CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

genome sequence of X. axonopodis pv. citrumelo and Xac Aw

Proposal Title

Identification and characterization of critical virulence and copper resistance genes of Xanthomonas axonopodis pv. citri & related species

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/14/11	Citrus Research and Development Foundation	Unclassified

REPORT UPDATE (500 words; summarize your accomplishments)

Citrus canker is a serious disease of most commercial citrus cultivars in Florida.The goal of the proposed research is to identify and characterize novel and critical genes involved in pathogenicity and copper resistance present in X. axonopodis pv. citri (Xac) and related strains. Identification of critical virulence factors is a crucial step toward a comprehensive understanding of bacterial pathogenesis, host-species specificity, and invasion of different tissues thus to design new management strategies for long term control. Treatment of citrus with copper-based bactericides is one of the most common practices used for control. However, there is potential for horizontal gene transfer of copper resistance genes from other closely and distantly related bacterial strains, which will drastically reduce the efficacy of copper bactericides. Currently, copper resistant strains of other xanthomonads, including X. axonopodis. pv. citrumelo, the citrus bacterial spot pathogen, have been isolated from fields in Florida.Understanding the potential mechanisms of copper resistance in Xac and potential horizontal gene transfer of this resistance to Xac is also important for the long-term management of citrus canker.Currently, five Xac related strains are being sequenced with FL-1195 completed, XacAw close in completion (chromosome completed, plasmids in progress), while three more genomes are in the gap closing stage.

Genome sequence of Xac strain Aw was completed. It is currently being checked for any problematic regions. The GenePrimp analysis of the annotation showed 1728 anomalies. Manual curation is currently in progress. Multiple gaps still exists for the two plasmids. Primer walking,cloning and sequencing are being used to close those gaps.The genome sequence has been compared to the reference strain XAC A 306 using MAUVE and global rearrangements were observed. Analysis of T3SS effectors revealed presence of unique XopF1 and avrGf1 effector genes which are absent from Xac A 306 strain. Mutant of xopF1 and double mutant of xopF1/avrGf1 are being constructed to determine their roles in host specificity.

Complete genome sequence of X. axonopodis pv. citrumelo FL-1195 was completed. At least 48 regions, which were found incorrect during annotation analysis, were rechecked and corrected. Correction was done using the 454 and Illumina read data as well as PCR sequencing of the questionable regions. The CDS prediction was curated using the GenePrimp pipeline. 1485 anomalies were checked and 570 CDS were corrected after being identified as long, short, broken or new genes. The genome sequence has been compared to the reference strain Xac A 306 using MAUVE and global rearrangements were observed. In detail analysis of T3SS, other secretion systems, effectors, LPS, EPS, motility clusters and unique regions different from Xac A 306 strain is currently in progress.

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Organization Citrus Research and Education Center

Contract Number 233

Project Duration (years) 3 *Year of Project* 2

Total Funds (current year) $107,054.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.