CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2014

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Exploiting the Las and Lam phage for potential control of HLB (Year 2)

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
7/30/14	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. All Las bacteria examined to date have been found to carry prophages in their genomes. In periwinkles, but not in citrus, lytic phage particles are formed and can be visualized. 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. We now report that the Las prophage holin not only suppresses growth, but kills E. coli when overexpressed, indicating that strong expression of this gene alone in Las cells would be sufficient to kill the cells, whether or not phage particles were formed.

Four differently sized potential promoter-active regions upstream of the Las holin (SC1_gp110) were cloned in front of a promoterless lacZ gene to form reporter constructs. E. coli colonies carrying these plasmids were nearly white on X-gal media, indicating a lack of holin promoter activity to drive lacZ expression. One of these was fused to a promoterless GUS reporter gene, cloned in a wide host range shuttle vector and used to transform L. crescens (Lcr) strain BT-1. This reporter gene exhibited unusually strong GUS activity in Lcr strain BT-1; in fact, this GUS activity was stronger than that observed using the constitutive lacZ promoter in Lcr. Significantly, in both qualitative and quantitative (MUG-based) assays, diluted psyllid extracts strongly and specifically inhibited GUS reporter activity in Lcr expressed by the holin promoter, as compared to the untreated controls. This suppression activity was heat labile (ie., heat treatment of the psyllid extracts destroyed the inactivating activity). In the same quantitative activity assays, psyllid extracts at the same treatment levels failed to inhibit the constitutive lacZ promoter driven GUS activity to any significant level. This work has been accepted for publication in Applied and Environmental Microbiology and is now available.

The lack of holin promoter activity in driving the LacZ reporter in E. coli indicates the absence of a transcriptional activator that is present in Lcr and necessary to drive the expression of typically repressed downstream gene. An alternative but less likely explanation is that E. coli carries a repressor that recognizes the holin promoter and silences the expression of the reporter gene. Both hypotheses are being tested in an effort to identify the activator or suppressor.

It seems likely that one or more chemical ligands in the psyllid extract is able to penetrate Lcr cells, bind to, and interfere with, phage late gene transcriptional activator(s), (for example, a MarR-like transcription factor) leading to suppression of phage formation in psyllids. Based on the increased transcriptional activity of the holin promoter in periwinkle, (a) similarly acting ligand(s) is/are also likely to occur in citrus. The strong activity of the holin promoter in the absence of psyllid or plant extracts may help explain why liberibacters other than BT-1 have not been cultured to date. Regardless of whether or not phage particles form, expression of the Las holin alone is sufficient to kill Las. Once Las bacteria are separated from any plant or insect host, any host-provided ligand that may suppress transcriptional activity will become increasingly dilute, and prophage late gene expression derepressed. We speculate that factors present in hosts may suppress phage activation and may be necessary, but not alone sufficient, for free living culture of Las bacteria. The assay developed here provides a high-throughput basis to screen for chemicals that may activate Las holins or interfere with holin suppression.

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

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