CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Characterization and manipulation of the prophages/phages of 'Candidatus Liberibacter asiaticus' for the control of citrus huanglongbing

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/15/15	Citrus Research and Development Foundation	Pathogen/Disease Detection

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

The objective of this research will 1) characterize Pr-D (FP3) and its role and disease suppression; 2) investigate the dynamics of the prophages/phages in Las bacteria by revealing the variations in gene expression and recombination; and 3) identify critical elements, such as heat and chemical stress that facilitates lytic activities of the prophages. In addition, we will demonstrate whether or not if the "cross protection" using mild strains of Las bacteria will work for the HLB pathosystem along with quantitative detection protocols for prophage-based strain differentiation.

We have propagated more Las-infected periwinkle and citrus plants that contain high titers of prophage/phage FP3, which will be used for isolation and characterization of prophage/phage FP3. Phages from infected periwinkle tissues have been enriched using a refined phage purification pipeline consisting of differential centrifugations and PEG precipitation. The partially purified phage preparation showed an enrichment of phage DNA by 20~50 folds and a trace amount of Las genomic DNA as determined by PCR. While phage-like particles were observed under electron microscope, the images were not conclusive due to the large amount of contaminating host materials present. Further purification will be carried with sucrose density gradient and/or CsCl equilibrium density gradient centrifugation.

Different varieties of citrus plants inoculated with a mild strain have been evaluated in greenhouse. Intriguingly, different varieties showed different response to the "mild stains/isolates". However, in a given variety, the mild strain status was maintained after three consecutive propagation. We are evaluating the factors that affect the symptoms and titers and determining if a mild strain can be maintained in major commercial citrus varieties. Over one hundred of pre-inoculated sweet orange and grapefruit plants have been subjected to field evaluation. The first group planted in the field show their consistency of mild symptoms as those in the greenhouse after 7 months.

We have developed a digital PCR (dPCR) system for early detection of HLB and tracking of lysogenic and lytic activities of the Las prophage/phage. We show that as few as 1 to 2 copies of the targeted DNA molecules per microliter can be detected, with the prophage probe providing the best sensitivity. With this tool, we were able to detect the lytic activities of the Las bacteriophage in some sample. For example, 20 times more phage DNA than bacterial DNA was present in sample EL L2VC2 than other samples, indicating active induction and replication of the Las phage in the infected citrus.

RNA-seq analysis for bacterial transcripts from Las-infected plants subjected to heat stress using the same parameters as those currently used in thermotherapy has been completed. This analysis utilized a novel technique developed by the Duan Lab to detect bacterial transcripts in mixed eukaryotic/ prokaryotic samples at set time points throughout a typical course of thermotherapy treatment. Overall, the analysis revealed that, depending upon the time at which the samples was taken, between 4% and 9% of the total predicted genes for Las appear to be differentially regulated during the thermotherapy process compared to a sample taken at time zero. These genes provide initial evidence of how the bacteria itself is modifying its transcriptional activity in response to the increase in temperature. Although a majority of the regulated genes found are defined as hypothetical, several do have a predicted function and their contributions to the effects of heat therapy are now under investigation. Reverse-transcription PCR is currently being used to confirm the results of the RNA-Seq studies. Additional plants subjected to a traditional course of thermotherapy are being used to verify that Las genes found to be regulated under heat stress are consistent amongst various trees. Alternative forms of heat stress will also be tested for a specific subset of the genes identified to determine their role in the bacterial/plant interactions.

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