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	5/19/10

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 two complete circular lytic phage genomes, SC1 and SC2, with the copy number of SC1 replicating an average 10X higher in citrus and 20X higher in periwinkle, than when the phage is integrated into the chromosome in infected psyllids. [Gabriel & Zhang, 2009. Phytopathology 99 (6): S38]. Neither phage were previously reported. A fosmid DNA library from curated Las strain UF506, isolated from an infected Florida citrus tree, was surprisingly biased towards phage-related DNA inserts. Two highly related circular phage genomes (SC1 and SC2) were assembled from the UF506 library, revealing 5 new genes not previously identified in psy62. Annotation revealed multiple genes on SC2 which were pathogenicity related; since SC2 also appeared to lack lytic cycle (ie., lysis) genes, SC2 may be involved in lysogenic conversion of Las to become more virulent. Phage particles associated with Las were found in the phloem of infected periwinkles by transmission EM. Southern blot and PCR analyses were used to: 1) confirm the presence of replicating SC1 and SC2 circles in Las infected citrus and periwinkle and replicating SC2 circles in Las infected psyllids; 2) map the cos sites and confirm gene order on both SC1 and SC2, and 3) determine the genomic DNA integration sites of both SC1 and SC2 as prophage. Semi-quantitative RT-PCR revealed that the copy number of (lytic cycle) SC-1 in infected citrus and periwinkle averaged 10X and 20X higher, respectively, than in (lysogenic cycle) infected psyllids. The SC-2 phage DNA appeared to stably replicate as an excision plasmid at a level 2-3X higher in planta than in psyllids.

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 1,095,921 bp of partially confirmed Lam genomic DNA sequence obtained by 454 sequencing in 318 contigs, which corresponds to 86% of the predicted Lam genome. A high degree of syntenic gene order was observed between Las and Lam. Interestingly, both the SC1 and SC2 Las phage were found in Lam, and the gene order of the phage was also highly conserved. The 5 new and potentially pathogenicity related genes found in SC2 were also found on the equivalent Lam phage. This may be further evidence of the importance of these phage in lysogenic conversion of Liberibacter to become more virulent. We are in the process of making another full 454 sequencing run, and are on track to meet target deadlines of the original proposal.