CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2013

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)
8/7/13	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.)

All 4 goals were completed. These were: 1) cloning of previously identified early/late gene promoter regions fused with lacZ as a reporter in E. coli; 2) cloning and expression of both Las and the Lam repressors and determining responsiveness of the lacZ reporter constructs in E. coli, and 3) cloning and expression of all 4 Las and the one possible Lam anti-repressors, and determining responsiveness of the reporter and clones from Milestone 2. The primary goal was Goal 4: development of a assay for Las phage late gene expression. We used quantitative, real time PCR (qRT-PCR) assays to determine levels of expression of the Las SC1 late genes in both citrus and periwinkle.

In an effort to functionally confirm that those genes annotated as being lytic cycle genes were in fact lytic cycle (late) genes, we functionally characterized one of them, SC1_gp110, annotated as a holin). Heterologous expression of SC1_gp110 in E. coli revealed bacteriostatic activity and functionally confirmed the annotation of the expressed, predicted protein product as a holin. We used RT-qPCR to monitor the expression of this holin and 4 other putative phage lytic cycle genes in Las- infected periwinkle and citrus. Surprisingly, holin mRNA levels increased significantly in citrus 12 to 24 hours after leaves were detached, indicating a potential lysis triggering mechanism in citrus. In citrus, expression was monitored before and after heat treatment at 42°C for two days, a level demonstrated to cure plants of Las infection. The relative expression of four of the five putative lytic cycle genes examined, SC1_gp025, SC1_gp110, SC2_gp095, and SC2_gp100 were much more highly expressed in periwinkle than citrus. However, heat treatment of citrus leaves failed to increase expression of any of these genes, indicating that lytic cycle induction of these prophages is not tied into a presumed heat stress response of Las in citrus. The possibility of activation by biochemical inducer(s) specific to periwinkles is currently being examined.

Conclusions from this year's funding are:

1) Functional holin activity of the predicted SC1_gp110 gene confirms the annotation.
2) Expression analyses revealed much higher levels of expression of phage late genes, particularly the holin, in periwinkle as compared to citrus, possibly indicating a phage response specific to periwinkle.
3) The significant increase in holin mRNA levels in citrus, 12 to 24 hours after being detached, regardless of temperature, indicates a potential lysis triggering signals in citrus. The holin promoter region is being developed into a reporter gene construct that may be useful for monitoring lytic cycle activation by potential inducers in citrus.
4) The mode of action of thermal therapy (heat curing of Las) in infected citrus does not seem to be connected to phage induction.

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Form PR-12 Quarterly Reports SUBMIT REPORT

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