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	7/15/09

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

Developing diagnostic method for the detection of citrus greening pathogen (HLB)

TITLE and CONTACT INFORMATION

Proposal Title Development of sensitive, non-radioactive and rapid tissue blot diagnostic method for the large scale detection of citrus greening pathogen

Principal Investigator Siddarame Gowda	*PI Last Name* Gowda
Email gowda@ufl.edu	*FDACS Contract Number* NAS ID 79
Phone (863) 956 1151 EX 1394	*Project Duration (years)* 2 *Year of Project* 1
Organization CREC- IFAS, University of Florida	*Total Direct Funds (current year)* $ 41,040.00

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

In the citrus trees infected with HLB, the bacterium is present in low concentrations often with erratic distribution even within individual trees. To detect the onset of HLB infection and its progression in citrus trees in the field, we used tissue specific hybridization technology. The region corresponding to the ribosomal DNA of Candidatus Liberibacter asiaticus was PCR amplified and cloned into transcription vector, pGEM-T. In a similar way outer membrane protein (OMP), the RNA polymerase beta subunit, and DNA polymerase regions for the greening bacterium were cloned into pGEM-T. Based on the orientation of HLB specific sequence, the SP6 RNA polymerase or T7 RNA polymerase was used to generate digoxigenin-labeled minus-strand RNA molecules which will hybridize with the corresponding plus-strand DNA molecules of HLB sequence. In addition, we produced digoxigenin-labeled PCR products using specific primers for the OMP, RNA polymerase beta subunit, DNA polymerase region, the r-DNA region, and the 23S and 16S ribosomal RNA intergenic regions. In the initial dot blot hybridizations using the known amount of HLB specific DNA from the infected plants we observed a linear relationship with input DNA and the degree of hybridization. However, in the tissue blots we failed to observe hybridizations. The reason probably is the low titer of pathogen and the concomitant reduction of the HLB specific DNA. We are currently trying to increase the specific activity of the probes. One way is to multiple probes in an effort to increase sensitivity. Additionally, we have designed the primers corresponding to the EFTU region of the HLB DNA. The amplified product is highly specific for the HLB DNA from HLB-infected citrus plants but not psyllids. In psyllids, we see amplification of non-specific bands. Therefore, we are exploring different amplification regimen and DNA polymerase to increase the specificity.

Based on the sequence information deposited in the GenBank (contigs NZ_ABQW01000001 to NZ_ABQW01000034, total of 34 contigs), by Yongping Duan and his colaborators at USDA-ARS-USHRL, Fort Pierce, we designed a total of 30 sets primers for contigs 1-3, 5, 6, 8,10-13 (total of 10 contigs), that would amplify ~1- 1.5 Kb specific HLB sequence. These primer pairs amplified specific amplicons from the DNA isolated from HLB infected plants and no products were amplified from the healthy plants. Currently we are using these primers sets to amplify HLB specific DNA from HLB infected plants and to prepare non-radioactive DNA probes and strand specific riboprobes to use in tissue blots of infected citrus plants. However, we used some of these primer sets designed for the contigs 1-3 to amplify HLB specific products from adult psyllids both healthy and psyllids carrying HLB bacterium. The primers amplified products from both healthy psyllids and infected psyllids which suggested that non-specific amplification could be due to endogenous bacterial population in psyllids. We are currently designing primers for remaining contigs in an effort to identify specific primer pairs to use with psyllid DNA.