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	1/12/10

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

A super sensitive, cost effective and rapid detection of HLB bacteria developed.

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)* $381,222.00

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 metagenomics approach, and published in MPMI 22:1011-1020, 2009. In collaboration with Dr. Hong Lin in USDA-ARS, Parlier, California, we have obtained approximate 1.2Mb, a nearly complete genome of Ca. L. psyllaurous with less than 20 contigs, which has ca. 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. Preliminary comparison revealed significant difference between Ca. L. asiaticus and Ca. L. americanus.

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, Philippines, Thailand 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.

We have characterized the ATP/ADP translocase of Las, and proved its function in the heterologous E. coli system, and published in J. Bacteriol. doi:10.1128/JB.01279-09. We are currently developing a antibody-based "drug" to target this protein, aiming to disrupt the life cycle of the Las bacterium.

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.

A super sensitive qPCR detection technology has been developed, which increased the sensitivity at least by 100 fold and thereby eliminated the need of DNA isolation and increased the throughput of detection. The cost savings can be up to 200%. Because of the detection is based on HLB bacteria-specific primers, the detection data confirmed our previous results on seed transmission and the HLB disease phenotypes with low bacterial titer.

A BAC library (61,440 clones with insert size 30Kb to 200Kb) of Las-infected psyllids was constructed. BAC clones of Las bacterium are being used for multiple genomics projects.