Title of report:
Exploiting a chitinase to suppress *Xylella fastidiosa* colonization of plants and insects

Principal Investigator:
Rodrigo Almeida
Dept. Environmental Science, Policy and Management
University of California, Berkeley
Berkeley, CA 94720
rodrigoalmeida@berkeley.edu

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Abstract
Previous research showed that *Xylella fastidiosa* has a chitinase (ChiA), which is required for sharpshooter vector colonization, transmission to plants, as well as plant colonization. The goals of this project are to understand the function(s) of ChiA so that it can be exploited as a tool for control of Pierce's disease by disrupting *X. fastidiosa* interactions with both plant and insect hosts. This report summarizes recent efforts aimed at experimentally determining carbon sources that can be used by *X. fastidiosa* in this context, as well as continuing our work to try to determine why the *chiA* knockout mutant is deficient in plant colonization.

Lay Summary
The previously identified *X. fastidiosa* chitinase (ChiA) represents a unique opportunity to try to disrupt *X. fastidiosa* interactions with both insect and plant hosts, as well as sharpshooter transmission, because all of these processes are affected in the mutant strain that does not have this enzyme. The goal of this project is to better understand how ChiA impacts plant and insect colonization so that it can be exploited to limit Pierce's disease spread.

Objectives
Efforts during the report period focused on experimentally determining ChiA substrates, and if ChiA is involved in evading the plant immune system.

Results and Discussion
Chitinase substrates
Biolog plates containing 190 different carbon sources have been tested with both the WT cells and the chitinase mutant cells. A few amino acids (N-acetyl-L-glutamic acid, L-arginine, L-ornithine, L-phenylalanine and L-pyroglutamic acid) were negative when tested with the chitinase mutant strain (which should indicate that no oxydo-reduction activity occur in the corresponding wells) and positive while tested with the WT strain. However, when using L-phenylalanine as a sole carbon source, growth was observed for both the chitinase mutant and the WT strains. L-arginine and N-acetyl-L-glutamic acid were also tested as sole carbon sources but the results were inconclusive.

Plant carbohydrates degradation

As the chitinase mutant is not able to move within grapes - no cell was found at 15 cm above the inoculation point in the 20 plants inoculated with the chitinase mutant whereas they were found in 15 out of the 19 plants inoculated with the WT cells - we are currently tested whether this lack of movement is due to the inability of the mutant to degrade components of the pit membrane. *X. fastidiosa* has indeed been shown to move from one xylem vessel to another by degrading pit membranes. The ability of the WT strain, the chitinase mutant and the complemented strain to degrade cellulose, xylan and pectin are currently under investigation.

Another potential role of the chitinase

Another potential function of the chitinase - evoked in the previous report- could be to degrade a bacterial defense elicitor to evade the plant immune system. If this is the case, there should be some differences between the expression of certain plant defense genes in plants infected by the WT cells and the ChiA mutant cells. This hypothesis is now being tested by measuring by qPCR the expression of some defense-associated genes plants infecting by the WT strain, the *chiA* mutant strain or mock-inoculated. We have started extracting RNA from the petioles taken 3 ½ months post-inoculation for the WT strain, the chitinase mutant, and the mock-inoculated plants.

Conclusions

Research has identified a series of new carbon sources that may be utilized by *X. fastidiosa*, efforts are now focusing on experimentally confirming these results; some appear to not be used as carbon sources by the *chiA* mutant strain. We note that a no-cost extension was requested for this project because we are seeking a new researcher to perform the work. A search for a qualified postdoctoral researcher failed, so a qualified staff research associate was hired in late May 2016. That person is now trained, so we hope the project will move forward faster.

References Cited

Funding Agencies

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