CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Functional genomics of Liberibacter in a model system

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
2/12/16	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.)

Our goal to examine expression of transcripts in host Sinorhizobium meliloti (Sm) due to introduced Candidatus Liberibacter asiaticus (CLas) transcription factor genes. These data will lead us to identify targets to screen for novel anti-bacterials. Between September and December 2015, we have analyzed the first Affymetrix GeneChip experimental data to reveal targets for Clas RpoH in S. meliloti. We have also completed construction of clones and mutants for several more of our target genes.

RpoH results: Using RNA from the S. meliloti rpoH1H2 mutant strain expressing CLas rpoH or from control strains, we prepared cDNA and probed a genomic AffyChip. We found 20 genes activated over 2-fold by CLas RpoH and 39 genes activated by Sm RpoH1. There were 14 genes shared between the two, that is, expressed by both types of RpoH1. Based on these results, we can identify strong candidates for CLas RpoH target gene fusions. Our top 3 candidate promoters for fluorescent fusions to use in high-throughput compound screening are: ibpA, clpB, and groES5. We already have uidA (GUS) and mCherry (red fluorescent protein) transcriptional fusions to these genes.

We have also made progress in constructions for analyzing 6 transcription factors from CLas . We have constructed visRN, ldtR, and lsrB mutants in S. meliloti. Each of these is being systematically tested for growth and other functions with the cloned (synthetic) corresponding CLas gene. As previously reported, we found some function of CLas LsrB copmlementing slow growth of S. meliloti ΔlsrB in complementation of slow growth. We further discovered that S. meliloti ΔlsrB is sensitive to the cell envelope disrupting agent, deoxycholate (DOC); we demonstrated that overexpression of CLas lsrB partially restores growth of the Sm ΔlsrB mutant on DOC. In another case, we found that Sm ΔvisNR has a severe motility defect in culture. Overexpressing CLas visNR restores motility, demonstrating that the synthetic CLas visNR is functional. We have successfully constructed phrR1 and phrR2 single mutants and are in the process of constructing the double mutant.

As we described in the last report, construction of a ctrA deletion strain is complicated since ctrA is an essential gene. Our recent work revealed another surprise, which is that the cloned CLas ctrA gene is deleterious to survival of S. meliloti wild type cells when grown on standard rich medium (LB). We can maintain the plasmid in the cells despite this, because the CLas ctrA gene is on a regulatable promoter. Without IPTG inducer, no CLas CtrA protein is produced and the S. meliloti cells are viable; at high IPTG, the cells die. By using alternative media and low levels of inducer, we are currently testing whether the CLas ctrA gene can be modulated to supply appropriate function to S. meliloti. While this surprising result complicates our strategy, it opens up another possible avenue for chemical discovery. We might ask the question, are there any compounds in our chemical library that would interfere with the toxicity of CLas CtrA to S. meliloti? We will explore this possible design.

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