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

Exploiting the Las and Lam phage for potential control of HLB

Today's Date: 9/30/16

Sponsoring Organization (drop-down): Citrus Research and Development Foundation

Category (drop down): 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.)

Las appears to have acquired key genes for plant adaptation by way of its phage and these phage genes are highly regulated; repressed in psyllids, and derepressed only in plants. 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 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. Four different green fluorescent protein (GFP) reporter constructs were made using both the chromosomally encoded, bidirectional promoter regions of the phage repressor cloned in both directions, and the phage encoded late and early gene (divergent) promoter regions. In Lcr, phage late (lytic) gene promoter activity was quantified and was significantly (7X) higher than the early gene or lacZ promoter (used as a control), both of which were strongly and comparably expressed. The LexA-like repressor promoter exhibited low but significant expression levels, as expected. This work established a baseline for the reporter assays in Lcr using repressor expression plasmids. For repressor expression, 1) the full length chromosomal LexA (CLIBASIA_01645) repressor gene, 2) a naturally occurring truncated version of the LexA repressor gene, and 3) a phage encoded C2-like repressor gene were all cloned to be driven by the constitutive LacZ promoter to examine transcription repression of GFP following co-transformation with the GFP containing promoter reporter constructs in Lcr. These experiments are underway.

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. This Wolbachia gene was cloned in an expression vector and used for in vitro protein synthesis. The expressed Wolbachia protein was added to an Lcr culture expressing the holin promoter/GUS reporter and exhibited partial but strong inhibition of the holin promoter. This work provides confirmation of a specific, now identified repressor target, and also indicated that complete suppression of holin promoter activity required an additional partner.

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). We identified and cloned two chromosomally encoded peroxiredoxins, one secreted, in addition to the phage peroxidase. These peroxiredoxins are highly conserved among all Liberibacters (including the Las Ishi strain which does not have a phage). Three separate experiments to interrupt either or both genes in Lcr were unsuccessful, indicating that we will have to use a rescue plasmid that can be cured in order to conduct the small molecule repression screen on any of these targets.

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