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	3/15/10

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

Genome sequencing of Liberibacter species from limited media cultivation

TITLE and CONTACT INFORMATION

Proposal Title Cultivation and Identification of the Causal Agent of Huanglongbing Disease of Citrus

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Organization FDWSRU NAA ARS USDA	*Total Direct Funds (current year)* 227,000.00

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

The main focus thus far has been to improve the current media to increase growth of the group of bacteria known as Candidatus Liberibacter. To quantify differences between media, microscopy is being used to calculate the number of cells/colonies present on the different media. We are also quantifying differences between different DNA extraction methods using phase contrast and light field microscopy from culture material. This methodology will also establish a correlation between cell/colony count and the Ct value of RT-PCR assays.

In collaboration with Ft. Pierce, FL, the testing of Koch's postulates under field conditions has continued this quarter. Symptoms have not appeared, and a second inoculation will be started this quarter.

The second focus has been sequencing the genomes of the three Liberibacter species from pure culture. Using amplified DNA from cultured cells and Illumina Solexa second generation sequencing technology, we sequenced L. asiaticus strain China1 previously shown to be pathogenic. A collection of 21M trimmed paired-end 90mer reads obtained from this sequencing was aligned to all published bacterial genomes at NCBI using GSnap. Notably only 2,220 reads aligned to the current reference for L. asiaticus CP001677.2, primarily to regions identified as rDNA. Some alignment to chromosome 1 of Ralstonia pickettii and Ralstonia megaplasmid was observed. Reads also demonstrated alignment to the family Rhizobiaceae. Over 90 percent of reads were novel, not aligning to any bacterial genome at NCBI. The read set was used to construct a draft Phase 1 assembly (contigs unordered and unoriented) resulting in 481 contigs, indicating a genome size of 3.8M bp. These contigs demonstrate local similarity to bacterial sequences at NCBI, but contain much novel material and in many cases ORFs similar to known genes or conserved domains. Sequence-derived evidence was used to confirm the presence or absence of these sequence contigs in 8 culture samples and 34 diseased tissue samples. The presence of Rhizobiaceae-like sequence in all samples confirmed the successful sequencing of the genome from cultured cells of Liberibacter. Ralstonia plasmid sequence was found in 25% of Asian samples, but not in North and South American samples. Unique sequences showed two patterns; sequence present in all samples suggesting bacterial chromosomal DNA and sequence present in a percentage of the samples suggesting plasmid DNA.

The genomes of L. asiaticus and L. americanus are currently being assembled and annotated. The genome of L. africanus is still in the sequencing phase.