CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 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)
4/16/15	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 to separate the Las bacteriophages from contaminating host materials.

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.

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 trees.

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. Bacterial RNA was purified from these samples using a novel technique that we newly developed with depletion of microbial 23S and 16S rRNA for use in RNA-Seq analysis. Overall both the quantity and purity were measured and the samples are being sent for RNA-Seq analysis. The development of this technique will provide a more rapid approach than originally planned since the effects of heat stress on all of the phage-encoded genes can be investigated simultaneously. Data obtained via RNA-Seq will also be broader than that obtained from looking at the phage encoded genes individually through PCR-based assays, and thus may also lead to insights related to the regulation of the phage encoded genes through trans-acting factors.

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

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