Analysis of Beneficial Bacterial Populations from Chinese Longbeans

Alexis Zaide, Jeff Hillyer, Scott Holt*, Western Illinois University, Biology, Macomb, IL

Specific Goals

understand the biodiversity of natural beneficial plant bacteria

build a collection of beneficial bacteria & assess their effectiveness for boosting plant growth

harness microbial biodiversity for development of natural agricultural biologicals that support the growth of crop plants

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Model Plant Species: Common Names

Yardlong Bean, Chinese Long Bean, Asparagus Bean

https://www.tbo.com/storyimage/TB/20131023/ARTICLE/131029832/EP/1/2/EP-131029832.jpg

Model Plant Species Scientific Name

Vigna unguiculate (L.) Walp. Ssp. Sesquipedalis (L.) Verdc.

Origin / Distribution:

originated from domestication of Cowpea
originated in southern Asia
now grown in Asia, Europe, & North America.

Status = Legume (seed pod-forming, dicots)

symbiosis w/ N2-fixing bacteria (root nodules)
the plant makes its own food & enriches the soil

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http://www.worldformarket.com/wp-content/uploads/2016/02/Yard-Long-Bean-White-Yard-Long-Bean.gif

https://www.tbo.com/storyimage/TB/20131023/ARTICLE/131029832/EP/1/2/EP-131029832.jpg

https://petalsandwings.files.wordpress.com/2012/08/longbean-7-1.jpg

USDA Plant Guide

Food Source

pods, young leaves, & stem tips edible

humans & wildlife (Deer)

Commercial

harvested sold farmers markets & local distributers

Ornamental

large violet-blue flowers & draping pods

useful city parks, office buildings, homes

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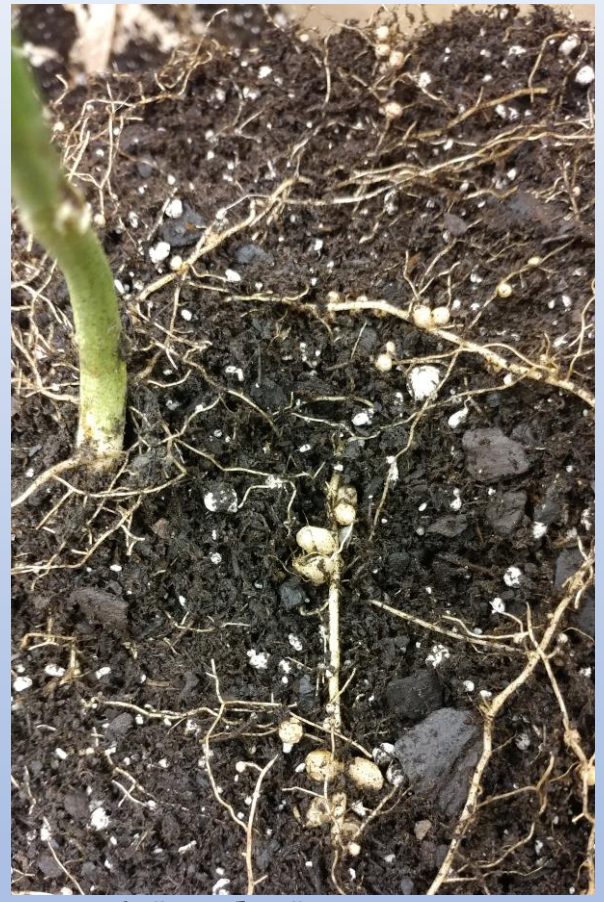

Project Goals

assess the beneficial bacterial populations on longbeans
leaves & nodules

use culture dependent & culture independent methods

Leaves

Methylobacterium species
beneficial for plants

Nodules

symbiotic rhizobia spp infect root nodules

i.e. = *Bradyrhizobium?*

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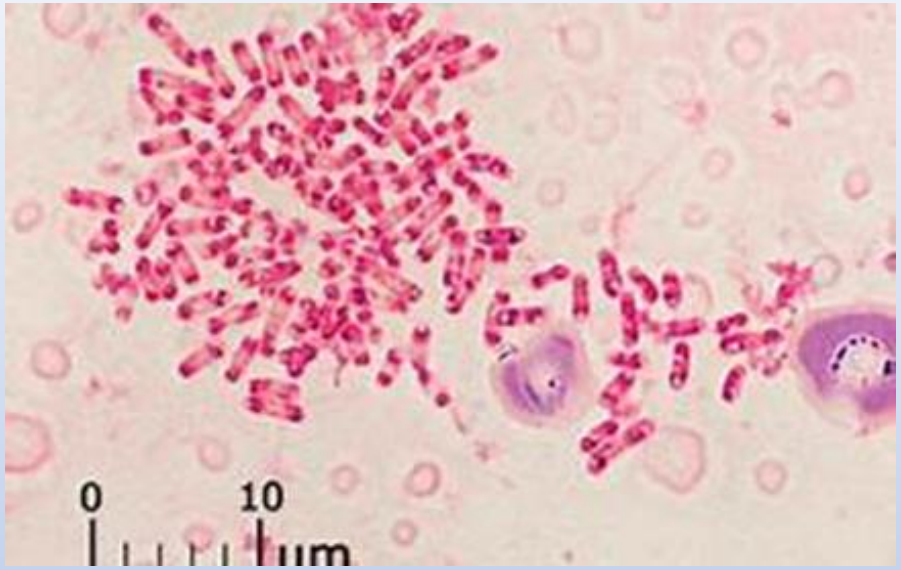


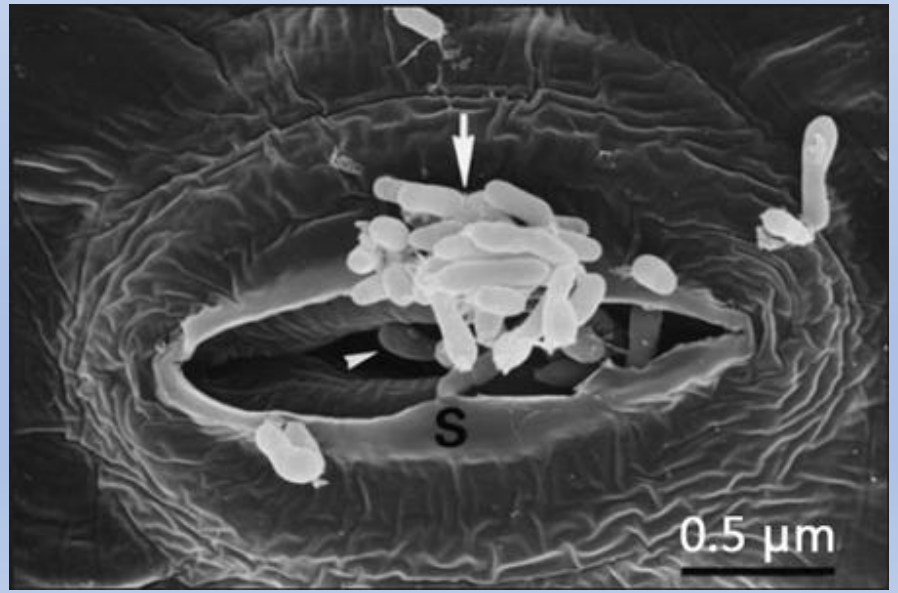
Leaves: *Methylobacterium* species

gram negative, rod shaped cells

live on plant leaf surfaces

metabolize C1 = methanol (dehydrogenase)

liberated from plant during growth



Stroma (100s / mm)

Significance = Provide Benefits to Many Plants

improve seed germination & crop yield, ...

pathogen resistance & drought stress tolerance

excrete plant growth hormones & osmoprotectants

positively influence plant growth & health.

used as natural seed inoculates by ag biotech co.

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Experimental Outline

Leaves (Methylobacterium) or Nodule (rhizobia)

Culture Dependent

Culture Independent

Extracts
Leaves & Nodules

1. Total Bacterial Count
2. *Methylobacterium* Count
3. Rhizobia count (nodule)

Culture Collection
Store at -80°C

Microbial DNA

PCR

16S rDNA Amplicons

Sequencing

Main Bacterial Phyla
Proportion to each other

Composition of Beneficial Bacterial Populations on Plant Species

Isolation of Total Bacteria & Methylobacterium from Leaves

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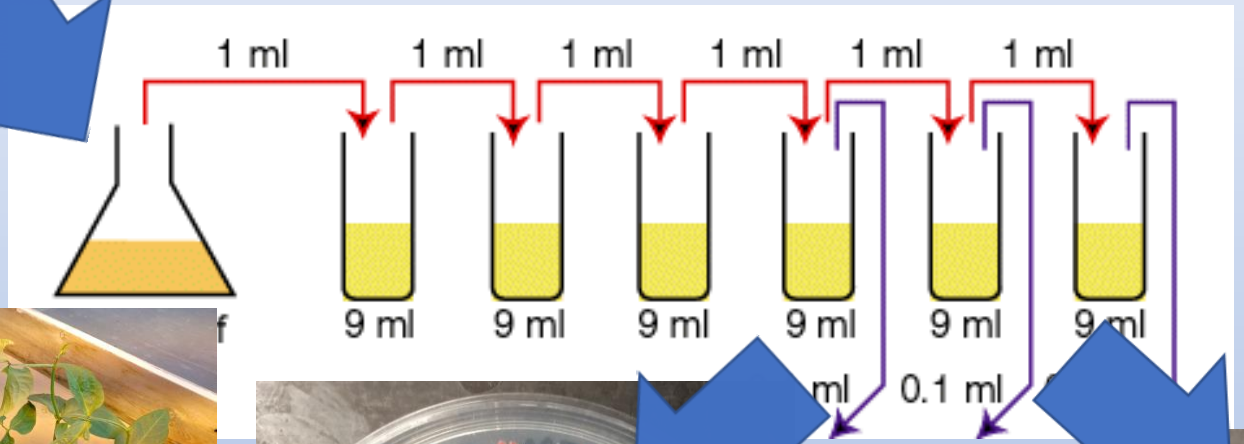
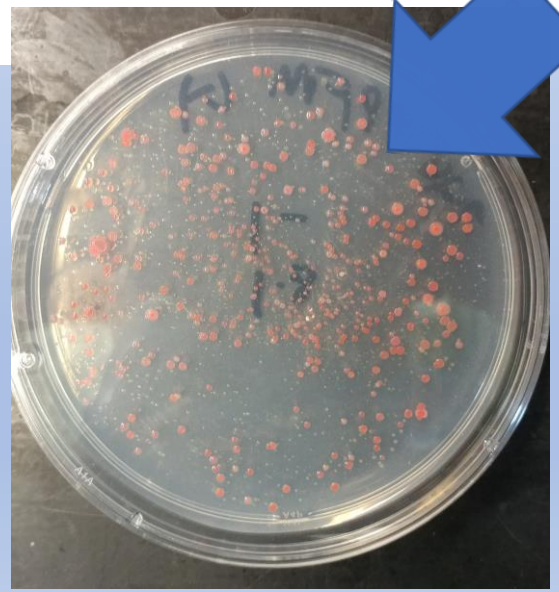
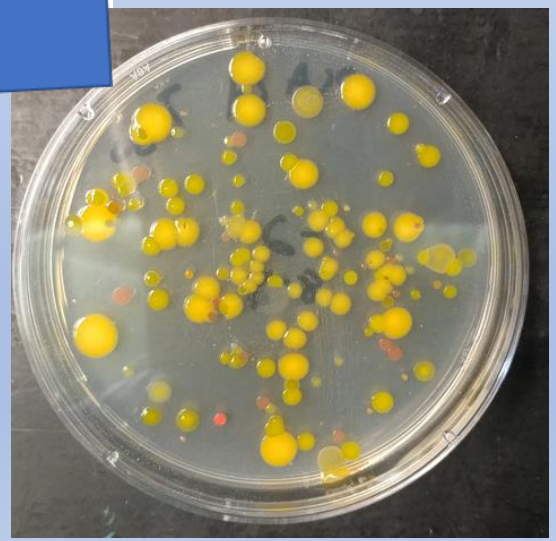




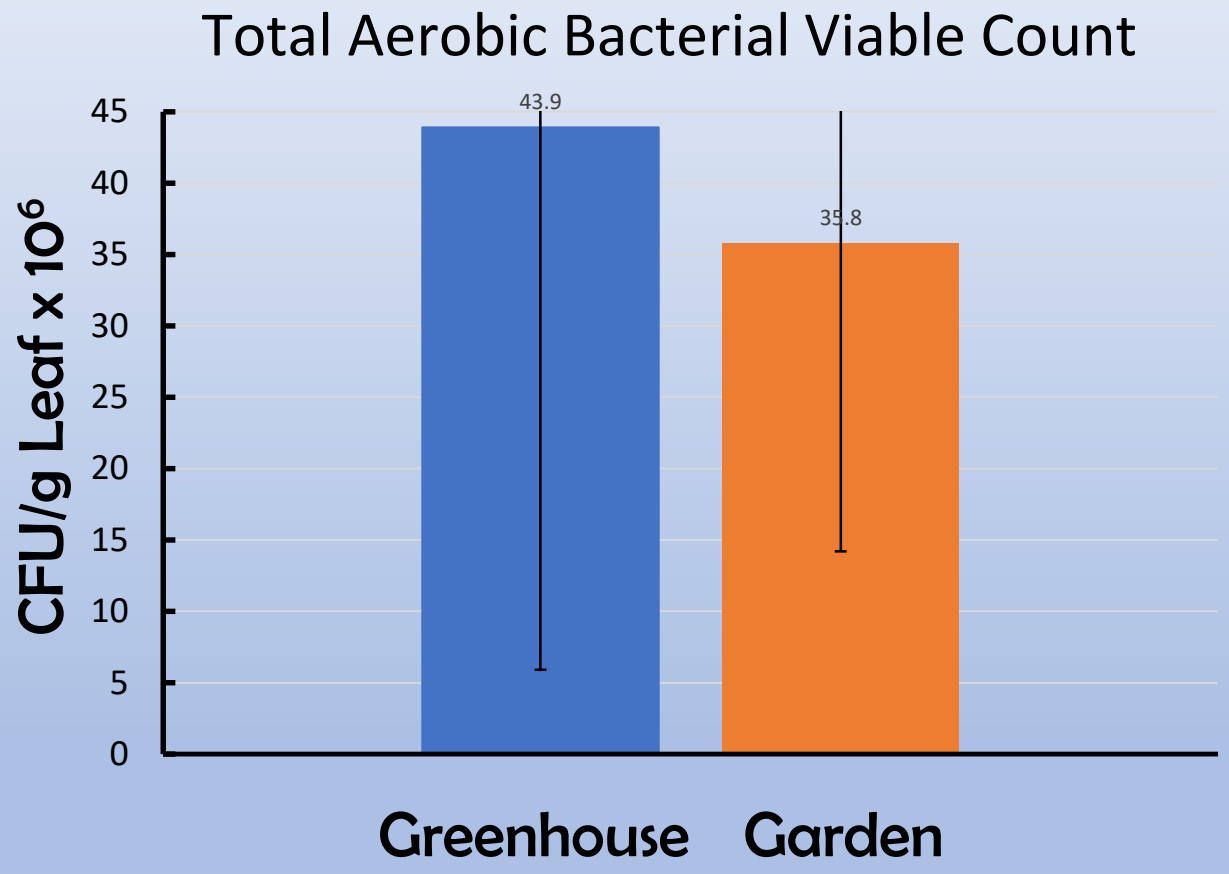
Methylobacterium



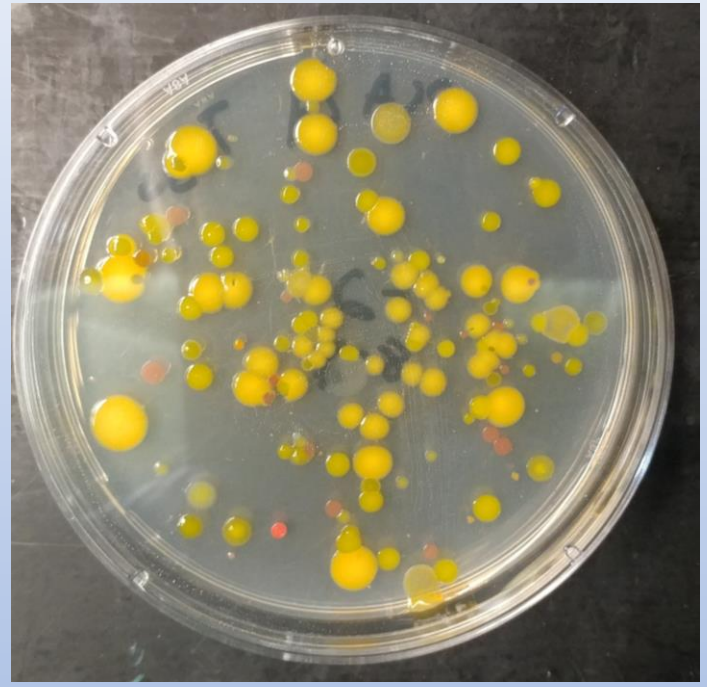
Total Bacteria

Pilot Study: Total Aerobic Bacterial Viable Count on Longbean Leaves

Greenhouse Beans Vs Garden Beans



mean value from 3 leaves



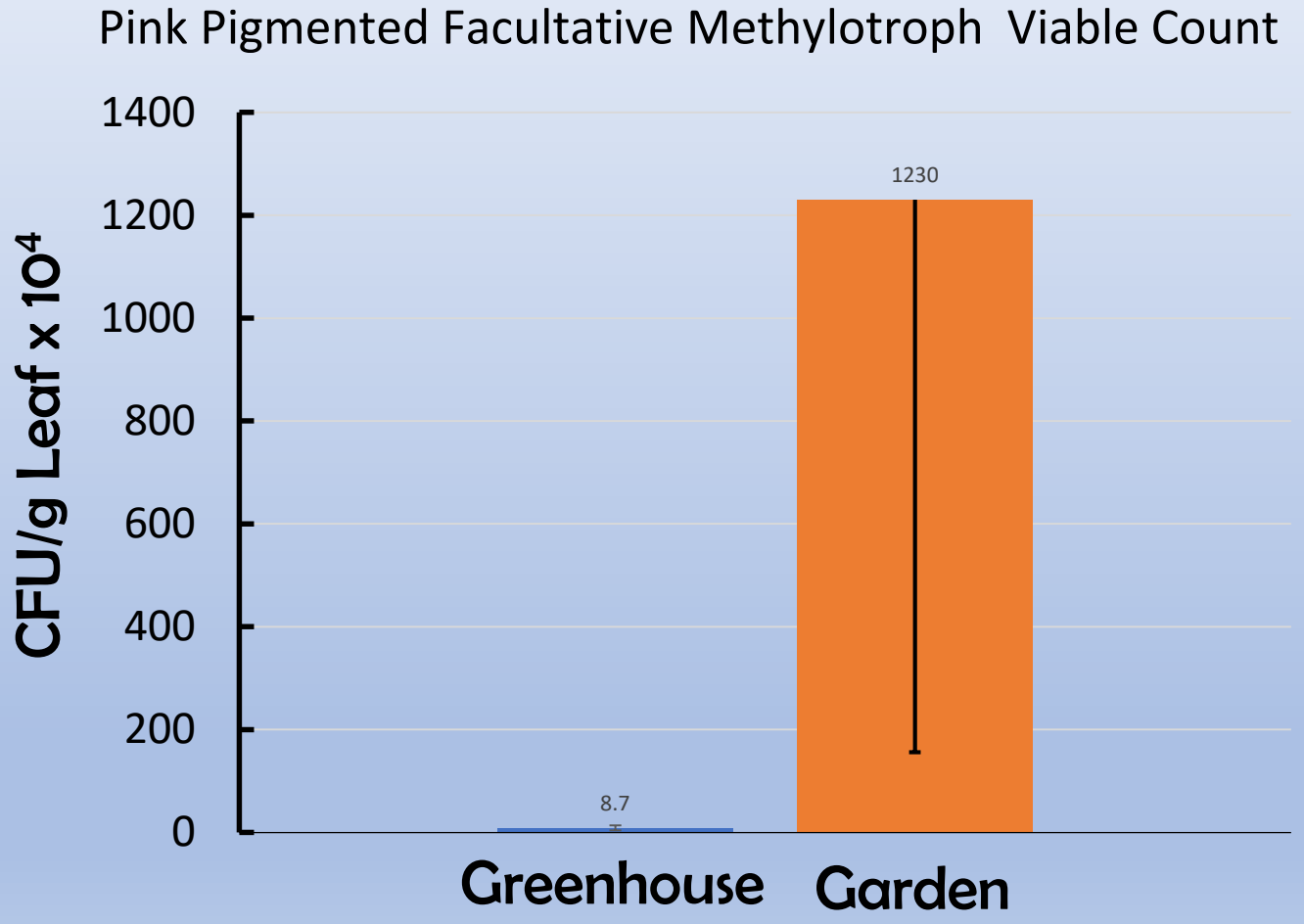
Heterotrophic Bacterial Count
Plate Count Agar

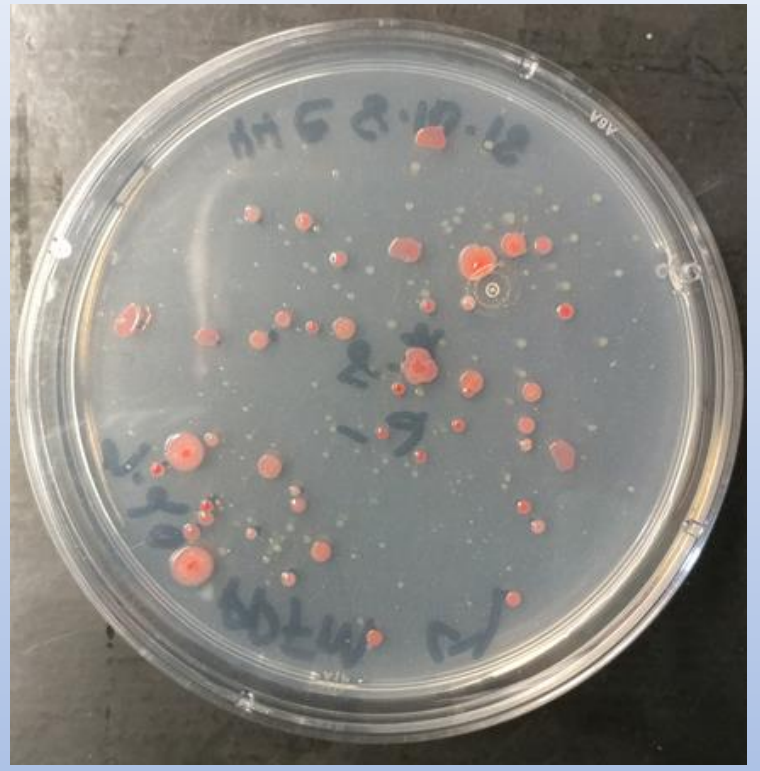
☺ tremendous variability

☺ no significant difference

Pilot Study: Total *Methylobacterium* Viable Count on Longbean Leaves

Greenhouse Beans Vs Garden Beans





Methylobacterium on AMS w/ methanol
Pink colonies

☺ tremendous variability

☺ no significant difference

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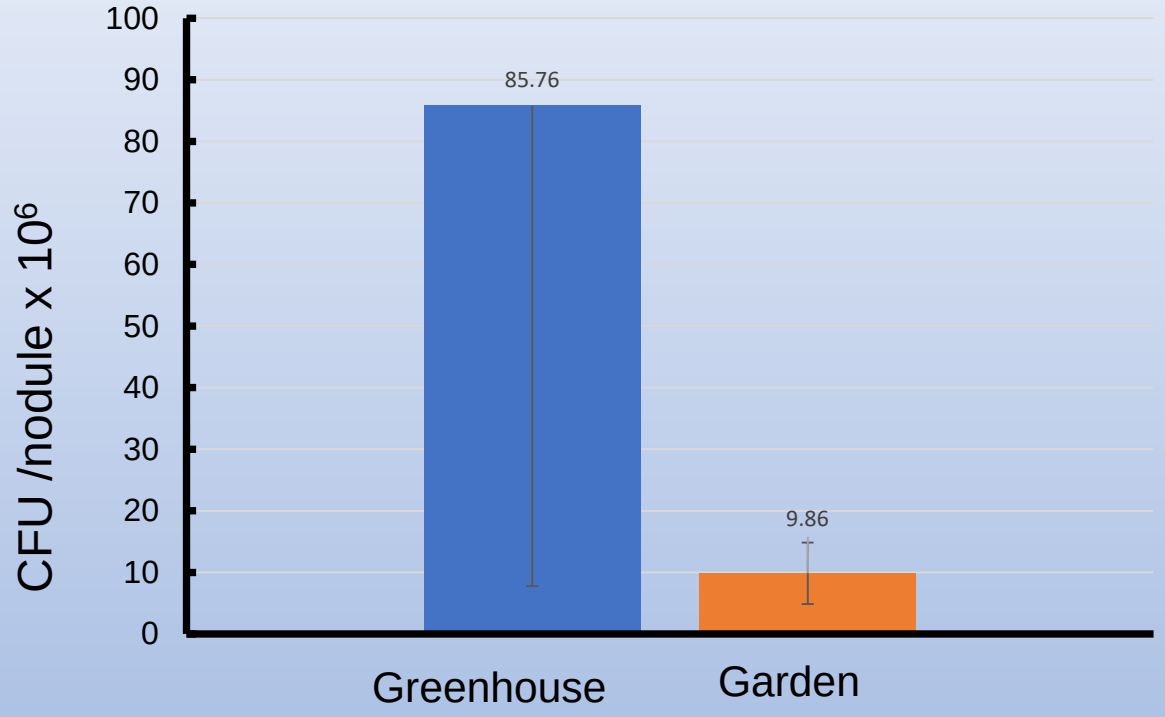
rhizobia viable count from root nodules



Nodule Rhizobia on CRYMA
2 weeks incubation @ room temperature

☺ **tremendous variability**

☺ **no significant difference**

CFU / mg better than CFU / nodule?

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Table 1. Identification of Rhizobia from root nodules	
Cultural Characteristics	cream or pinkish, mucoid colonies 14 days incubation on *CRYMA
Cellular Characteristics	Gram negative, rod-shaped granules of poly-B-hydroxybutyrate
Molecular (16S V4 region)	“Bradyrhizobium” typical (B. elkanii) “Bradyrhizobium” atypical (B. japonicum or B. radiobacter)

Table 1 Summary

Two types of Bradyrhizobium spp. were isolated from nodules but only one sp. type per nodule.

Bradyrhizobium “typical” (elkanii) was found in a majority of nodules.
Bradyrhizobium “atypical” (japonicum) was found in an occasional nodule.

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Table 2. Identification of Methylobacterium from Leaves	
Cultural Characteristics	pink-pigmented colonies 5-7 days grown on *AMS
Cellular Characteristics	Gram negative, rod-shaped lipid inclusions or volutin granules
Molecular (16S V4 region)	“Methylobacterium” (extorquens or populi)

Table 2 Summary

Methylobacterium species were detected on leaves

Larger sequencing length needed for species

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Project Results Summary

This is the first report on detection and identification of beneficial *Methylobacterium* and *Bradyrhizobium* spp. associated with the productive alternative food crop Chinese Longbeans.

It is also unusual to find two different rhizobia species form a symbiosis with the same crop plant.

The beneficial bacterial species isolated from productive Longbeans may be used to develop natural microbial inoculants that support the growth of other crop plants.