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

Molecular and Cellular mechnisms that drive psyllid vector-Liberibacter interactionsin the transmission pathway

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
9/22/14	Citrus Research and Development Foundation	Infection Consequences

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

Obj 1A: To identify target psyllid effectors, the mining capabilities for the single (sTCW) and multi-(mTCW) [C. Soderlund, W. Nelson, M. Willer and D. Gang. (2013) TCW: Transcriptome Computational Workbench; PLOS ONE: 8(7), e69401] psyllid transcriptome databases have been finalized and released on line. Thus far over 500 candidates are available for hypothesis-testing using the database tools available at the website, including Gene Ontology and differential expression (GOSeq, EdgeR) profiles. Two manuscripts have been submitted. Obj 1B: Yeast-2 hybrid studies to capitalize on in vitro protein-protein interactions important in psyllid-Liberibacter interactions are ongoing. Previously, we reported 17 CLas candidate genes from a list of 25 putative candidates (identified in silico using the db, obj. 1A), were cloned into the Yeast 2 Hybrid (Y2H) mating experiments using the ACP gut and salivary gland libraries. To date 24-gut and 24-salivary gland library matings have been performed. From these experiments we discovered a number of potentially lucrative ACP gene products ('prey'), and have moved them into the dsRNA station in the pipeline. Based on predicted functions, including some shared in common with other host-pathogen/parasite systems, it is becoming possible to hypothesize a pathway of CLas invasion into ACP tissues and organs (circulative). Among the promising candidate effectors are an ACP protein that comprises domains annotated as having antibacterial defense and quorum sensing functions. The knockdown of genes involved in quorum sensing processes could interfere with biofilm formation or signaling pathways that are essential for CLas establishment and survival. To identify potential CLas factors involved in lucrative points in the circular, propagative transmission pathway, ACP genes are used as "bait" in mating studies with the CLas library. We last reported results from 14 ACP candidates mated against the CLas prey library. Thus far, 25 ACP gene candidates have entered the Y-2H station to test for interactions using the CLas prey library. Data analysis has been completed for 19 of these experiments, with the remaining being in various stages (PCR, cloning, sequencing, etc.) and moving towards completion. More than one third of mating experiments have produced potentially, biologically relevant prey products. The most interesting thus far are ACP bait genes with annotations to endocytosis/phagocytosis pathways, with one in particular that interacts with a CLas receptor-like protein known to be required for CLas invasion. Further biochemical analysis (in silico) of this 'prey' protein has confirmed it to be the aqueously soluble domain/portion of the protein. A second interesting candidate is also an endocytosis-related ACP gene that interacts with a CLas prophage protein. The CLas prophage/CLas/ACP interaction highlights another possible avenue for abating vector transmission. A third candidate has been identified, which may function as a regulatory protein that interacts with a microtubule attachment protein. This suggests it may have a role in Liberibacter adhesion and invasion of the psyllid host. Confirmation of Y-2H interacting proteins, and identification of additional interactors using immunoprecipitation (IP) and co-immunoprecipitation (Co-IP) are underway. Two CLasY-2H candidates predicted to be involved in adhesion and quorum sensing were used to optimize IP and Co-IP assays owing to the abundance of these ACP interactors identified in Y-2H assays. About 50% of the adhesion-related candidate was found to be expressed in the soluble protein fraction. Optimization of pulldown assays is underway. Obj 2: RNAi studies are underway to functionally validate candidate effectors. To this end, good quality dsRNA has been synthesized for thirteen psyllid genes predicted to be involved in cytoskeleton formation, defense response, vesicle transport or transcytosis, and nutrition. Knockdown experiments for 10 candidates have been completed using oral delivery and microinjection, with knockdown frequencies ranging from 50-100%. Five genes showed reduced transmission frequencies ranging from 18-25%. Confirmation of knockdown (qPCR), followed by transmission bioassay of additional candidates identified in silico (transcriptome) and those identified using in-vitro Y-2H studies, is ongoing.

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