Giant Jamboree 2018

Transformation of Endophyte *Enterobacter ludwigii* with ACC Deaminase Related to Ethylene Stress Response

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Project Goal

Due to impending climate change related variation in rainfall, increased drought and soil salinities are predicted to become serious problems that lead to reduced crop yields from osmotic stress. In environments with high salinity and dry soil, plants respond by producing more ethylene, which engages regulatory pathways leading to premature senescence. By diverting the ethylene pathway responsible for stress response and ultimately crop dormancy, crop yields will increase. Ethylene's precursor, 1-aminocyclopropane 1 carboxylate or ACC, can also be broken down into ammonia and α-ketobutyrate. By harnessing the power of ACC deaminase, the ethylene pathway can be diverted and ultimately prolong the growing season. This project focuses on identifying which naturally occurring bacteria can be transformed to produce ACC deaminase, and then successfully reintroduced into the root environment of our subject plant, *Brachypodium distachyon*. Persistence will be measured using GFP tagged ACC deaminase and imaging using confocal microscopy. Protein modeling will be done using Phyre2, MATLAB, Chimera, and Pymol to analyze protein kinetics, structure and homology.

Overview

There are many different types of endophytes: bacteria, fungi or archaea that live without symptoms inside the plant host. Each plant species has their own group of endophytes which live in different parts of the plant. By focusing on the endophytes growing in the roots we can target ethylene, a hormone that regulates stress response in plants. This is because endophytes inside the roots of plants are known to secrete the enzyme ACC deaminase (ACCD), which converts 1-aminocyclopropane-1-carboxylate (ACC) into ethylene. The degradation of ACC into α-ketobutyrate and ammonia reduces the formation of ethylene, thereby delaying the onset of senescence. Our team began by screening for many different endophytes that are ACC deaminase deficient by collecting wild grass samples and performing root bacterial isolations. After isolating many different bacterial species with unique morphologies, colony PCR and Sanger sequencing of 16S rRNA was used for sequence identification. Strains already expressing ACC deaminase function were eliminated from the list. We attempted multiple transformation protocols on strains lacking ACC deaminase and native antibiotic resistance. The endophyte most receptive to transformation was *Enterobacter ludwiggii*. This bacterium was then used as a chassis for ACC deaminase transformation with a plasmid containing a *Pseudomonas sp.* gene for ACC deaminase + eGFP. After transformation, our model grass *Brachypodium distachyon* was inoculated with the transformed bacteria by soaking the seeds in the bacterial culture before planting. The seeds were then grown in sterile soil and microscopy was used to determine if the GFP tagged bacteria was growing in the roots.

Results and Developments

Ethylene Signaling Pathway and Bacterial Isolations

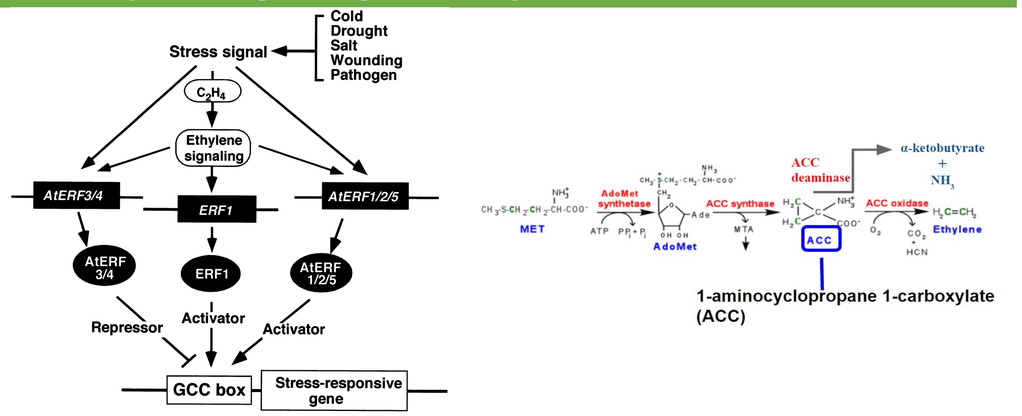

Figure 1. The diagram on the left shows the ethylene pathway involved in drought response, and on the right is the ethylene biosynthesis pathway and derivatives

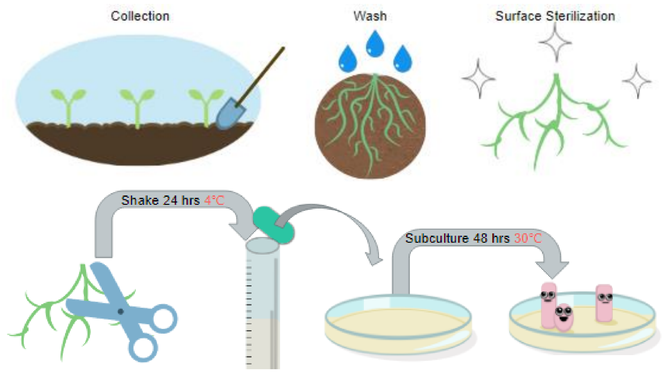

Figure 2. The diagram above shows the root endophyte isolation and culturing process starting with collecting the samples and ending with an isolated bacterial strain.

Endophyte Persistence Assay

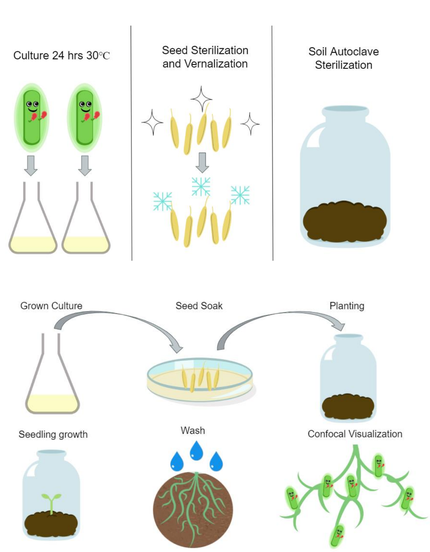

Figure 3. Method of testing persistence of endophytic bacteria in roots of *B. distachyon.* Seeds are soaked in bacterial culture and grown in a sterile environment, then visualized

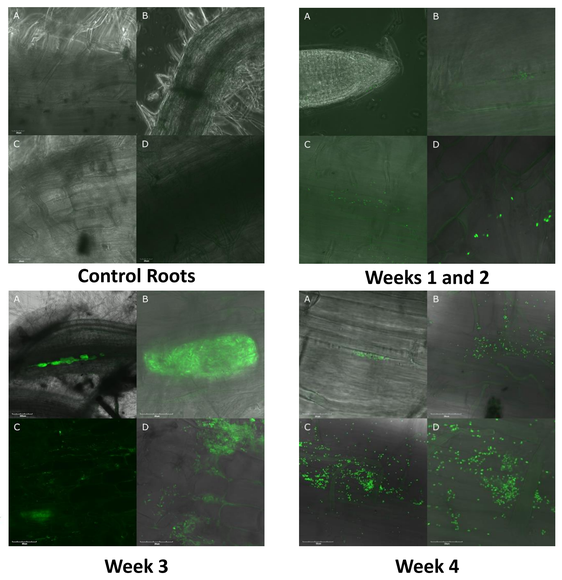

Figure 4. *B. distachyon* roots at 1-4 weeks after endophyte inoculation by seed soaking and our control. The bacterial endophyte expresses an *acdS*-eGFP fusion, allowing for visualization of the endophyte in and on roots.

Transformation Plasmid Map and Gene Target

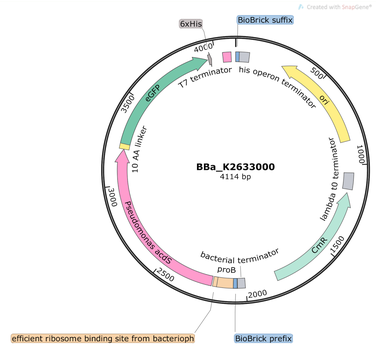

Figure 5. Plasmid map of BioBrick BBa_K2633000. This expresses a fusion of *Pseudomonas* sp. *acdS* and eGFP for plant growth promotion and fluorescent labeling.

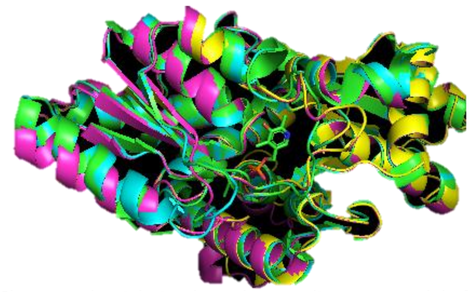

Figure 6. Shown at the right is a homologous alignment model of 1TYZ – ACC deaminase and three other ACC deaminase gene homologs. The green ring shown in the middle is PLP, the cofactor which binds to ACC.

Summary of Conclusions

- Identified a expansive library of endophytes culturable from root tissue, and identified by Sanger sequencing.
- *Enterobacter ludwigii* FCP2-01 (Bba_K2633006) was successfully transformed with our target plasmid, and eGFP was expressed in the plant tissue.
- Persistence of bacteria in roots of *B. distachyon* was seen for up to 4 weeks.

Future Work

- Create a database of endophytes as possible transformation targets for different crops
- Continue testing plants with endophyte to determine if increased yield under drought stress
- Test host range of FCP2-01.

Acknowledgements

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References: [1] Farrar et al. 2014 *Plant Biotechnology* 12(9) **[2]** Argueso et al. 2007, *Plant Growth Regulation* 26(2) **[3]** Thibodeaux, C. J., & Liu, H. (2011). Mechanistic Studies of 1-Aminocyclopropane-1-carboxylate Deaminase (ACCD): Characterization of an Unusual PLP-dependent Reaction. *Biochemistry*, *50*(11), 1950–1962. http://doi.org/10.1021/bi101927s