Florida Citrus Advanced Technology Program

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

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2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	HLB Pathology	7/14/09

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

Progress Report (April - June, 2009)

TITLE and CONTACT INFORMATION

Proposal Title Development of SSR markers for detection, genotyping, phenotyping and genetic diversity assessment of "Candidatus Liberibacter" strains in Florida

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Organization USDA, ARS	*Total Direct Funds (current year)* $105,342

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

The objectives of the research during this period is to (1) perform genome-wide sequence analysis to identify Simple Sequence Repeat (SSR) loci from genomic sequences of "Candidatus Liberibacter asiaticus" (Las). (2) Design and develop PCR-based SSR molecular markers for detection and genetic analysis. We carried out research experiments and successfully met milestone of the objectives. Followings are the summary of research activities and accomplishments

1. Genome wide sequencing analysis to identify Simple Sequence Repeat loci

We conducted genome sequence analysis to identify simple sequence repeat (SSR) loci. A complete "Candidatus Liberibacter" asiaticus" (CLas) genome sequences has been obtained by pyrosequence technology using the DNA extracted from a single Las-infected psyllid (Duan, et al., 2009). The bacterial has genome size 1.23 M base pairs represented in a circular chromosome with average 36.5% GC content. The software, "Tandem Repeat Finder" was used to search SSR loci genome wide. These searches included mono, di-, tri-, tetra-, penta-, hex- type of perfects, in-perfect and compound types of simple repeats. The criteria to identify SSR loci for designing primer were as followings: 1) for each type of repeat, we selected loci containing at least 5 or more of SSR motifs. 2) each locus has one copy per genome. These researches led to the identification of 123 loci that met the criteria and were potentially useful to design SSR markers.

2.In silico CLas sequence validation. To ensure that sequence loci selected for SSR marker development are unique to CLas, in-silico sequencing analyses were carried out using BLAST to compare the 123 SSR candidate loci with all the available microbe sequences in the NCBI databases. Next, selected SSR sequences were aligned by CAP3 program to remove any duplicate loci. Ten of SSR loci were found to be duplicate and therefore were removed from the list. About 200 bp up and down stream of flanking sequences of each selected loci were then selected for SSR primer designs.

3. SSR primer design. "Molecular Beacon" software (V. 7.0) was used for primer design. As much as possible, the same parameters were applied for all SSR primer designed, which included, ~50% GC, Tm=55oC, primer length ≈ 20bp, self dimer/cross dimer ΔG= -5 kcal/mol., and amplicon sizes ranging from 150 to 350 bp (Lin et al., 2005). Under these criteria, all designed primers will work in the same PCR conditions thereby facilitating SSR primer validation. Of 113 SSR selected loci, 45 of them passed the requirements for SSR primer design. To further evaluation multiplex detection, computational algorithms were used to search possible multiplex primer sets that had the least formation of self and cross primer dimmer under multiplex conditions. Using above criteria, 24 SSR primers had been designed.

4. PCR validation. To evaluate the usage of SSR markers, PCR assays were conducted using multiple CLas strains collected from Florida, Brazil, China and India. Specificity assays also performed to check if these SSR primers interact other citrus related pathogens such as Xylella fastidiosa 9a5c, Xanthomonas axonopodis pv. Citri, Citrus tristeza virus, "Ca Liberibacter africanus, "Ca Liberibacter americanus" and "Ca Liberibacter solanacearum". PCR products were then resolved by a 5% polyacrylamide gel and visualized by the silver staining. Of 24 primers we designed, 20 SSR primers showed clean and expected fragment sizes. Seven SSR primers are capable of differentiating CLas strains among various collections.

(1)Duan et al., 2009. Complete genome sequence of citrus huanglongbing bacterium, "Candidatus Liberibacter asiaticus" obtained through metagenomics. Molecular Plant-Microbe Interaction. 22:1011-1020. (2) Lin, et al., . 2005. Multilocus Simple Sequence Repeat Markers for Differentiating Strains and Evaluating Genetic Diversity of Xylella fastidiosa. Applied and Environmental Microbiology 71:4888-4892.