CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 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)

Citrus Research and Development Foundation

Category (drop down)

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: We last reported that all components of the user-friendly web versions of the single (sTCW) and multi-(mTCW) psyllid transcriptome databases had 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 and tissue specificity (e.g., midgut and salivary glands). To date, the expression of 19 transcripts predicted to be important for nutrition, adhesion, immunity and defense has been validated in ACP by RT-PCR.

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 12 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 15 gut library matings and 13 salivary gland library matings have been performed. Data analysis has been completed for 26 of those experiments with the remaining being in various stages (PCR, cloning, sequencing, etc.) moving towards completion. Through those 26 experiments we have thus far discovered over 50 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 5 mating projects (4 midgut and 3 salivary glands) with the matings of one CLas bait highlighting the importance of tissue specificity when identifying candidate effectors. 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 a transporter-like protein that is known to be critical in host-pathogen interactions. The knockdown of this gene may inhibit CLas dissemination in the psyllid. An ACP gene product similar to an enzyme involved in apoptosis was also identified. The knockdown of this gene may interfere with psyllid tissue integrity, thus CLas's ability to adhere properly which is likely required for successful transmission. To date, nine candidate ACP genes putatively involved in bacterial adhesion, nutrition, and defense have been mated against the CLas prey library. Several interacting gene products ("prey" inserts) have been identified with many still being analyzed. One ACP candidate with putative functions in endocytosis and an adhesion-related transcript interacted with two different CLas proteins, both of which are associated with extracellular matrices. Another ACP bait with putative peptidase activity interacted with a CLas pilus-associated protein. Due to the potential impact on virulence of the identified CLas prey, the knockdown of the ACP interacting partners is being investigated.

Obj 2: RNA interference (RNAi) studies are underway to functionally validate candidate effectors. To date, good quality dsRNA has been synthesized for six psyllid genes predicted to be involved in cytoskeleton formation, defense response, vesicle transport, or transcytosis and nutrition. Previously we reported on the impact of the knockdown of two of the cytoskeleton-related genes on Liberibacter transmission using both oral delivery and microinjection methods. The preliminary data suggested that the method of dsRNA delivery is crucial, and should be optimized accordingly. To date, dsRNA for 6 genes have been oral delivered and/or microinjected into psyllids. Confirmation of knockdown by qPCR has been confirmed for two, one of which was tested in the transmission bioassay and showed a reduction in transmission by 18%. Confirmation of knockdown, followed by transmission bioassays of the remaining genes, is ongoing.

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.