Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

| 2009-2010 REPORT | CATEGORY (drop-down) | TODAY'S DATE (m/d/yr) |
|--|--|-----------------------|
| Quarterly Report Annual Report Final | Psyllid 💿 | January 19, 2011 |
| WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished) | | |
| | | |
| Novel beta-proteobacteria discovered in the asian citrus psyllid. | | |
| TITLE and CONTACT INFORMATION | | |
| <i>Proposal Title</i> Genome-enabled metabolic reconstruction of Ca. Liberibacter asiaticus and its use in culturing and controlling the pathogen. | | |
| Principal Investigator Eric W. Triplett | PI Last Name Triplett | |
| Email ewt@ufl.edu | FDACS Contract Number 336 | |
| Phone 352-392-5430 | Project Duration (years) 3 Year of Project 1 | |
| Organization University of Florida, IFAS | Total Direct Funds (current year)249,000 | |
| | | |

REPORT UPDATE (650 words; provide details about your headline)

Our efforts to culture Liberibacter continue but there is no progress to date. Further, efforts to reproduce a published method for culturing Liberibacter have also failed. In addition to continue to modify our current medium as our metabolic reconstruction is refined. Another strategy has been recently adopted. We are determining the metabolome of citrus phloem. Based on this, we will design media to culture Liberibacter.

We have also determined the microbes that live on or within the psyllid. DNA was extracted from eleven Liberibacteri PCRpositive psyllids and ten PCR-negative psyllids. The highly conserved 16S rRNA gene was amplified from each of these psyllids. The amplified 16S rRNA products were sequenced using Illumina technology. Just over 192,000 sequences were obtained from each psyllid.

The microbial diversity of these insects is surprisingly vast. We expected to see a dominance by the alphaproteobacteria, Wolbachia and Liberibacter. However, a total of 543 bacterial genera were found in these psyllids.

Liberibacter represented 1.59% of all sequences in the PCR-positive psyllids and only 0.009% of sequences in PCR-negative psyllids. Other genera higher in PCR-positive psyllids were Chelatococcus, a methylobacterium and Paraholospora. The dominant genera found in the PCR-positive psyllids were Wolbachia, Liberibacter, and Anaplasma. The dominant genera in the PCR-negative psyllids were Wolbachia, Anaplasma, and Oxalobacter.

The most striking observation on these psyllids is the dominance of the population by betaproteobacteria that have not yet been classified to genus. These betaproteobacteria represent nearly half of the total bacterial population of the psyllid. Understanding the role of these betaproteobacteria may allow novel control mechanisms of the insect.