CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 2016

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Functional genomics of Liberibacter in a model system

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
9/27/2016	Citrus Research and Development Foundation	Model Systems

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

In our work through March, we ramped up the RNA expression assay portion of our project. For study of LdtR, we compared RNA transcripts from S. meliloti (deletion)ldtR bearing a plasmid with Plac (IPTG-inducible) Ca. Liberibacter asiaticus ldtR with a WT control bearing the empty vector. Cells were induced for 1 hours at 0.5 mM IPTG to induce expression of the regulator genes. These RNAs were converted to cDNA for hybridization to gene chips.
Background information on ldtR and ldtP genes showed that the Sinorhizobium LdtR protein binds to its own promoter and to that for ldtP.
As shown by Pagliai et al, ldtR and ldtP are conserved in S. meliloti; their version of CLas LdtR, encoded by a sequence amplified from Ca L. asiaticus strain psy62, binds to a characteristic promoter motif including promoters for LdtR and LdtP. Our transcription assays showed that in S. meliloti, the expression level of the Sm ldtP gene does not go up in response to th plasmid-borne CLas LdtR. We did see high variation in results among three biological replicates. We found that overall 8 genes went up by 1.5-fold or more. Some of these are ctrA controlled, some are cell cycle related, and some related to transcription effects of a (deletion) podJ mutation or of NCR-peptide exposure. Of these 8 genes, only 2 had CLas orthologs. We observed that ldtR is less motile than WT and expression of CLas LdtR may further reduce motility. Tests for stress sensitivity showed there is no differential growth for the engineered strain on plates containing the detergent deoxycholate at concentrations of 0.1% or 0.2% (the highest level tried).
As reported in January, we constructed mutations for the visR and visN genes. The VisR-VisN proteins function as a heterodimer to constitute a LuxR-type transcription factor. We found the (deletion)visR visN double mutant has a motility defect. A plasmid carrying the cloned CLas visNR genes was able to complement the mutant defect of the S. meliloti (deletion)visR visN double mutant. Preliminary transcription results show that the CLas visNR plasmid activates expression of several dozen S. meliloti genes.

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