CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 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, 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. Furthermore, the holin promoter was constitutively "on" in L. crescens (Lcr) strain BT-1, and was strongly suppressed by diluted psyllid extracts and the suppression was heat labile (ie., heat treatment of the psyllid extracts destroyed the inactivating activity).

We now report that the psyllid extract suppression activity is also abolished by proteinase K treatment, suggesting that psyllids produce a protein inhibitor of the phage holin. The inhibitor was precipitated with acetone, and size fractionation demonstrated an inhibitor in the size range of 10-50 kDa. This reporter system may be developed into a high-throughput chemical screen for treatments that may interfere with psyllid or plant regulation of the phage lytic system.

In addition, two additional phage late genes, SC2-gp095 ("peroxidase"), SC2-gp100 ("glutathione peroxidase"), were functionally characterized. Given that the highly reduced Las genome encodes no known defense against host generated reactive oxygen species (ROS), the putative phage-related ROS scavenging functions annotated as peroxidase (SC2_gp095) and glutathione peroxidase (SC2_gp100) may represent important "lysogenic conversion" genes whose expression may increase bacterial fitness and delay symptom development in the host plant. Both SC2_gp095 and SC2_gp100 were expressed at significantly higher levels in periwinkle than in citrus or insects. SC2_ gp095 alone, and in tandem with SC2_gp100 were separately cloned in a wide-host-range (repW) shuttle vector pUFR071 (under control of the lacZ promoter), and transformed into E. coli and Lcr, a culturable proxy for Las. The transformed Lcr cells showed enhanced in vitro resistance to H2O2, 23% higher enzymatic activity and faster growth rates in culture as compared to Lcr cells transformed with only pUFR071. Moderate enzymatic activity was also evident in transformed Lcr culture supernatants, but not E. coli supernatants, confirming a predicted non-classical secretion potential for SC2_gp095, and suggesting such secretion from Las.

Experiments are underway to further characterize the role of SC2_gp095 in planta. We hypothesize that Las peroxidases: 1) mitigate the direct antibacterial effect of reactive oxygen species (ROS) on Las cells, and 2) disrupt systemic cell-to-cell self propagation of ROS (H2O2) -mediated signaling in the host plant. The latter idea may explain the surprisingly long incubation period before symptoms appear. Las peroxidase(s) may be secreted effector(s) that function to suppress host symptoms, a tactic used by most biotrophic plant pathogens.

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

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