CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2016

(●) *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)
7/15/2016	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 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 detected the 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.

Based on RNA-seq data, we further verified some of the transcriptome using Las infected citrus plants after heat treatment at 5 time points: T0/T3/T6/T9/T12 hours. Comparing the effects of heat stress among the samples, we identified differentiation on gene expression level between the plants treated with heat, at all set points T3/T6/T9/T12, compared to no treatment T0. We found genes that constantly overexpressed and downregulated in Las bacteria after heat treatment. For example, superoxide dismutase gene was overexpressed after 3/6/9 and 12 hours after heat treatment. Interestingly, the dnaK, heat shock proteins gene was also overexpressed. While some genes were consistently down regulated in the samples of T3/T6/T9 and T12 treatments, some were only downregulated after 12 hours of heat treatment. These genes are in particular related to motility, such us flgF-Flagellar basal-body rod protein, flagellar C-ring protein, and components of type IV pilus. In addition, some factors that promote growth and protein synthesis were downregulated, they are methionyl-tRNA synthetase, RNA polymerase sigma factor RpoD, and DNA-directed RNA polymerase subunit. These results indicates that the heat treatment had a strong effect on Las bacteria gene regulation, and possibly induced the lytic cycle, reducing growth and motility of Las bacteria after heat treatment. Through these analyses, we may have identified key genes involved in the lytic induction after heat stress that can be eventually manipulated to induce lytic pathways. Based on the variations of Las prophages/phages, we recognized certain molecular mechanisms behind the symptom variations and their association with "mild strains" of Las bacteria and host tolerance/resistance.
Construction of a transcriptional reporter system is also currently in progress for the final verification of the genes identified as being involved in stress response to heat in plants subjected to thermotherapy. This system will also allow future experimentation to rapidly identify other catalysts that can produce the same reduction in bacterial numbers as thermotherapy.
Purification of the ~10Kb FP3 region has been achieved from both periwinkle and citrus, though the amount purified from citrus appears to be less than that from periwinkle (as would be expected from the lower bacterial titer found in citrus). A library as been constructed from the DNA resulting from the FP3 purification for sequencing of this important region.

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Organization USDA-RAS-USHRL

Project Number 803

Project Duration (years) 3 *Year of Project* 2

Total Funds (current year) $165,463