Analysis of Beneficial Bacterial Populations from Chinese Longbeans

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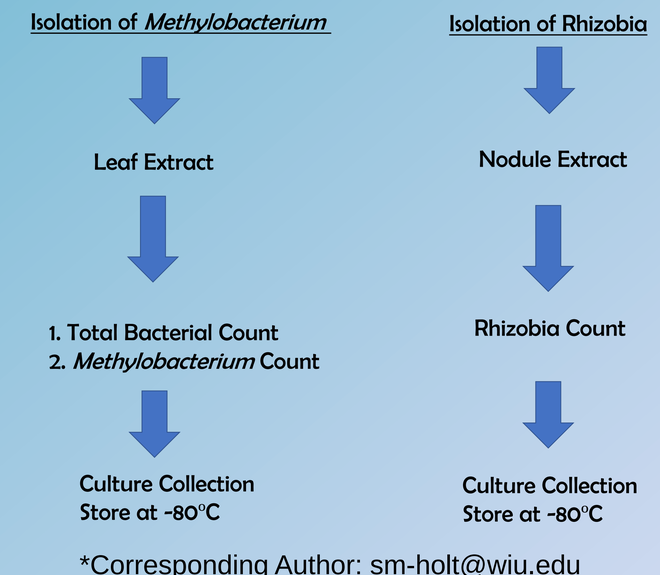
ABSTRACT

Chinese Longbean (Vigna unguiculate (L.) Walp. Ssp. Sesquipedalis (L.) Verdc.) is a crop plant that originated in the southern Asia region from domestication of Cowpea. Longbeans are now grown in Asia, Europe, and North America as a food source and for ornamental applications. Longbean pods, leaves, and stems are edible and the violet-blue flowers with draping pods are a useful ornamental. Despite their popularity, little information is known about the beneficial bacteria associated with this productive alternative food crop. The objectives of this project were to detect, enumerate, and identify beneficial Methylobacterium spp. on the leaves and symbiotic rhizobia spp. in the root nodules. The beneficial bacterial species isolated from productive longbeans can be used to develop natural microbial inoculants that support the growth of other crop plants.

METHODS

Longbean plants were divided into two groups and cultivated in a garden setting (summer 2018) and a greenhouse setting (fall 2018). For *Methylobacterium* detection, macerated leaves were plated on selective agar containing methanol and incubated for seven days at 25ºC. Pink colonies typical for *Methylobacterium* spp. were counted and identified using cultural, microscopic, and molecular identification. Leaf samples were also plated on Plate Count Agar to determine total bacterial count. For rhizobia detection, root nodules were surface sterilized, crushed, plated on Congo Red Yeast Extract Mannitol Agar (CRYMA), and incubated for fourteen days at 25ºC. Suspected rhizobia colonies were counted and identified using cultural, microscopic, and molecular identification.



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RESULTS: Identification of Methylobacterium & Rhizobia

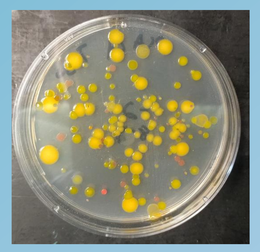
Table 1. Identification of Rhizobia from root nodules	
Cultural Characteristics	cream or pinkish, mucoid colonies 14 days incubation on *CRYMA
Cellular Characteristics	Gram negative, rod-shaped granules of poly-B-hydroxybutyrate
Molecular (16S V4 region)	“Bradyrhizobium” typical (B. elkanii) “Bradyrhizobium”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

*CRYMA, congo red yeast extract mannitol agar

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)

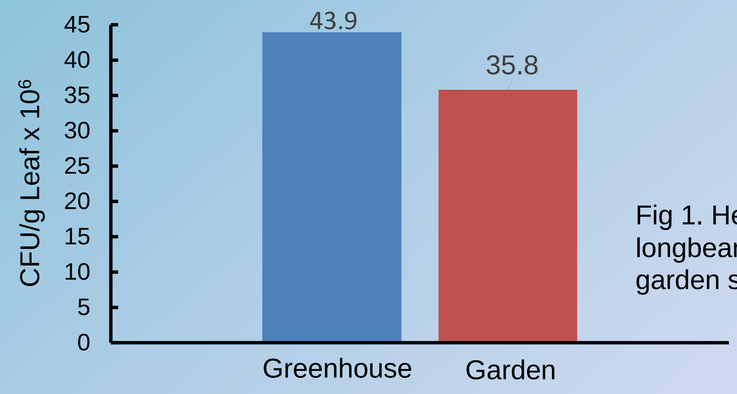
*AMS = Ammonium Mineral Salts Medium supplemented w/ 0.5% methanol

RESULTS: Longbean Microbial Counts



Total Bacterial Count

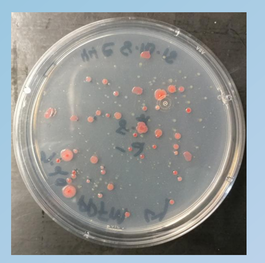
Fig. 1 Total Aerobic Bacterial Viable Count From Leaves



RESULTS SUMMARY

Table 1. 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.

Fig 1. Heterotrophic bacterial counts for greenhouse longbeans were slightly higher (43 CFU /g leaf x 10^6) vs garden setting (36 CFU /g leaf x 10^6).



Methylobacterium Count

Fig. 2. Methylobacterium Viable Count from Leaves

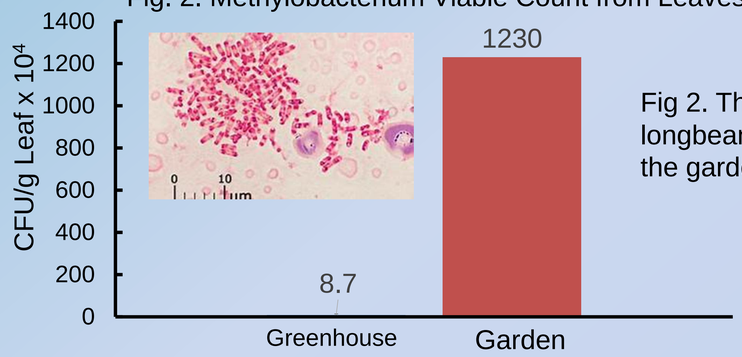


Fig 2. The *Methylobacterium* counts for greenhouse-grown longbeans (8.7 CFU /g leaf x 10^4) were much lower versus the garden longbean plants 1230 CFU /g leaf x 10^4).

Although none of the bacterial count comparisons were statistically significant due to variability, there was clearly a trend that growth conditions impact *Methylobacterium* counts on longbeans.

Bradyrhizobium Count

Fig. 3. Rhizobia Viable Count from Root Nodules

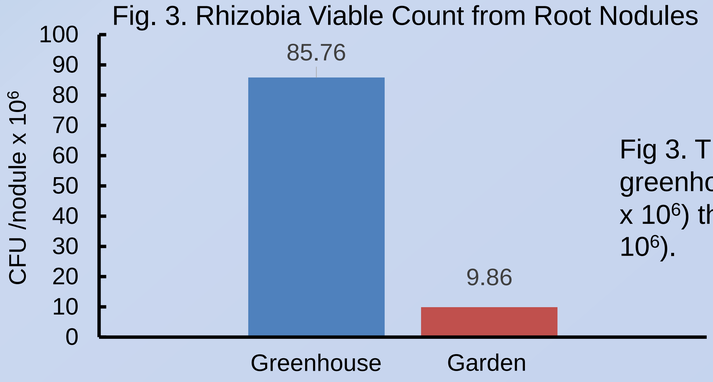


Fig 3. The root-nodule rhizobia bacterial count for greenhouse longbeans was slightly lower (9.8 CFU / nodule x 10^6) than the garden longbeans plants (16.0 CFU/ nodule x 10^6).

CONCLUSIONS

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 can be used to develop natural microbial inoculants that support the growth of other crop plants.