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	10/2/09

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

Understanding the difference between the bacterial communities associated with different varieties of HLB diseased citrus

TITLE and CONTACT INFORMATION

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

Principal Investigator Nian Wang	*PI Last Name* Wang
Email nianwang@ufl.edu	*FDACS Contract Number* 231
Phone 863-956-1151 extension 1430	*Project Duration (years)* 2 *Year of Project* 1
Organization Citrus Research and Education Center, UF	*Total Direct Funds (current year)* 145114

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

Citrus Huanglongbing (HLB) is one of the most destructive diseases on citrus. Very little is known about the causal pathogen and the microbiome associated with the infected trees. The goal of the proposed research is to characterize the bacteria (endophytes and plant pathogens) associated with HLB positive citrus, the Asian citrus psyllid, dodder, and periwinkle. The specific objectives of this proposal are: (1) Comparison of the microbiomes in leaf midribs and roots of HLB pathogen positive and free citrus of different varieties; (2) Characterization of the microbiomes that can be transferred among citrus, psyllid, dodder, and periwinkle; and (3) Understanding how Candidatus Liberibacter asiaticus colonizes and moves inside the phloem. 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:

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. The result has been published in the following publication: Uma Shankar Sagaram, Kristen M. DeAngelis, Pankaj Trivedi, Gary L. Andersen, Shien Lu, and Nian Wang 2009 Bacterial diversity analysis of Huanglongbing pathogen-infected citrus using PhyloChips and 16S rDNA clone library sequencing. Applied and Environmental Microbiology 2009 75: 1566-1574.
2. Samples (leaf and root) from different varieties including grapefruit, Murcott, and Hamlin were collected from Florida. Both healthy and infected trees were sampled. Bacteria associated with the root system will be studied comprehensively.
3. Samples (leaf and psyllid) from three different locations were collected. The bacterial diversity study of the HLB infected trees and the psyllids in those groves will reveal potential competitive microorganisms for the control purpose.
4. 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.
5. Development of a proper in vitro screening system that provides repeatable and reliable results in shorter periods of time is an important step for isolation of efficient bacterial antagonists. We have developed a method to screen antagonistic bacteria against unculturable HLB pathogen. The method uses the discrimination of live-dead cells by EMA and speed and sensitivity of QPCR. Six bacterial isolates were found to reduce the number of viable Las cells.