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

Exploiting the Las and Lam phage for potential control of HLB (Year 3)

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
6/15/15	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.)

One of the primary goals of this work is to identify a small molecule treatment that can be used to activate the phage lytic cycle genes encoded by Las prophage, thus bringing about the death of Las bacteria carrying these prophage. The majority of Candidatus Liberibacter asiaticus (Las) strains described carry bacteriophage similar to SC1 and SC2 of Las UF506. Lytic phage particles are formed and have been reported in both periwinkles and in citrus. We previously reported that relative mRNA expression levels of prophage late genes SC2-gp095 ("peroxidase"), SC2-gp100 ("glutathione peroxidase") and particularly SC1-gp110 ("holin") were much higher in periwinkle than in citrus. We also reported that both the prophage holin (SC1_gp110) and endolysin (SC1_gp035) were functional, and that strong expression of the holin gene alone in Las cells would be sufficient to kill the cells, whether or not phage particles were formed. This could be part of the explanation as to why Las has to date not been cultured (Fleites et al. 2014). The holin promoter (a "late" phage gene promoter that is switched on only when the lytic cycle is activated) is strongly suppressed by psyllid extracts. Identification of the repressor protein is underway.

We now report that the peroxidase on SC2 is a reactive oxygen species (ROS) scavenger and confirm that it has peroxidase activity. SC2-gp095 was cloned in a shuttle vector and transformed into L. crescens (Lcr). Transformed Lcr cells showed 20-25% enhanced resistance to hydrogen peroxide on agar plates and 47% higher enzymatic activity than Lcr. Non-classical secretion potential was confirmed by enzymatic and Western blot analyses in Lcr. Transient expression of SC2_gp095 in planta resulted in strong transcriptional down-regulation of RbohB, the key gatekeeper of the H2O2 mediated defense signaling in plants, helping explain the surprisingly long incubation period (years) before HLB symptoms appear in Las-infected citrus.

In psyllids, stringent control of all lytic cycle genes, including SC1 excision and replication (early genes) as well as the lysis genes and the peroxidase (late genes), appears to be critical to prevent Las from being detected by the insect innate immune system. Upon examination of SC1 and SC2 promoter regions, we found strong matches to a predicted Las chromosomally encoded repressor, and driving the lytic cycle (late) genes on SC1 and the peroxidase (late) gene on SC2. This likely ties these phage genes into the bacterial SOS response, which is activated by stress, particularly DNA damage. This is the second phage promoter / repressor region we have identified, and we are working to confirm the functionality of the predicted Las repressor. We have sequenced five PCR amplified UF506 repressor genes from citrus, and all encode a full-length protein. In contrast, all eight sequences derived from UF506 infected periwinkle had a truncated version of the repressor, indicating Las survival in periwinkle depends on this mutation. If so, this repressor represents an excellent molecular target for control. Even if only a small amount of phage late gene expression is activated in psyllids, it would likely trigger a strong insect innate immune response that can become systemic and result in aborting the infection process.

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Project Number 723

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

Total Funds (current year) $129,369.00

Form PR-12 Quarterly Reports SUBMIT REPORT

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