CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2014

(●) *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)
1/19/15	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. 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 stain 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.
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. The copy number measurement of the targeted DNA molecules can be statistically differentiated from the healthy sample and negative water controls. We are optimizing dPCR-based assay for differentiation of Las populations that carry different prophages/phages.

New primers were designed to amplify the Las phage region iFP3. These new primers contained a thiophosphate modification between the last two nucleotides at the 3' end to increase their stability during rolling circle amplification (RCA). Samples from a Las positive citrus plant, a Las positive periwinkle plant, and a Las negative control plant were used for RCA. After several attempts at amplification via commercially available RCA kits, a protocol that amplified the DNA approximately 7 fold was developed. The specificity of the product obtained with the designed primers is currently being tested. In addition, Las-infected plants were subjected to heat stress using the same parameters as those currently used in thermotherapy. Sampling at various durations of time while the plant is enduring this stress will allow for the effects of heat stress on phage-encoded genes to be investigated.

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Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.