CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ SELECT PERIOD March 2013

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Genome-enabled metabolic reconstruction of Ca. Liberibacter asiaticus and its use in culturing and controlling the pathogen.

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/2/13	Citrus Research and Development Foundation	Culture, Molecular Biology, Koch's Postulates

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

Since the completion of the metabolic model of L. crescens we have been focusing on the development of a genetic system in this Liberibacter model. Specific genes involved in carbon metabolism, regulation, and cell wall recycling have been selected as targets for knockout in L. crescens. These genes are present in L. crescens but not L. asiaticus and through the development of knockouts in L. crescens we hope to better understand the constraints on L. asiaticus growth in culture. Electroporation using several plasmids / cosmids, such as p15TV-L, pLAFR1, pGS9, pHRGFPGUS, and pUFR071, were performed to test the transformation efficiency on L. crescens. It was found that only the vector pUFR071 was able to transform and replicate in L. crescens, which leads to an assumption that plasmids of smaller or similar size of pUFR071 should be able to electroporated into L. crescens. The gene targetted for knockout will be cloned into a suicide vector (such as p15TV-L) with a kanamycin cassette inserted in the middle of the gene and transformed into Escherichia coli DH5a. The construct will be confirmed by sequencing and the development of kanamycin resistance. The final construct will be electroporated into L. crescens. Chromosomal crossover will occur and mutants will be selected for kanamycin resistance. The knockout mutant will be confirmed by sequencing of the target gene.

PI First Name Eric

PI Last Name Triplett

Email ewt@ufl.edu

Phone 352-392-5430

Organization University of Florida, IFAS

Project Number 336

Project Duration (years) 3 *Year of Project* 1

Total Funds (current year) $249,000.00

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.