CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 2017

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Developing a culture medium for Liberibacter asiaticus through comparative multi 'omics analysis with its closest cultured relative, L. crescens

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
09/05/2017	Citrus Research and Development Foundation	CLas culture, genomics, molecular biology, and Koch's

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

Media continued to be modified to improve the growth of L. crescens and use those results to develop better media for CLas culturing. During this period the Killiny lab provided an enormous amount of metabolomic data from citrus phloem to enhance our media formulations. Results from this work has suggested components that should be added or added at a higher concentration to the medium for the culturing of CLas including carbohydrates (α-ketoglutarate, galactose, glucose, fructose, maltose, sucrose, xylose), amino acids (alanine, arginine, asparagine, aspartate, cysteine, 2-aminobutyrate, glutamate, glycine, isoleucine, phenylalanine, proline, serine, threonine, and valine), sugar alcohols (iso-inositol, sorbitol, xylitol), organic acids (citrate, fumarate, malate, and succinate), micronutrients (B, Cu, I, Mn, Mo, Zn), vitamins (ascorbate, cobolamine, diaminopemilate, pyridoxal phosphate, riboflavin, thiamine). In past media formulations, some of these molecules were in particularly low levels, particularly thiamine which was 100-fold below the level found in media.

For our defined media for L. crescens, we have always used a commercial source of Grace's insect medium as part of the formulation. In the past, we mistakenly assumed that the levels of compounds reported by the manufacturers are the actual levels present in the Grace's medium. The components of three commercial sources of Grace's medium were examined by metabolomics and none of them were very close to the standard composition of Grace's medium as listed on their labels. One of them, made by a manufacturer in Mubai, India, was very different and provided remarkable growth of L. crescens with no other added components. The growth obtained with this medium was very similar to that obtained by culture in BM-7, the standard, undefined medium used for this organism. The two other sources of Grace's medium from Gibco and Sigma did not support L. crescens growth and a version of this maedium made by our lab with ingredients from Sigma also did not support growth. We were unable to culture CLas on the Indian-made Grace's medium, referred to as Hi-GI.

Metabolomics analysis showed that Hi-GI medium contains 10-fold higher levels of various vitamins compared to what would be expected in Grace's medium. Sugars and organic acids (including glucose, fructose, sucrose, turanose, maltose, fumarate, alpha-ketoglutarate, malate, maleate and succinate) were also higher. To date, we haven't been able to make a defined medium based on Hi-GI that provides the same high level of growth as does Hi-GI. This implies the chemically defined medium still requires improvements. Liberibacter crescens requires compound or combinations of compounds for optimal growth and these have not yet been discovered.

Meawhile, we have a large stock of Hi-GI on hand and are modifying it in order to culture CLas.

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Organization UF/IFAS

CRDF Project Number 15-027

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

Total Funds (current year)

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