CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2013

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Exploiting the Las and Lam phage for potential control of HLB (Year 2)

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
8/7/13	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.)

Liberibacter crescens BT-1 (Lcr, isolated from mountain papaya), has been cultured and sequenced (GenBank CP003789, Leonard et al., 2012) and has the potential to be developed into a model Liberibacter system. for functional analyses of Las, Lam and Lso genes. To become a model system, BT-1 needs to be 1) reliably and stably transformed with a shuttle vector so that different genes can be added in; 2) gene knockouts in BT-1 must be reliably made, and 3) the strain needs to be introduced into a plant and be shown capable of growth in planta. The first two of these 3 goals have already been met.

To determine if BT-1 might be tractable for functional genomics studies, the minimum inhibitory concentrations (MICs) of several antibiotics commonly used for plasmid selection were determined. BT-1 was found to be quite sensitive to: chloramphenicol, < 4 mg L-1; gentamycin, < 1 mg L-1; kanamycin, < 2.5 mg L-1; and tetracycline, < 0.3 mg L-1.

BT-1 cells were readily transformed at high frequencies with two different wide host range replicons tested: pUFR071 (RepW; Al-Saadi et al., 2007) and pUFJ05 (Bordatella replicon; Reddy et al., 2007). The transformation frequencies were estimated to be 3000 transformants / mg of DNA for pUFJ05 and ~ 20× greater for pUFR071.
Stability of pUFR071 was evaluated; this plasmid was >95% stable, without selection, when grown in BM7 medium for over 20 generations. pUFR071 was extracted from BT-1, retransformed into E. coli and appeared from restriction analysis to be unchanged. BT-1 cells transformed with pUFR071 showed only a marginal loss of plasmid, even when grown for more than 70 generations in the absence of antibiotic selection. The results are in line with the expected stability of RepW origin in the presence of stabilizing partition locus parA (De Feyter et al. 1990).

A gene knockout in BT-1 was successfully demonstrated, following the strategy outlined in Castañeda et al. (2005). Briefly, a partial internal fragment of the BT-1 gene encoding the Type I restriction-modification system restriction subunit R (GenBank B488_07050) was PCR-amplified and cloned into TOPO® 2.1 (Invitrogen, Carlsbad, CA). The resulting plasmid was used for electroporation of BT-1 cells. A single homologous recombination event at the target site duplicated the cloned region and integrated the vector between incomplete copies of the target gene. The antibiotic resistant colonies were analyzed by PCR to confirm interruption of the target gene and integration of the plasmid backbone.

Attempts to artificially inoculate marked BT-1 strains into tobacco, citrus and periwinkle are currently in progress.

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

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