CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ SELECT PERIOD March ▼ 2017

◉ *Quarterly Report* ○ *Final*

Proposal Title

Control citrus Huanglongbing using endophytic microbes from survivor trees

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/14/2017	Citrus Research and Development Foundation	Epidemiology and Cultural Control

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.
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. The DNA and RNA samples were sequenced. Around 10 Gb clean reads data was generated per metagenome sample. 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.
As shown in Objective 1, Bradyrhizobium and Burkholderia are the most abundant bacteria that have shown dramatic changes between survivor trees and HLB diseased trees. Members of Burkholderia and Bradyrhizobium have been known to benefit plants. We determined the contribution of Burkholderia to the citrus hosts. We isolated multiple Burkholderia strains. We selected two representative strains A53 (Burkholderia metallica) and A63 (Burkholderia territori) to inoculate citrus plants using the soil drench method. The results demonstrated that the two strains could successfully colonize the root surface and maintain a relative high population even seven months after inoculation. We then conducted a greenhouse study to evaluate the effects of the selected strains on the plant fitness. Salicylic acid (SA)-mediated ISR is an important benefit of beneficial bacteria to the plant host. We determined the expression of three SA mediated ISR marker genes, SAM, PR1 and PR2, of the inoculated trees. Plants treated with strain A53 exhibited a significant upregulation of PR2 gene at 3 dpi compared with negative control plants. A63 induced expression of the SAM gene at 5 dpi and the PR1 gene at 7 dpi. Similarly, Actigard induced the PR1 and SAM gene expression at 5 and 7 dpi.
In addition, we grafted the roots from survivor trees to healthy and HLB diseased trees in greenhouse to check the effect of endophyte changes on the grafted trees. Since endophytes appear to be enriched from the rhizosphere, we also used the soil from the survivor trees to plant both healthy and HLB diseased trees in the greenhouse.
We are testing the effect of application of isolates on plant defenses and attractiveness to psyllids.
Two manuscripts are being prepared to report the findings.

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