CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2012

(●) *Quarterly Report* ( ) *Final*

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)
1/30/13	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.)

Research results are very similar to those reported in the September 2012 quarterly report. We ran additional experiments similar to those described in the previous quarterly report. Because of the season, seeds were mature and more difficult to homogenize completely with or without enzyme treatment. We continued investigation with two experimental approaches: 1) enzyme treatment without subsequent physical pulverization of the tissue and 2) enzyme treatment followed by physical pulverization of the tissue. Fractionation on Percoll gradients yielded results similar to previous. Percoll fractions with the lowest Ct values (most bacteria) were analyzed by Fluorescence In Situ Hybridization (FISH) microscopy. The most intense fluorescence was observed with spherical bodies and a few bacilliform shapes. Because of the manner in which the experiments have to be performed, contamination is possible though precautions are take to minimize this occurrence. As before, the numbers of cells calculated from qPCR values for the Percoll and preceding fractions indicated more cells should be present than were observed in the sample observed by microscopy. We know that the disruption of the vascular bundles is not complete since filtration prior to Percoll fractionation traps tissue fragments that contain Liberibacter DNA; and, most likely Liberibacter cells. We have expanded our isolation work to include adult psyllids and psyllid nymphs. Psyllids are softer bodied and preliminary experiments suggest they homogenize more efficiently than plant tissues. Real-time PCR analysis of fractions from Percoll gradients suggest Liberibacter cells band differently when extracted from psyllids. Currently, fractions are being analyzed by FISH.

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.