CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 2013

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Molecular and cellular mechanisms that drive psyllid-vector-Liberibacter interactions in the transmission pathway

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
9/16/13	Citrus Research and Development Foundation	Infection Responses

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

Obj 1A: All components of the user-friendly web versions of the single (sTCW) and multi-(mTCW) psyllid transcriptome databases have been finalized in preparation for the public release which will allow researchers to quickly identify targets, not only based on expressional differences but in combination with predicted functions. Recent data obtained from the sTCW databases containing salivary glands and midgut tissue transcripts show that the salivary glands contain more transcripts that are differentially expressed in response to Liberibacter than midgut tissues. Also there are drastically more 'biological process' Gene Ontology categories that contain a significant number of differentially expressed transcripts in the salivary glands compared to the midgut. These data highlight the potential importance of tissue specificity, specifically the role of the salivary glands in Liberibacter transmission. Previously, it was reported that members from 3 OrthoMCL-defined clusters (mTCW) were validated in both POP and ACP. To date, the expression of transcript members from an additional cluster predicted to be important for bacterial nutrition has been validated in both ACP and POP by RT-PCR using primers designed from conserved regions. Obj 1B: Yeast-2 hybrid studies were initiated to study protein-protein interactions important in psyllid-Liberibacter interactions. Previously it was reported that a total of 9 CLas candidate genes from a list of 19, had been moved into the Yeast 2 Hybrid (Y2H) mating experiments using the ACP gut and salivary gland libraries. To date 12 gut library matings and 12 salivary gland library matings have been performed. Data analysis has been completed for 20 of those experiments with the remaining being in various stages (PCR, cloning, sequencing, etc.) moving towards completion. Through those 20 experiments we have thus far discovered roughly 44 ACP gene products that have high levels of interest making them good candidates for RNAi and will be moved into that phase of the project if they fit the criteria specific for RNAi. Previously we reported the findings from the first 3 mating projects (1 midgut and 2 salivary glands). Since the last update, we have discovered more interesting candidate effectors that will be moved into the RNAi phase of the project many of which are predicted to be important in Liberibacter adherence to host tissues. One interesting candidate is an endopeptidase putatively involved in the dissolution of extracellular matrices. The overexpression of this gene could greatly interfere with CLas transmission. An ACP gene product similar to enzymes involved in signaling cascades important for bacterial communication and subsequent biofilm formation was also identified. A CLas "bait" with protease function, identified an ACP gene important for bacterial nutrition and is made available to the bacteria after processing. An interesting finding came from using the CLas "bait" FlgL protein. No interactors were discovered from gut matings, only from salivary gland mating experiments which suggest the motility of Liberibacter is most important in this tissue. To date, one candidate ACP gene putatively involved in bacterial adhesion has been mated against the CLas prey library interacting gene products ("prey" inserts) identified are currently being analyzed. Obj 2: RNA interference (RNAi) studies are underway to functionally validate candidate effectors. To date, good quality dsRNA has been synthesized for five psyllid genes predicted to be involved in cytoskeleton formation, defense response, vesicle transport, or transcytosis. Previously we reported on the impact of the knockdown of one of the cytoskeleton-related gene on Liberibacter transmission using the oral delivery method. Recently, both microinjection and feeding studies have been conducted for actin, the other cytoskeleton-related gene whose knockdown is expected to result in high psyllid mortality and is used as a control to optimize our studies. Mortality results from oral delivery of dsActin show that 37% more psyllids died after feeding on dsActin-treated diet in comparison to the control. Mortality results from microinjection delivery of dsActin show that 15% more psyllids died from dsActin injections in comparison to control injections. These preliminary data suggest that the method of dsRNA delivery is crucial, and should be optimized accordingly.

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