Inoculation of Baldcypress with Salt-tolerant Endophytes

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Abstract

Baldcypress trees are highly important in coastal swamps, especially in the Gulf Coast region, due to their ability to buffer the effects of major storms. These populations are being threatened by increased salinity and flooding due to sea level rise. Endophytes (bacteria and fungi) are known to improve their host plant's response to various stressors, which leads us to believe that endophytes may improve the resilience of these threatened baldcypress populations. Our lab has inoculated baldcypress seedlings with endophytes we have determined to be substantially salt tolerant, and the objective of this research is to gain a better understanding of the longevity of inoculated salt tolerant endophytes in the roots of baldcypress trees, and how these symbionts influence seedling growth. This was accomplished through identification and analysis of reisolated fungi and bacteria from the roots of the inoculated seedlings. Results indicate that the method of inoculation was ineffective, as root samples collected both 3 days after inoculation and 1 month after inoculation typically did not yield endophytes that matched the ITS1 region of the DNA of the strain used to inoculate the seedlings.

Introduction

Baldcypress trees (*Taxodium distichum*) dominate coastal flooded forests (swamps) and are crucially important to the Gulf Coast region as major buffers against storm damage. Salinity and flooding have known negative effects on baldcypress physiology individually, and these effects are increased in combination, demonstrating an interactive effect which may influence baldcypress populations (Allen et al. 1996, Krauss et al.1998, Krauss et al. 1999). It has been well documented that endophytes can substantially improve their host plant's responses to both biotic and abiotic stresses such as increased salinity, temperature variation, herbivory, and fungal pathogens

(Friesen 2011, Rodriguez et al. 2009). Because of these benefits, plant-endophyte interactions have the potential to confer a competitive advantage to their host plants under stressful conditions.

In previous work, our lab has screened bacteria and fungi isolated from mangroves, smooth cordgrass and baldcypress for salt tolerance. Additionally, we have examined how salinity and flooding influences the microbial communities in the roots of baldcypress trees (Kimbrough et al. 2019, Lumibao et al. 2020a and 2020b) in the field. Since then, we have been conducting salinity assays with our collection of endophytic strains from four mangrove species, smooth cordgrass and baldcypress. From assays with 90 strains, we have identified 46 strains that grew at 35ppt salinity and 11 strains that grew at 200 ppt salinity. This *in vitro* work is currently being followed up with *in planta* inoculations.

We are beginning to inoculate these strains into baldcypress seedlings, challenging them with salinity, and measuring plant performance. After inoculating baldcypress seedlings with bacteria and fungi, we are challenging them with salt stress and comparing their performance to controls with freshwater. Specifically, this sub-project consists of gathering and analyzing the molecular data (i.e., sequencing) to identify the strains of bacteria and fungi found in experimental seedling roots, as well as confirm that the inoculations worked.

This work is based on two hypotheses. First, if endophyte inoculations result in long-term residency of endophytes in root systems, then they can be reisolated from roots after salinity treatments and plant growth trials. The expectation is that the majority of bacteria and fungi that we isolate after one month will be the strain used to inoculate the plants. Second, if endophytes increase baldcypress resilience to stress, then a high salinity treatment will have a differential influence on baldcypress

seedlings. We predict there will be less of a negative salinity effect on plant growth for inoculated versus noninoculated seedlings.

Methods

To understand the effectiveness of the inoculation regime, endophyte cultures were reisolated and identified from 13 trials (each with a different inoculant strain). These cultures came from root pieces gathered at two times: pre-planting and post-harvest. The endophyte cultures were reisolated on individual agar plates and allowed to colonize until the plate was mostly or completely full. Samples from each trial were then sorted separately into groups based on morphology and photographed.

DNA was extracted from each plate using the Extract-N-Amp protocol from Tellez et al. (2020) and samples were subsequently stored in freezing temperatures. The PCR, gel electrophoresis, and sequence editing processes followed that of Kimbrough, Berlow, & Van Bael (2018.) For fungi, we used primers ITS1F and LR3 to amplify the nuclear ribosomal internal transcribed spacers (nrITS) and 600 bp of the large ribosomal subunit (partial LSU). For bacteria, we used primers 27F and 1492R to amplify the 16S rDNA gene DNA. PCR products were verified with gel electrophoresis, and all viable products were sent with original primers to GENEWIZ for Sanger sequencing. A total of 10 96-well plates, 2 fungal and 8 bacterial, were sent for sequencing.

Contigs were assembled in Mesquite v3.6 using Chromaseq package v1.5 and edited with Sequencher v5.4.6. Contigs were grouped by trial and collection time (pre-planting or post-harvest) and compared to one another and to the sequence of the original inoculant. Sequences with 97% similarity were considered to be part of the same operational taxonomic unit (OTU). Representative sequences from each OTU were put through BLAST searches to assign taxonomic identities from NCBI archives.

Results & Discussion

Out of all the sequences from the 13 trials, most did not match the target strain. The highest rate of target strain matches was in the post-harvest M1-10964 samples, with 22% of sequences matching the target (8 out of 36.) Many families were identified, the most common being Bacillaceae, Enterobacteriaceae, Rhizobaceae, Nectriaceae, and Paenibacillaceae. However, because the vast majority of samples taken did not have a 97% match with the target sequence, this inoculation method has been determined to be ineffective. Currently, the Van Bael lab has moved away from inoculating with liquid culture and fungal spore solution and has turned toward different techniques that may inoculate baldcypress roots with endophytes more effectively.

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