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	6/27/09

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

Candidatus Liberibacter asiaticus is the pathogen responsible for HLB disease in Florida.

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)

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:

Candidatus Liberibacter asiaticus (Las) as the pathogen responsible for HLB disease in Florida. Our data indicates that Ca. Liberibacter asiaticus is the pathogen responsible for HLB disease in Florida. The bacterial diversity associated with citrus leaf midribs was characterized for citrus groves that contained the HLB pathogen. We employed a combination of high-density phylogenetic 16S rRNA gene microarrays and 16S rRNA gene clone library sequencing to determine the microbial community composition for symptomatic and asymptomatic citrus midribs. PhyloChip analysis indicated that 47 orders of bacteria in 15 phyla were present in the citrus leaf midribs, while 20 orders in 8 phyla were observed with the cloning and sequencing method. PhyloChip arrays indicated that nine taxa were significantly more abundant in symptomatic midribs than in asymptomatic midribs. Only Las was detected at a very low level in asymptomatic plants but was over 200 times more abundant in symptomatic plants. The PhyloChip analysis results were further verified by sequencing 16S rRNA gene clone libraries, which indicated the dominance of Las in symptomatic leaves. On average, more than 86% of the clones from symptomatic trees were Las. These data implicate Las as the pathogen responsible for HLB disease in Florida.

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. We used a detailed approach for screening novel isolates by conducting qualitative and quantitative assays for traits related to mineral nutrition (phosphate solubilization, siderophore production), development (phytohormone synthesis), health (production of antibiotics), induction of systemic resistance (salicylic acid production), and stress relieve (production of 1-amino-cyclopropane-1-carboxylate deaminase). Qualitative screening showed that for all of these criteria, asymptomatic plants harbor a significant greater diversity of potentially beneficial bacterial strains. In vitro quantitative assays showed that the isolates from the asymptomatic trees have significantl greater beneficial abilities.