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*	Genetics	10/1/09

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

Genomic sequencing of Ca. Liberibacter americanus from Brazil and two circular phage genomes of L.asiaticus.

TITLE and CONTACT INFORMATION

Proposal Title Genomic sequencing to closure of a curated Florida citrus greening strain of Candidatus Liberibacter asiaticus

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Phone 352-392-7239	*Project Duration (years)* 3 *Year of Project* 1
Organization University of Florida, Gainesville	*Total Direct Funds (current year)* $246,601.00

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

This project has three objectives: 1) gap closure of Ca. Liberibacter asiaticus (Las) found in Florida; 2) complete genomic sequencing to closure of Ca. L. americanus (Lam) strain São Paulo from Brazil, and 3) comparative genome analysis of Las and Lam to attempt to determine common factors enabling pathogenicity to citrus.

Objective 1 Progress: Within the recently published Las strain psy62 chromosomal genome (Duan et al. 2009), many unique genes of unknown function are found, and several phage related genes are found integrated into the chromosome, but no replicating phage DNA was found. We reported last quarter the finding of at least one complete circular phage genome and a large contig that appeared to represent another phage. Neither were previously reported. We have now quantified the copy number per cell of the two phage DNAs relative to the main chromosome, and have confirmed that the SC-1 phage DNA appears to replicate in Las when infecting citrus to a level averaging 10X higher than the Las chromosome when Las is extracted from infected psyllids, and that SC-1 replicates to a level averaging 20X higher than the Las chromosome when Las is extracted from infected periwinkle. The SC-2 phage DNA appears to replicate in Las to a level 2-3X higher than the Las chromosome in both citrus and periwinkle. These data strongly indicate that we have discovered two lytic phage and was reported at the last APS meeting [Gabriel & Zhang, 2009. Phytopathology 99 (6): S38].

Since some of the SC-1 and SC-2 phage genes were found by Duan et al (2009) to be integrated into the Las genome, both phage likely have a lysogenic cycle. Lysogenic phage are known to mobilize bacterial genes when they repackage into infectious particles and then horizontally transfer these genes between different bacteria that they infect. Any genes carried on a phage that replicates to a level 10X higher than the host chromosome also replicates the copy number of all genes it carries to a level 10X higher than the rest, dramatically increasing gene expression levels, possibly affecting virulence.

Following that lead, several new Las genes were found on SC1 and SC2, encoding: 1) a hemagglutinin; 2) an ABC dipeptide secretion component of a Type I secretion system; 3) a porin type major outer membrane protein; 4) a lysozyme and 5) a fatty acid desaturase, any of which may contribute to pathogenicity. None were reported in the psy62 genome. The hemagglutinin was found on both SC-1 and SC-2. Hemagglutinins are secreted by Type I secretion systems and can be effectors of pathogenicity. Complete Type I systems are found in the Las genome. Since this new hemagglutinin is encoded by two different phage, and since the phage can replicate to ca. 10X-20X copy number in plants per Las cell, this hemagglutinin will be investigated experimentally for a potential role in HLB disease.

Objective 2 Progress: In collaboration with Fundecitrus in Brazil, Lam strain "São Paulo" DNA samples extracted from citrus and purified on pulsed-field gels by Dr. Nelson Wulff has resulted in 78 kb of partially confirmed Lam genomic sequence obtained by 454 sequencing assembled into contigs greater than 1 kb. Additional sequencing is currently being performed, and will be analyzed together with data from our previously reported Lam fosmid libraries in comparison with the Las genome and the yet to be published sequence from the Liberibacter causing "zebra chip" disease of potato (Objective 3). We are on track to meet target deadlines of the original proposal.