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*	Management	7/14/09

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

Full genome sequence of the HLB bacterium, 'Candidatus Liberibacter asisaticus' completed.

TITLE and CONTACT INFORMATION

Proposal Title Dissecting The Disease Complex of Citrus Huanglongbing in Florida

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Phone 772-462-5840	*Project Duration (years)* 2 *Year of Project* 1
Organization USDA-ARS	*Total Direct Funds (current year)* $381,222

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

The objective of this project is 1) to complete Las genome sequence and to conduct comparative genomics of the Liberibacter species; 2) to explore the potential role of the microbial community and genetic diversity of Las bacteria in HLB development; 3) to confirm if Las bacteria are seed-transmissible and their role in HLB development.

A complete circular genome of Candidatus Liberibacter asiaticus has been obtained using multiple displacement amplification and 454 pyrosequencing technologies from a single Las-borne Asian citrus psyllid (Diaphorina citri). Annotation of the 1.23 Mb genome sequence uncovered 1136 predicted coding sequences (CDS), and all but 300 of these CDS could be assigned to one of the defined Clusters of Orthologous Groups of proteins (COGs). Phylogenetic analysis of the full genome sequence has shown that within the Rhizobizles, Las is closely associated with members of the Rhizobiaceae family. Completion of this genome has also revealed that Las has a limited capacity for aerobic respiration, is likely auxotrophic for at least five amino acids, lacks Type III and IV secretion systems although the Type I secretion machinery appears to be present and 40 ATP-binding cassettes (ABCs). With such a large number of ABCs in relation to the small size of the Las genome and the nature of ABCs, it is quite possible that some of these transporters affect virulence, host range and/ or symptom elicitation. A more extensive discussion can be found in our paper entitled "Complete genome sequence of the citrus huanglongbing bacterium, 'Candidatus Liberibacter asiaticus' obtained through metagenomics, which is published in MPMI 22:1011-1020, 2009.
In collaboration with Dr. Hong Lin in USDA-ARS, Parlier, California, we have obtained a nearly complete genome of Ca. L. psyllaurous with 34X coverage. We have also obtained the draft genome (approximately 70%) of Ca. L. americanus using multiple displacement amplification and 454 pyrosequencing technologies. We are currently confirming the sequence of these contigs both in the psyllids and host plants.

The information from our genome sequence allowed us to design new primers and probes that target various regions of the bacterial genome. Using these new primers and probes, we revealed the genetic diversity of Candidatus Liberibacter asiaticus (Las) collected from Florida, Brazil, China and Japan. The relationship between the diversity and disease phenotypes were partially correlated. A putative insect transmission determinant gene was identified. The role of this gene is being investigated.

The seed transmission of Las is tested in grapefruit, sweet orange and trifoliate orange. Relative high titer of Las detected from seed coat and inner seed coat of the seeds collected from HLB-affected citrus plants. Very low titer of Las was detected from the seedlings, ranging from 3 to 42% using nested PCR. Most, if not all the seedlings did not have typical HLB symptoms and the threshold of the bacterial titer for HLB, even in the three year old seedlings. The results indicated that the seed-transmitted Las could not cause HLB by themselves. The role(s) of these seed-transmitted Las is under investigation.