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)

Understand the virulence mechanism of HLB pathogen by genome sequencing and functional genomics approaches

TITLE and CONTACT INFORMATION

Proposal Title Original: Genome sequencing of Candidatus Liberibacter asiaticus. Revised: Understand the virulence mechanism of Ca. Liberibacter asiaticus by genome sequencing and functional genomics approaches

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Organization Citrus Research and Education Center, UF	*Total Direct Funds (current year)* 160916

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

Citrus Huanglongbing (HLB) is the biggest threat to the Florida citrus industry. Florida accounted for 70% of the total U.S. production in 2006-07. HLB, known as citrus greening, can debilitate the productive capacity of citrus trees with losses of 30-100% reported. HLB was found in Florida in 2005 and has spread throughout thirty of the citrus producing counties in Florida by January 2008.

The overall goal of this proposed work is to characterize the virulence mechanisms of Candidatus Liberibacter asiaticus (Las), the citrus Huanglongbing (HLB) pathogen, thus to come up with new management strategies by genome sequencing and functional genomics approaches. The original goal of the proposed research is to further complete the genome sequencing of Candidatus Liberibacter asiaticus, for which a draft sequence is available. The goal was modified to meet the current progress in genome sequencing of Candidatus Liberibacter asiaticus with the advice and permission from program manager of FCPRAC. The tile has been changed to the following to better suit the goal: Understand the virulence mechanism of Candidatus Liberibacter asiaticus by genome sequencing and functional genomics approaches.

Comprehensive metabolic reconstruction is being used to further understand the biology of Liberibacter asiaticus and identify potential genes for targeting using small molecules and other chemicals.
Bioinformatics analysis was performed to identify potential virulence factors. The SignalP v3.0 program was used to predict the presence of signal peptide within the proteins. The secretomeP 2.0 program was used to predict the non-classical secretion proteins without signal peptide. ORF containing transmembrane domains was predicted by TMHMM2.0 program. Smart and other programs were used to further mine the genome sequence of Candidatus Liberibacter asiaticus.
The genes CLIBASIA_03170 and CLIBASIA_02180 were cloned into the vector TMV30bGFP by PCR amplification and digestion with the restriction enzymes Pac1 and Xho1. The insertion of the two genes in the TMV vector were confirmed by PCR amplification using gene specific primers, followed by sequencing.
CLIBASIA_01555, CLIBASIA_00965, CLIBASIA_02215, CLIBASIA_03975, CLIBASIA_02975, CLIBASIA_00880, CLIBASIA_00470, CLIBASIA_05460, CLIBASIA_05315, and CLIBASIA_02120 were cloned into pGEMT-easy vector. The insertion was confirmed using PCR with gene specific primers, and will be sequenced for further confirmation.
In vitro transcription and plant inoculation:
CLIBASIA_03170 was inserted into TMV30bGFP, and the vector control TMV30bGFP were linearized using kpn1 and in vitro transcription was done using T7 RNA polymerase. The transcript was then used for inoculation of 4-5 week old Nicotiana benthamiana plants. The plants were observed for symptom expression about 7 days after inoculation. The symptoms shown by the empty vector and the vector containing CLIBASIA_03170 were being monitored.