Isolation and Characterization of Plant Growth Promoting Seed Endophytic Bacteria

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Background

Endophytes are bacteria that actively colonize the internal tissues of host plants and establish lifelong associations without causing any apparent damage or harm. Some endophytes directly promote plant growth through processes like nitrogen fixation, pathogen resistance, and plant hormone production. Additionally, seed endophytes are of special interest as they may be transmitted from generation to generation. Maize (*Zea mays*), also known as corn, is not only a staple food in many parts of the world, but is also used as a feed crop, to produce ethanol, and is the largest source of agricultural income in the US. Switchgrass (*Panicum virgatum* L.) is a perennial warm season grass that is used for forage, bioremediation, and bioenergy, increasing its importance to agriculture and soil health [1]. The goal is to isolate, identify, and characterize bacterial endophytes that reside within maize and switchgrass seeds to extend our knowledge of PGP (plant growth promoting) bacterial endophytes. In order to isolate a wide range of endophytes from the seeds, various media were tested and pure isolates were characterized and taxonomically identified using the 16s rRNA gene.

Methods

Figure 1. Seeds used for bacterial isolation (in order from left to right), Alamo Switchgrass, Blackwell Switchgrass, Maize 3382, Maize 3489, Maize PI 550473, Maize PI 629258, Maize 2015 Hybrid, Maize 354, and Haas Avocado.

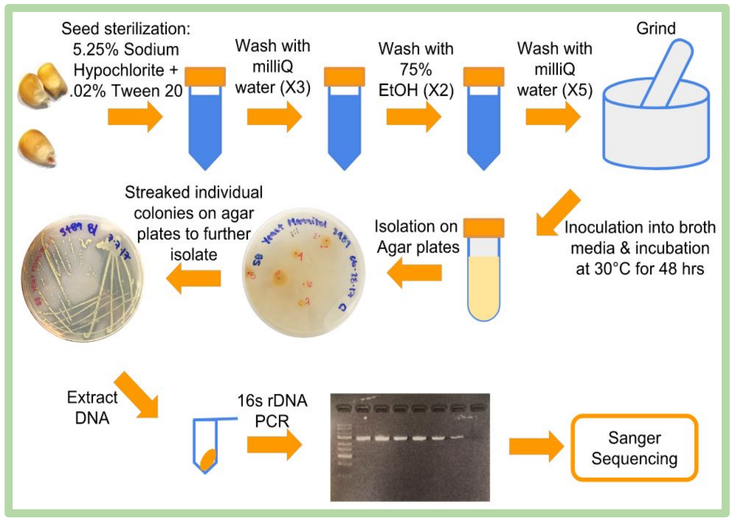

Figure 2. Experimental design to surface sterilize seeds in order to specifically grow seed endophytes by eliminating all epiphytic bacteria. Media used: Yeast Mannitol Broth, NFb, HGB, Modified NFHO, and Jensens. All media besides Yeast Mannitol Broth are nitrogen deficient and are suspected to cultivate nitrogen fixing bacteria. To further isolate nitrogen fixers, agar plates were incubated in anaerobic jars at 30° C.

Motivation

- Chemical nitrogenous fertilizers are applied to promote plant growth. However, they have several adverse effects, such as:
  - Environmental Effects:
    - Eutrophication
    - Greenhouse gas emissions
    - Decreased soil quality
    - high energy manufacturing
  - Human Health Effects:
    - Methemoglobinemia [2]

Results

- Identification and characterization of avocado seeds are in process.
- No growth observed for switchgrass seeds in any nitrogen deficient media under anaerobic conditions.
- 13 isolates from maize seeds were successfully cultured using Yeast Mannitol Agar media plates.
  - 6 isolates were successfully amplified with 16s rRNA gene primers, followed by sanger sequencing and closely matched sequences were obtained using the nucleotide BLAST program.
  - Among 6, 5 isolates are closely related to genus Arthrobacter, while 1 is related to genus Kocuria.

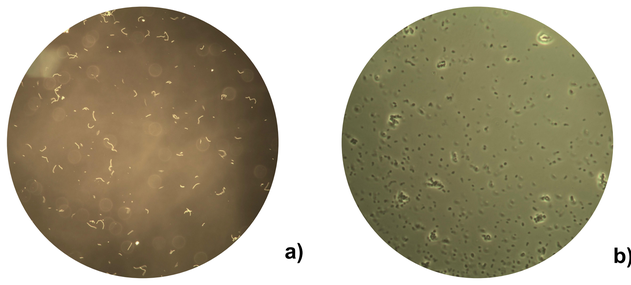

Figure 3. Microscopic Investigation: 100X view under a compound microscope of Yeast Mannitol Media inoculated with surface sterilized and ground (a) Haas avocado seed and (b) maize seeds. Movement was observed, indicating microbial growth.

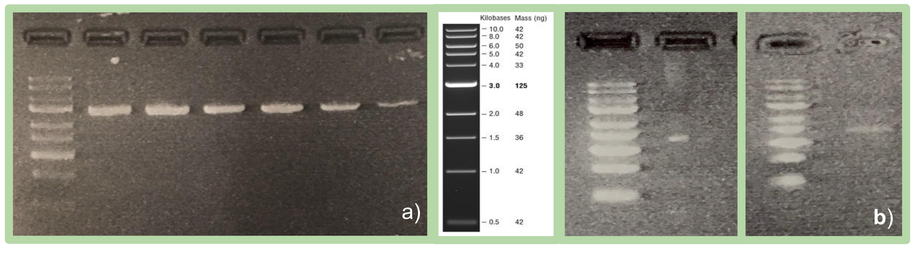

Figure 4. PCR products obtained by gel electrophoresis from endophyte isolates obtained through DNA extraction from cultured isolates in Yeast Mannitol Broth from Maize 3489 and 3382. a) 16s rRNA indicated at 1,400 bp in length. b) NifH indicated at approximately 360 bp in length.

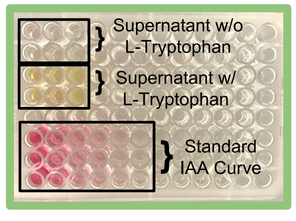

Figure 5. Indole-3-Acetic Acid (IAA) Assay indicates PGP characteristics through production of PGP hormones. Addition of Salkowski reagent to supernatant of cultured bacteria turns red or pink, then OD is taken after 30 minutes, indicating the presence of IAA. 92-YM3382-B1, 93-YM3489-C2, and 95-YM3489-C5 incubated in Yeast Mannitol Broth and 02-YM3489-C6 incubated in Yeast Mannitol Broth with L-Tryptophan indicated that they produced IAA. These isolates are related to *Kocuria rhizophila, Arthrobacter siccitolerans, and Arthrobacter oryzae*.

Results Continued

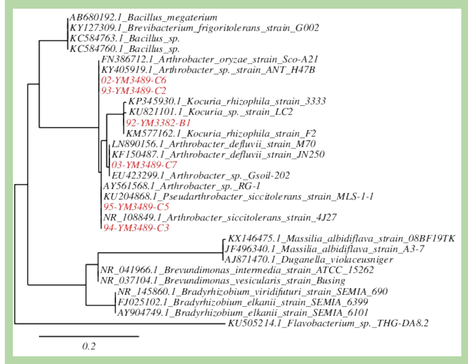

Figure 6. A phylogenetic tree constructed using partial 16s rRNA gene sequences (750 bases) of 6 isolates. The isolates are highlighted in red (92-YM3382-B1, 93-YM3489-C2, 94-YM3489-C3, 95-YM3489-C5, 02-YM3489-C6, 03-YM3489-C7). The tree was constructed using an online version of PhyML. (http://www.phylogeny.fr/documentation.cgi)

Conclusions

- 16s rRNA gene sequence analysis showed that 5 isolates belong to genus *Arthrobacter* and 1 isolate belongs to genus *Kocuria*.
- *Arthrobacter* has been found to be one of the most dominant bacteria in maize rhizospheric soil [4]. Also, they are found to be a polyphosphate-accumulating organism isolated from maize rhizosphere soil [5].
- The production of Indole-3-Acetic Acid by isolates 92-YM3382-B1, 93-YM3489-C2, and 95-YM3489-C5, as well as 02-YM3489-C6 (incubated in 0.5mg/mL of L-Tryptophan) indicated that the isolates related to *Kocuria rhizophila, Arthrobacter siccitolerans, and Arthrobacter oryzae* have PGP characteristics.
- Amplification of NifH gene PCR products shows that two of the strains (isolates 92-YM-3382-B1 & YM-3984-C4) may have nitrogen fixation abilities, as shown in Figure 4.
- Thus, seed endophytic bacteria can be successfully isolated from maize seeds and also are indicative of PGP characteristics. These bacteria may be transferred from the seed to the rhizosphere and could play a role in plant-microbe interactions.

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Support Information
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