# 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 | 3/18/10 |

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

Detection of Candidatus Liberibacter asiaticus was NOT necessarily equal to the presence of "HLB disease" in plants

**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, USHRL | *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 the Las genome sequence and conduct comparative genomics studies on 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 was obtained using a metagenomics approach and published in MPMI 22:1011-1020, 2009. In collaboration with Dr. Hong Lin at the USDA-ARS in Parlier, California, we have obtained approximate 1.25Mb of sequence from Ca. L. psyllaurous. This nearly complete genome contains less than 10 contigs and has ca. 34X coverage . We have also obtained a 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 in both psyllids and host plants. A preliminary comparison revealed significant differences 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, genetic diversity of Candidatus Liberibacter asiaticus (Las) samples collected from Florida, Brazil, China, Philippines, Thailand, India and Japan can be found. The relationship between the diversity and disease phenotypes were partially correlated. A putative insect-transmission determinant gene was identified and the role of this gene is under investigation.

We have characterized the ATP translocase from Las and proved its function using a heterologous E. coli system. This data was published in J. Bacteriol. 192:834-840, 2010. We are currently developing an antibody-based "drug" to target this protein, aimed at disrupting ATP import, which may be important for its survival. We have also characterized the individual genes of two putative zinc operons in Las, with an overall aim of interfering with the ability of Las to regulate zinc uptake.

Seed transmission of Las was tested in grapefruit, sweet orange, and trifoliate orange. Relatively high titers of Las were detected from both seed coats and inner seed coats collected from HLB-affected citrus plants. A very low titer of Las was detected from the embryos and seedlings using nested PCR and real-time PCR. Most, if not all the seedlings did not show typical HLB symptoms and contained a relatively low Las bacterial titer for HLB, even in the three to four year old seedlings. The results indicated that the seed-transmitted Las could not cause typical HLB disease by themselves, which suggested "Detection of Candidatus Liberibacter asiaticus was NOT necessarily equal to the presence of "HLB disease" in plants."

A super sensitive qPCR detection technology has been developed, which increased the sensitivity of detection by 100-2000 fold, thereby eliminating the need for DNA isolation and increasing the throughput of the detection method. The cost savings can be up to 500%. Because the detection is based on HLB bacterium-specific primers, the detection data further confirmed our results on seed transmission and HLB disease phenotypes with low bacterial titers. The role(s) of the seed-transmitted Las is under investigation.