CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 2016

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)
03/21/2016	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 the 769 project, we attempted to bring a group of people together to culture Ca. Liberibacter asiaticus (CLas). Mike Davis gave us his latest recipe for culturing CLas. Dean Gabriel did experiments that showed that the bacteriophage was not expressed in Mike Davis's cultures. Mark Hilf had some ideas but these did not contribute to the progress made to date.

Nabil Killiny was very helpful. He completed and published a metabolome of citrus phloem. He is now working on a similar metabolome of the psyllid gut. We used the citrus information to add several components to the medium we are now using.

A defined medium for L. crescens was developed. This has served as the foundation for optimizing a medium for CLas. Meanwhile, several approaches suggested refinements to the medium. Comparative genomics of various Liberibacter showed that CLas probably requires a high osmotic environment where L. crescens does not. As a result, we added various sugars to the medium at the levels Dr. Killiny observed in citrus phloem. A transcriptomic analysis of L. crescens showed a high expressed of an ascorbate transported in the medium suggesting that ascorbate was limiting in the medium. A small RNA analysis of L. crescens suggested that the potassium levels we were using was too high. We have since reduced potassium levels. Proteome analysis of L. crescens showed the expression of 50 proteins in L. crescens whose gene homologs are lacking in CLas. Of these, 30 are of known function, 20 are of unknown function. Determining the functions of these 20 proteins may be important in solving the riddle of CLas culturing. The 30 proteins of known function mainly code for amino acid synthesis suggesting that certain amino acids be added to the medium. In addition, proteomics taught us that nicotinate and zinc are needed in any CLas medium.

A new round of multi-omics analysis of L. crescens in a defined (that provides slow growth) and undefined medium (that provides faster growth) is in progress and should tell us more about factors limiting the growth of Liberibacter strains in culture.

An essential gene set was determined in L. crescens under BM-7 medium growth conditions. Mutagenesis of L. crescens identified 314 genes that are essential for growth in culture, of those, 238 have homologs in CLas. The 76 essential genes of L. crescens that are needed for growth of L. crescens in BM-7 medium likely code for functions required for growth of CLas on that same medium. These results suggested that folate, fatty acids, thiamine, and pantothenate are required for any CLas growth medium.

As a result of all of these studies, we now have a medium called AM13 that allows very slow growth of CLas in culture. We now need to confirm the purity of this culture and optimize it for faster growth. We also need to sequence the genome from CLas in culture since it is likely to be a more accurate genome sequence than was obtained from psyllid metagenomes in the past. This is in progress and we found a novel way to do this without amplifying the DNA.

The growth observed in AM13 is very slow and the cultures never become turbid. Thus, improvements to the medium are still needed to get the cell numbers necessary for many future experiments.

PI First Name Eric

PI Last Name Triplett

Email ewt@ufl.edu

Phone 352-392-5430

Organization University of Florida, IFAS

Project Number 769

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

Total Funds (current year)

Form PR-12 Quarterly Reports SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.