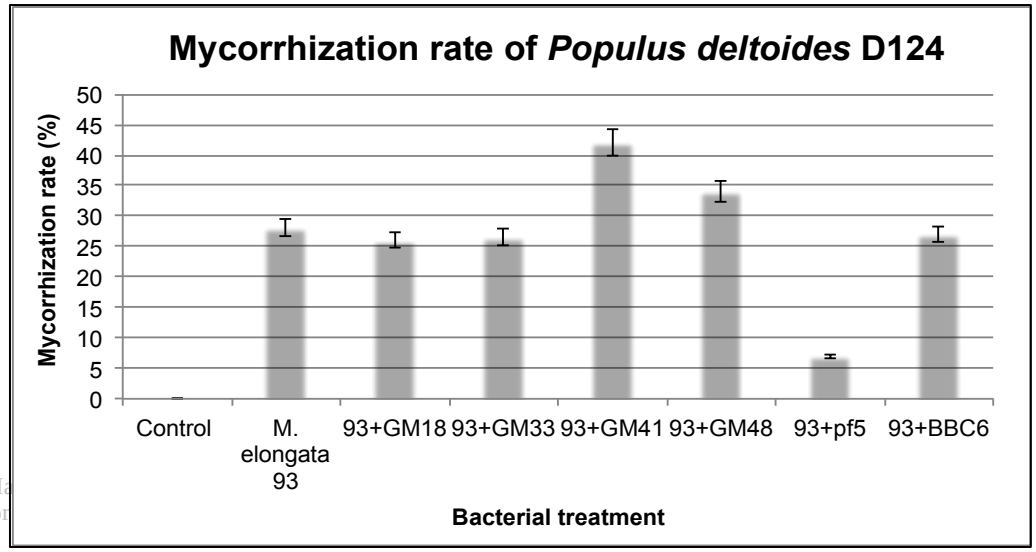



The *Pseudomonas fluorescens* strain GM41 has a significant effect on the growth of the mycelium (ANOVA, $p \geq 0.01$)

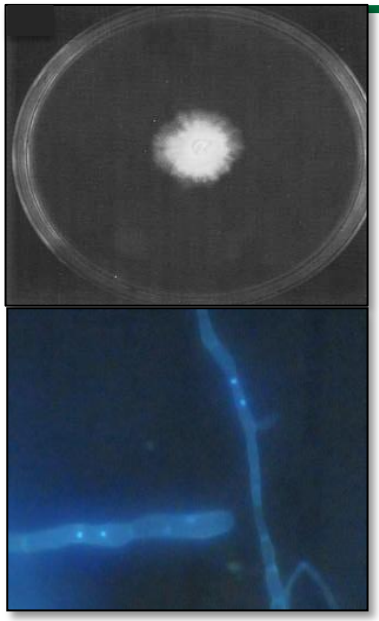
# Focus on the sequenced *Mortierella elongata* strain #93

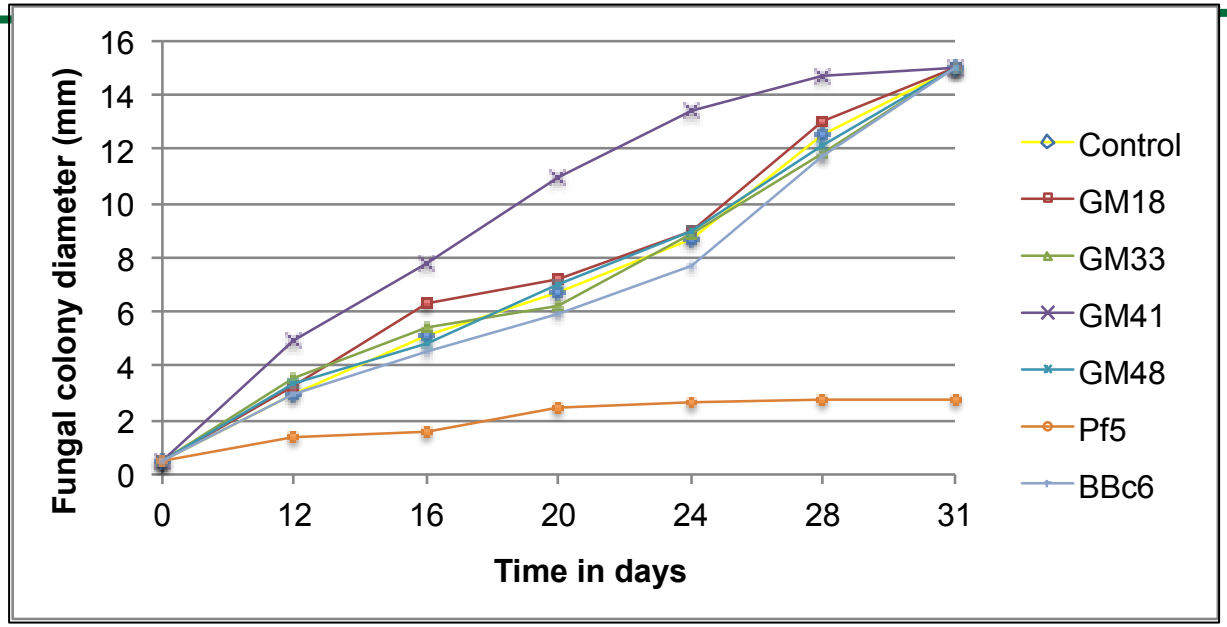


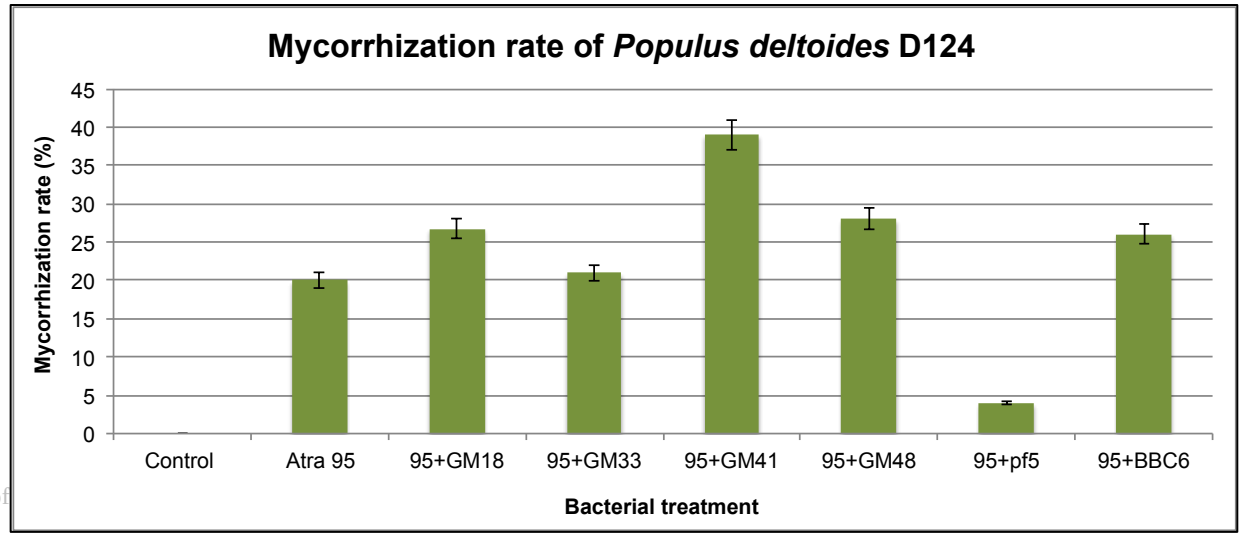




The *Pseudomonas fluorescens* strain GM41 has a significant effect on the growth of the mycelium and root colonization as well (ANOVA, $p \geq 0.01$)

# Focus on the sequenced Atractiella sp. strain #95

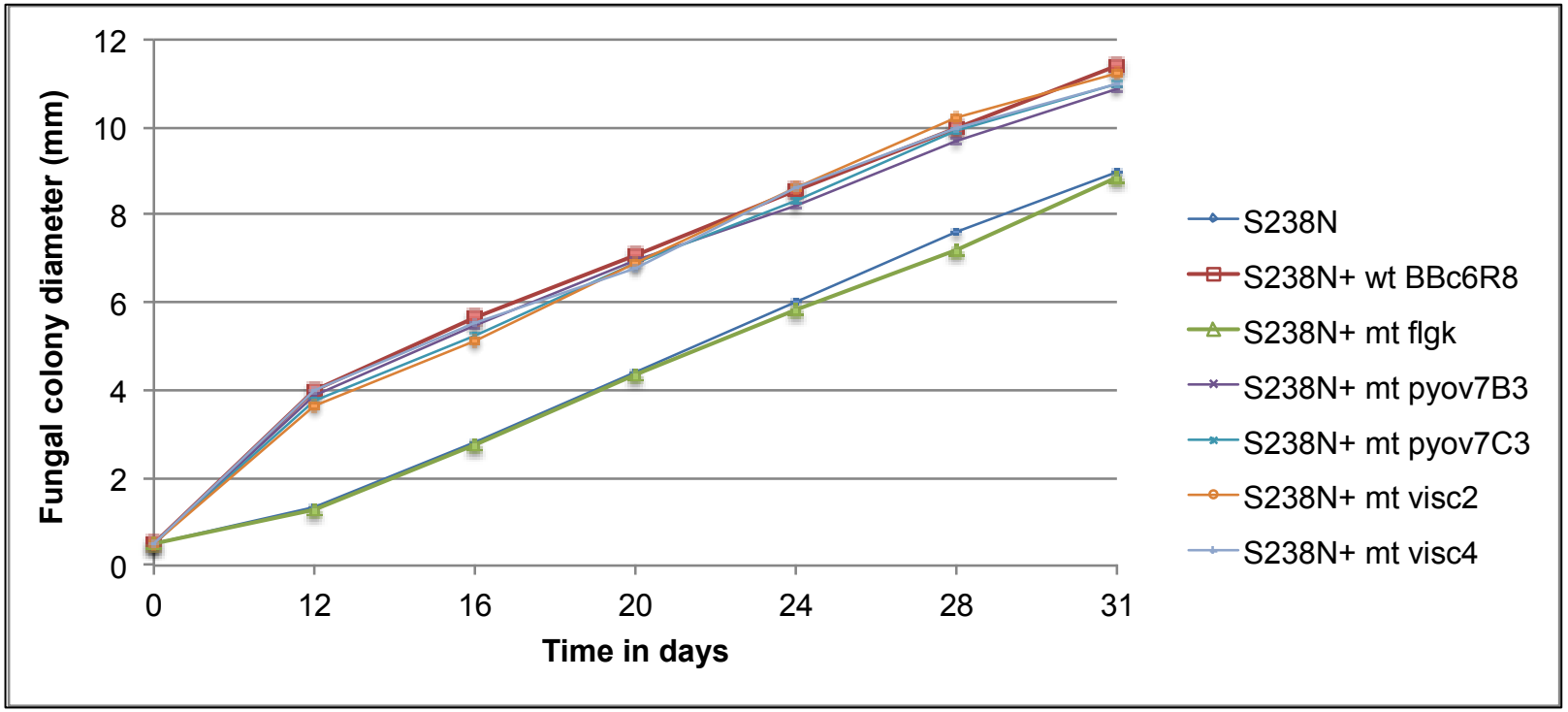




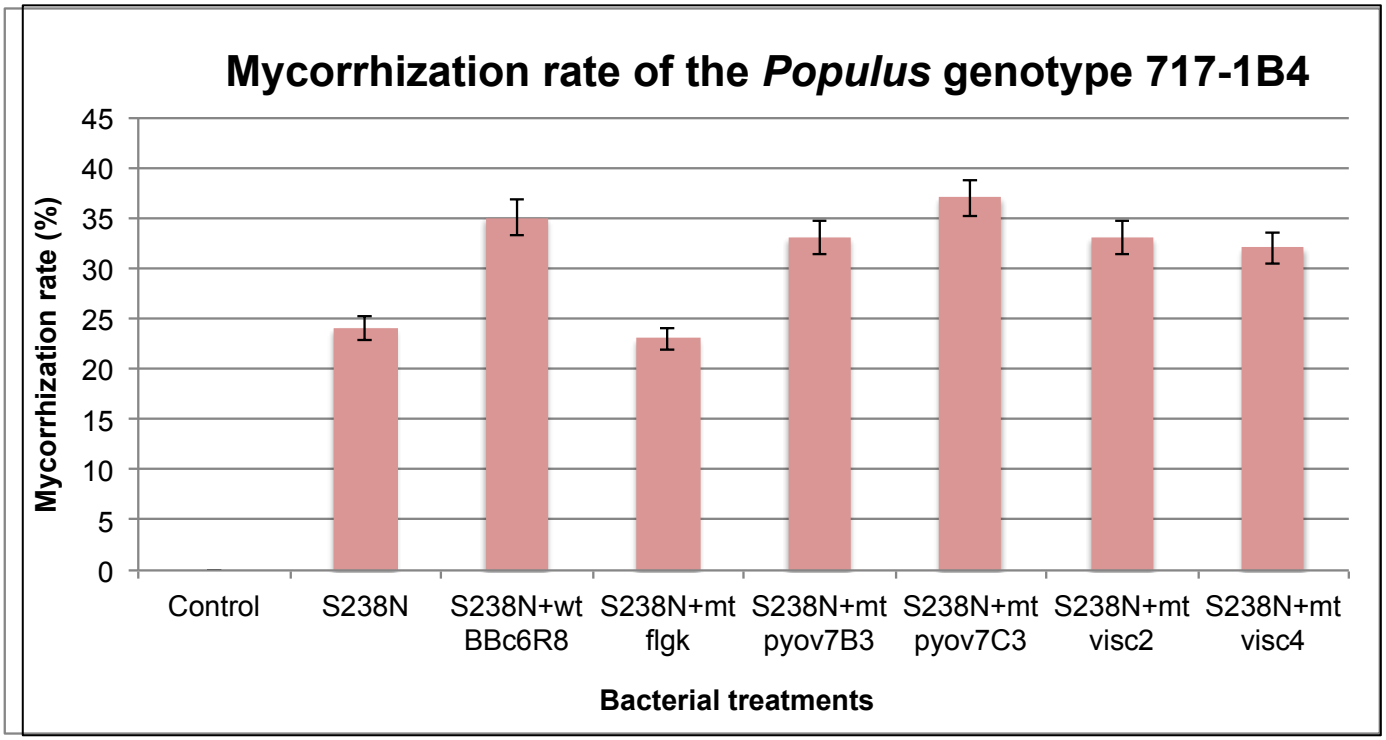
# Transition to the molecular determinants of MHB

## Effect of the BBc6R8 mutants on the *L. bicolor* S238N radial growth



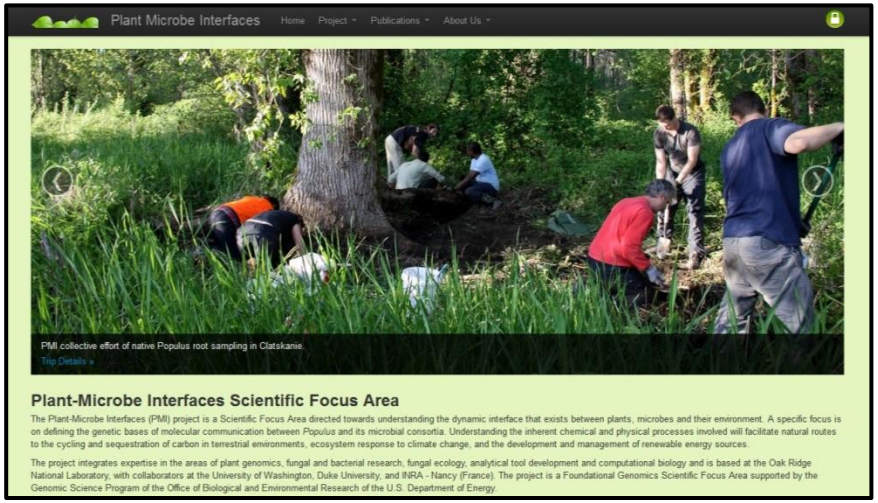

The *P. fluorescens* BBc6R8 mutant *Δflgk* has a significant impact on the *Laccaria* growth (ANOVA, $p \geq 0.01$)

# Effect of the BBc6R8 mutants on the colonization of *Populus* by *Laccaria* (*in vitro*)




The *P. fluorescens* BBc6R8 mutant *Δflgk* has a significant effect on the helper ability (ANOVA, $p \geq 0.01$)

# Acknowledgments




U.S. DEPARTMENT OF ENERGY
Office of Science

**pmi.ornl.gov**


F. Martin


J. Plett


R. Vilgalys


G. Bonito


C. Schadt


Jessy Labbe


D. Pelletier

INRA

Duke UNIVERSITY

OAK RIDGE National Laboratory

JGI DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE



OAK RIDGE National Laboratory