CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Characterization/Purification of Liberibacter from seed coats

Proposal Title

Analysis of the colonization of citrus seed coats by 'Candidatus Liberibacter asiaticus' the causal agent of citrus huanglongbing and their use as a concentrated, pure source of bacteria for research.

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
5/8/12	Citrus Research and Development Foundation	Culture, Molecular Biology, Koch's Postulates

REPORT UPDATE (500 words; summarize your accomplishments)

This research agreement became effective 1-Jun-11. A GS5-7 biological support technician was in place the first week of December, 2011. Preliminary results of foliar and seed coat tissue fractionation and filtration experiments were obtained both before and after the effective date of the Research Agreement. Analysis suggests that Liberibacter cells reside in a matrix within the phloem sieve elements and that a portion of this matrix is composed of bacterial DNA. Results from microscopy with Fluorescence In Situ Hybridization (FISH) indicated that individual bacteria or clumps of bacteria could be liberated by mechanical pulverization of source tissues, but numbers of cells were less than expected based on real time PCR (qPCR) data, and cell morphology appeared to be altered, which is a result consistent with published reports on purification of phytoplasma cells (also phloem colonizing bacteria) from plant tissue. A small library of prokaryote 16S rRNA gene sequences generated with degenerate primers indicated the prokaryote population in seed coat vascular tissue is 100% Liberibacter asiaticus. Experiments indicated that tissue pulverization methods which employ large or small steel ball bearings to dissociate tissue may not pulverize tissue adequately to release bacterial cells; a laboratory tissue homogenizer seems to be a better choice for tissue disruption. Experiments with fractionation of homogenized Liberibacter-containing foliar and seed coat tissues on Percoll gradients yielded distinct profiles of distribution of Liberibacter DNA (cells) for the two tissue sources, with seed coats providing a cleaner preparation. While real-time PCR (qPCR) assays indicate that ~35% of Liberibacter cells remain within seed coat tissue that moves only a short distance into the Percoll gradient as a highly visible band of debris, qPCR assays showed that ~15% of the Liberibacter DNA bands further down in the gradient at a position equivalent to where cultured E. coli cells band. This material is being examined with microscopy to ascertain the presence and character of bacterial cells. A portion of this research is being submitted for publication in Phytopathology and will be a poster presentation at the general meeting of the American Society for Microbiology, June 19-24, 2012.

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Organization USDA-ARS-USHRL

Contract Number 11-22-418

Project Duration (years) 2 *Year of Project* 1

Total Funds (current year) $89,650.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.