Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	HLB Pathology	12/29/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Characterize the microbiomes associated with Candidatus Liberibacter asiaticus infected citrus

TITLE and CONTACT INFORMATION

Proposal Title Characterize the microbiomes associated with Candidatus Liberibacter asiaticus infected citrus, psyllid, dodder, and periwinkle

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Organization Citrus Research and Education Center, UF	*Total Direct Funds (current year)* 145114

REPORT UPDATE (650 words; provide details about your headline)

Comparison of the microbiomes associated with HLB pathogen positive and negative citrus will illuminate the causal agent of citrus greening. Potential beneficial endophytic microorganisms could be identified from escape plants which survived in heavily infected citrus groove with HLB. Beneficial microorganisms have been shown in previous studies to have the capacity to control plant diseases by accelerating seedling emergence, promoting plant growth and development, and preventing the invasion of plant pathogens. The investigation of the microbiomes associated with different hosts will help understand the transmission of microorganisms between different hosts.

Major achievements: This research has resulted in three referred publications (two published, one submitted). 1. A comprehensive study of the bacterial diversity associated with healthy and HLB diseased citrus indicated that Candidatus Liberibacter asiaticus as the pathogen responsible for HLB disease in Florida. Phytoplasma was not found in any of the samples collected from Florida. Publication: Sagaram et al. 2009 Bacterial diversity analysis of Huanglongbing pathogen-infected citrus using PhyloChips and 16S rDNA clone library sequencing. Applied and Environmental Microbiology (AEM). 2. We characterized the effect of HLB on the bacterial community associated with citrus roots. This research has been summarized in the following manuscript "Huanglongbing, a systemic disease, restructures the bacterial community associated with citrus roots" which has been submitted to AEM for publication. In this study, 16S rDNA clone library analysis of the citrus roots revealed shifts in the microbial diversity in response to pathogen infection. The clone library of the uninfected root samples have a majority of phylotypes showing similarity to well known plant growth promoting bacteria including Burkholderia, Pseudomonas, Stenotrophomonas, Bacillus and Paenibacillus. Infection by Ca. L. asiaticus restructured the native microbial community associated with citrus roots and led to the loss of detection of most phylotypes while promoting the growth of bacteria such as Methylobacterium and Sphingobacterium. The relative proportions of different groups of bacteria changed significantly after pathogen infection. These data indicate that infection of citrus by Ca. L. asiaticus has a profound effect on the structure and composition of the bacterial community associated with citrus roots. 3.A method was developed to screen antagonistic bacteria against uncultured HLB pathogen. The method uses the discrimination of live-dead cells by EMA and speed and sensitivity of QPCR. This finding is published on European Journal of Plant Pathology which has attracted tremendous interest. 4. Isolation of plant growth promoting bacteria from potential escape citrus. Isolation of bacteria with the potential of plant growth promoting and biological control potential might reveal innovative ways controlling the HLB disease. Fifty-four morphologically distinct isolates were obtained from surface sterilized roots of symptomatic and asymptomatic (potential escape trees) citrus plants from a citrus grove with a HLB infection rate of more than 60% and an infection history of approximate five years. Qualitative screening showed that for all of these criteria, asymptomatic plants harbor a significant greater diversity of potentially beneficial bacterial strains. Six different bacteria have been shown to be able to reduce the population of Ca. L. asiaticus when tested in vitro. We are further characterizing the potential mechanism to utilize it to control HLB. Furthermore, we have also located tress which have survived HLB for more than 15 years in China. We plan to investigate whether beneficial bacteria could be isolated from those trees to inhibit the population of the HLB bacteria in citrus. 5.Samples (leaf and root) from different varieties including grapefruit, Murcott, and Hamlin were collected from Florida. Samples (leaf and psyllid) from three different locations were collected. The bacterial diversity of those samples are being studied.