# CITRUS ADVANCED TECHNOLOGY PROGRAM

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

**SELECT PERIOD** September 2016

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Exploiting the Las and Lam phage for potential control of HLB

| Today's Date | Sponsoring Organization (drop-down) | Category (drop down) |
|---|---|---|
| 9/30/16 | Citrus Research and Development Foundation | Culture, Molecular Biology, Koch's Postulates |

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

We have proposed targeting specific regulators of key phage encoded virulence genes (such as the Las peroxidase) as well as the peroxidase itself and key regulators of the (lethal) phage lytic cycle. Objective 1 is control of HLB using the putative Las chromosomally encoded LexA-like repressor protein LC1 (Clibasia_01645), potentially a key phage lytic cycle regulator. We had previously shown that LC1 binds specifically to its own promoter as well as to a key lytic phage SC1 promoter region midway between the divergent late and early gene promoter regions. Bioinformatics revealed several putative sites for LC1 binding. Electrophoretic mobility shift assays demonstrated that LC1 binds to its own bidirectional promoter and to another DNA region between SC1_gp125 and SC1_gp130 genes on SC1. Moreover, SC1_gp125 is predicted to encode a C2-like repressor (LC2), therefore, if functionally confirmed as a repressor, LC2 would constitute a second potential target implicated in lytic cycle activation. To evaluate the potential activity of the LC1 and LC2 repressors, promoter::GFP reporter constructs were transformed into Liberibacter crescens (Lcr) and E. coli. SC1_gp125 (LC2) promoter activity was significantly higher than SC1_gp130 and even the strong LacZ promoter. These promoter:: GFP reporters were cotransformed with vectors expressing the full-length LC1, truncated LC1 and LC2 repressors (all driven by LacZ promoter) in Lcr. Not only did LC2 self-repress its own promoter, but both full length and truncated LC1 repressors also repressed the LC2 promoter. Inactivation of either repressor would likely lead to Las cell lysis.

Objective 2 is control of HLB using a repressor protein of unknown identity from psyllids as target. We previously reported functional repression of a Las phage lytic cycle holin promoter by a predicted Wolbachia repressor protein found in aqueous psyllid extracts. The in vitro translated Wolbachia protein partially repressed the holin::GUS reporter in a dose-independent manner, as compared to the aqueous extract from the psyllid, thus indicating that complete suppression of holin promoter requires an additional partner. Heat-inactivated psyllid extract was unable to enhance the Wolbachia repressor- induced suppression of holin promoter activity, confirming the heat labile proteinaceous nature of the additional psyllid-sourced partner. Including Objective 1 results, we have identified 3 protein repressors and target promoters that are being used for high-throughput screening of combinatorial libraries for chemicals that might be used to activate the phage lytic cycle in Las, both in Las-infected psyllids and citrus.

Objective 3 is control of HLB using the Las phage peroxidase and Las lytic cycle activator(s) as targets. Bacteria use a variety of enzymes, including peroxidase, peroxiredoxin, catalase, and bifunctional catalase/peroxidases, to degrade Reactive Oxygen Species (ROS). In addition to the previously identified secreted peroxidase, we recently identified and cloned two chromosomally encoded peroxiredoxins, one secreted. Lcr transformed with the BCP-like peroxiredoxin showed a significant (200 fold) increase in tolerance to 100 µM hydrogen peroxide, as compared to a marginal 4-5 fold tolerance provided by either the PR2 peroxiredoxin or SC2 peroxidase. Most importantly, BCP-like peroxiredoxin augmented Lcr tolerance to 50 µM tert-butyl hydroperoxide (tBOOH; an organic oxidizing agent) by nearly 1000 fold. Las BCP-like peroxiredoxin may represent a second critical secreted effector, this one conserved across all pathogenic Liberibacter species (even in the phageless Las strains), that functions to suppress host symptoms.


*PI First Name* Dean

*PI Last Name* Gabriel

*Email* dgabr@ufl.edu

*Phone* 352-392-7239

*Organization* University of Florida


*Project Number* 15-009

*Project Duration (years)* 2 *Year of Project* 1

*Total Funds (current year)* $223,865

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.