Florida Citrus Advanced Technology Program

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

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2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Canker Pathology	12/30/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Identification and characterization of critical virulence and copper resistance genes of citrus canker pathogen

TITLE and CONTACT INFORMATION

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

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Organization Citrus Research and Education Center, UF	*Total Direct Funds (current year)* 190200

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

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 Xanthomonas 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. a. 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.
Major achievements: Currently, five Xac related strains are being sequenced, which includes Xac Aw and A* strains which have restricted host range compared to the A type strain, X. axonopodis pv. citrumelo strains (copper resistant and non-copper resistant), and Argentinian strain (copper resistant). Both 454 Titanium and Illumina (solexa) methods were used. Currently, a draft genome sequence is done for Xac Aw strain. Titration run was performed for the rest four strains. 1/8 plate of Titanium production run was completed. Illumina sequencing was completed. We are assembling all the data using the Xac A strain as the reference genome. We will decide whether further sequencing is needed based on the assembly. Primer walking is being used to fill the gaps. Comparative genomics analysis is being performed to identify novel genes which may contribute to host-species specificity and copper resistance.
In order to identify potential virulence factors, a X. axonopodis pv. citri mutant library mutant library constructed using EZ-Tn5™ <R6Kγori/KAN-2>Tnp Transposome™ kit is been screened for mutants affecting virulence. So far, 18,000 mutants have been screened and 215 mutants were selected for their phenotype changes in pathogenicity and virulence. Among them, 70 mutants have been characterized and complementation is underway. The rest of the mutants are being characterized. As a lot of those mutants were not able to grow in planta, we are also investigating the possibility of targeting those proteins for antimicrobial development. Two mutants with disrupted galU genes encoding UTP-glucose-1-phosphate uridylyltransferase have been studied extensively. This result has been submitted to AEM for publication (Guo et al. 2009). In that study, two non-mucoid mutants of Xcc 306 (F6 and D12), were identified. Rescue cloning and sequence analysis indicated that the two mutants have different transposon insertion sites in the galU gene. Further study showed that the galU gene was required for biosynthesis of extracellular polysaccharides, capsule, as well as biofilm formation. Mutation of the galU gene caused loss of pathogenicity on grapefruit. Although D12 showed a similar growth rate as the wild type strain 306 in nutrient broth medium, no population was established in the intercellular space. GalU represents a potential target for antimicrobial compounds screening to control citrus canker disease. GalU has been shown to be required for bacterial growth in planta in our study, antimicrobial compounds inhibiting its activity should potentially render Xac virtually avirulent. In addition, prokaryotic UTP-glucose-1-phosphate uridylyltransferases appear to be completely unrelated to their eukaryotic counterparts and have totally different structures even though they have almost identical catalytic properties. This interesting feature suggests that putative antimicrobial inhibitors of GalU will not be harmful for human and the citrus host.