CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD June 2016

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Control citrus Huanglongbing using endophytic microbes from survivor trees

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
7/12/2016	Citrus Research and Development Foundation	HLB epidemiology and mitigation of HLB by cultural pra

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

The goal of the proposed study is to characterize the effect of using endophytic microbes in controlling HLB. Our hypothesis is the outcome of the interaction among Las, psyllid and citrus is affected by the citrus phytobiome. In order to achieve the goal of this study, the following objectives will be conducted:

Objective 1. To characterize the phytobiomes and endophytic microbes from HLB survivor trees and HLB diseased trees. Three healthy and three HLB infected trees were selected for phytobiome analysis from Gapway grove based on the Las QPCR detection results.

The microorganisms collected from this experiment were classified as three types: rhizosphere, rhizoplane and endosphere communities. Rhizosphere, rhizoplane and endosphere DNA and RNA preparation was done using powersoil RNA extraction kit.

The DNA and RNA samples were sequenced.

Around 10 Gb clean reads data was generated per metagenome sample. After quality trimming and citrus host originated reads removal, the remaining 501,171,627X2 paired end reads for the 12 samples, totally around 120 billion bp (120 Gb) were pulled together for assembly.The final assembly was composed of 17,676,569 contigs longer than 200 bp, totally 10.8 Gb, the longest contig length was 536,098 bp, average length was 613 bp and the N50 was 651 bp. The statistics indicated the quality of the assembly was good. Multiple known beneficial microorganisms, such as Bradyrhizobium, Lysobacter and Variovorax showed significantly higher relative abundance and activity in rhizoplane microbiome despite of health status. However, several beneficial taxa, including Rhodopseudomonas, Achromobacter, Methylobacterium and Chitinophaga, showed higher relative abundance and activity in healthy rhizoplane microbiome compared with rhizosphere community in healthy trees but not in HLB samples. By performing comparison between healthy and HLB samples, we found several phyla, such as Proteobacteria, Acidobacteria and Bacteroidetes were enriched in healthy root-associated microbiome. HLB altered the rhizoplane microbiome by recruiting more functional features involved in autotrophic life cycle such as carbon fixation, and abandoning the functional genes involved in microbe-host interactions identified above, collectively resulting in downward spiral in rhizoplane microbiome-host interaction. This seems to suggest the manipulation of the root microbiome is necessary. However, the challenge is how to maintain a beneficial microbiome which is under study now.

Objective 2. To illustrate whether the endophytic microbes from survivor trees could efficiently manage citrus HLB.

We have begun to test different grafting methods of roots. Ten HLB-survivor citrus trees from Southern Gardens citrus grooves were selected and leaves and roots were collected for isolation of endophytes. In addition, we have isolated many beneficial bacteria from the survivor trees. Multiple isolates showed significant antibacterial activity against relatives of Liberibacter.

PI First Name Nian

PI Last Name Wang

Email nianwang@ufl.edu

Phone 863-956-8828

Organization University of Florida

Project Number 15-042

Project Duration (years) 3 *Year of Project* 1

Total Funds (current year)

Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.