Functional Studies of Genes Transcriptionally Regulated by Calcium in Xylella fastidiosa

By: Courtney Kloske, Sy Traore, Prem Kandel, Leonardo De La Fuente

Xylella fastidiosa (X. f.) is a bacterium that causes multiple lethal diseases in many economic crops including grapes, citrus, peach, plum, almond, coffee, pecan, and more recently olive. It was first described as Pierce's disease in the grapevines of California vineyards. *X. f.* lives only in xylem vessels, which carry water and nutrients from the roots to the rest of the plant. Proliferation inside the xylem leads to formation of a biofilm that obstructs the plant's vascular system, causing nutrient deprivation in the plant.

Previous research by our group showed that calcium increases the growth of the biofilm, and therefore, the virulence of the bacterium. Whole transcriptome analysis identified 17 *X. f.* genes that were consistently upregulated by calcium at different times, including 12 without an assigned function. One gene (PD0926) was selected and encodes for a hypothetical protein. The objective of this study was to determine the role of PD0926 in the virulence of *X. f.* To test its virulence, homologous recombination was used to cleanly delete the PD0926 and replace them with the chloramphenicol antibiotic resistance gene. Sequence verification was performed to verify successful knockout of the genes of interest in the *X. f.* genome.

We successfully knocked out gene PD0926 in the WM11 and Temecula strains of *X. f.* These mutated strains will serve as a resource for performing further *in vivo* and *in vitro* testing. Microfluidic chambers will be used to test the biofilm formation of the mutated strain, and twitching motility will be observed. An *in planta* test will be performed to show the progression of the disease within a tobacco plant. These tests will help us understand the virulence of the mutated strain. With this knowledge, a more holistic view of the bacterium will help with potential eradication of this bacterium and its related diseases.

Statement of Research Advisor:

Courtney's work was important to create mutants in X. f. in order to understand the biology of this pathogen. We decided to venture into unknown territory with the target genes chosen. Despite the fact that we could not finish what we originally planned, Courtney's research was an example of the challenges of doing research and how to deal with non-anticipated hurdles in the process.
—Leonardo De La Fuente, Plant Pathology