CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 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)
May 5, 2016	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.)

Accumulating evidence confirms our working hypotheses that Las acquired key genes for plant adaptation by way of its phage and that these phage genes are highly regulated; off in psyllids, and on only in plants. We have proposed targeting specific regulators of key phage encoded virulence genes (such as the Las peroxidase) as well as key regulators of the (lethal) phage lytic cycle. Direct targeting of the Las peroxidase enzyme itself is also proposed.

Objective 1 is control of HLB using the putative Las LexA-like repressor protein, potentially a key phage lytic cycle regulator. We had previously shown that this chromosomally encoded phage repressor (Clibasia_01645) binds specifically to its own promoter as well as to an SC1 promoter region midway between the divergent lytic cycle (late gene) and early gene promoter regions. Such binding is characteristic of a chromosomally encoded phage regulator/repressor, but did not prove functional repression of a phage gene. In order to demonstrate actual repression, multiple DNA constructs in two cross compatible bacterial vectors were made for use in L. crescens (Lcr) as a proxy host for Las. Lcr is missing all SC1 and SC2 prophage genes. Lcr-compatible vectors were used to 1) express the LexA repressor, and 2) separately express the multiple potential targets of the repressor using promoter activity reporter constructs. Four different GFP reporter constructs were made by translationally fusing an enhanced green flourescence protein, GFP with both 1) the chromosomally encoded, bidirectional promoter regions of the phage repressor in both directions, and 2) the phage encoded promoter regions, again in both directions. Only the phage SC1_gp125 promoter (lytic gene direction) GFP construct exhibited strong fluorescence in E. coli, whereas the remaining 3 constructs were largely inactive in E. coli. This confirmed our previously published work indicating that Liberibacter promoters don't function as well in E. coli as they do in Liberibacters. This also indicated strong constitutive activity of the lytic cycle promoter in the absence of repression.

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 an unknown repressor protein from aqueous psyllid extracts. The repressor protein has now been identified by liquid chromatography mass spectroscopy to be comprised at least in part, of a protein encoded by Wolbachia, a bacterial endosymbiont commonly found infecting psyllids, including all psyllids carrying Las in Florida. This repressor protein was also found to be uniquely encoded by Wolbachia found in Asian citrus psyllids and absent in Wolbachia found in the fruit fly (Drosphila).

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, some secreted, to degrade Reactive Oxygen Species (ROS), including peroxidase, peroxiredoxin, catalase, and bifunctional catalase/peroxidases. We identified two chromosomally encoded Las and Lcr peroxiredoxins, one secreted, in addition to the phage peroxidase. Hence, a small molecule based chemical repression screen for loss of peroxidase activity would be unlikely to work in their presence. These peroxiredoxins are highly conserved among all Liberibacters (including the Las Ishi strain which does not have a phage). We have cloned the peroxiredoxins and are in the process of creating Lcr deletion mutants of the corresponding genes in order to conduct the small molecule repression screen on the best target.

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