CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 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)
12/6/12	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.)

During summer, seeds generally are immature, relatively soft and easily homogenized. We tested two experimental approaches: 1) enzyme treatment without subsequent physical pulverization of the tissue and 2) enzyme treatment followed by physical pulverization of the tissue. For immature seeds, enzyme treatment followed by only extensive vortexing could cause extensive degradation of the tissues and release bacteria for further purification by filtration and centrifugation on a Percoll gradient. Enzyme treatment followed by physical disruption of tissue by treatment with a Polytron apparently gave at least equivalent tissue disruption if not slightly better based upon the calculated number of bacterial cells determined to be in the different portions of the Percoll gradient.

Percoll fractions that were indicated to contain the highest numbers of cells as determined by qPCR were assayed with Fluorescence In Situ Hybridization (FISH) microscopy. The most intense fluorescence was observed with spherical bodies (not seen in negative control preparations); the bacilliform shapes seen in fresh tissues disrupted and chemically fixed in place (paraformaldehyde) were infrequent, suggesting a change in the shape of the bacteria occurs during extraction from the tissue. Also, the numbers of cells calculated from qPCR values indicated more cells should be present than were observed the microscope. We do not know the basis of this discrepancy.

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Organization USDA-ARS

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Total Funds (current year) $85,350.00

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.