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	1/1/10

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)

The overall goal of this project is to characterize the virulence mechanisms of Candidatus Liberibacter asiaticus, 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 Ca. L. 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 Ca. L. asiaticus by genome sequencing and functional genomics approaches.

Comprehensive metabolic reconstruction is being used to further understand the biology of Ca. L. asiaticus and identify potential genes for targeting using small molecules and other chemicals. Some novel insights were acquired regarding its respiration, amino acids synthesis, Co-factors and nutrient transportation. Those information provide hints to cultivation and have been shared to groups to culture Ca. L. asiaticus.

In addition, genome comparison of Ca. L. asiaticus against Mycoplasma genitalium, one of the smallest bacterial genomes, has identified more than 200 homologs. Those shared genes might be close to the minimal set required for viability of bacteria and good candidates for screening antimicrobial small molecules. Several potential targets including SecA and GalU are being used to screen potential antimicrobial small molecules to control HLB.

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.

Totally 28 potential virulence genes were cloned into TMV30bGFP viral vector for transient expression on Nicotiana benthamiana plants. Out of the 28 genes cloned, 13 were successfully assayed on tobacco plants for symptom expression. Three out of the 13 showed interesting symptoms, and hence were selected for further characterization. The following symptoms were observed: LasA1 showed vein clearing after about a week after inoculation, and subsequent wilting and death of the whole plant within 2-3 weeks. LasA2 showed phyllody, stunting and very clear growth defects. LasA3 showed very severe yellowing. The symptoms were significantly different from the infection using the empty vector (TMV 30BGFP). The expression of the genes in planta were checked using RT-PCR. Microscopy analysis is being conducted to understand how those virulence factors cause symptoms. The leaf, petiole and roots of tobacco plants infected with the viral vector containing LasA1, LasA2, and LasA3 were collected after ~3weeks for observation under the light microscope.

To further confirm the result acquired using the TMV30bGFP vector, two binary vectors were also chosen to clone the genes of interest, pTLAB31 and pGR106. All three genes LasA1, LasA2, and LasA3 were cloned into pTLAB31 and confirmed by sequencing. The constructs, with the genes present in pTLAB31 were then introduced into two strains of Agrobacterium, AGL1 (for tobacco plants) and EHA105 (for citrus). The constructs in AGL1 were then used for making transgenic tobacco plants, and also for transient expression of the genes in tobacco plants. The constructs in EHA105 will be used for making transgenic citrus plants. Understand the virulence mechanism of Ca. L. asiaticus is critical for management of HLB.