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

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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 if ChiA is required for the degradation of one (or several) plant carbohydrates (movement and/ or carbon sources), and if ChiA is involved in evading the plant immune system.

Results and Discussion
Experimental results associated with work to determine if ChiA is involved in *X. fastidiosa* evading the plant immune system are ongoing but there are no new results to report. Although a substantial amount of information has been generated about the biology of *X. fastidiosa*, we still have very little experimental knowledge about the sources of carbon that *X. fastidiosa* can use. Because we hypothesize that the lack of *chiA* mutant strain colonization of host plants may be associated with reduced degradation of host polysaccharides, we screened for the ability of *X. fastidiosa* to utilize 192 different carbon sources. In the previous report we identified a few carbon sources used by *X. fastidiosa* using the Biolog screening system. We have now compared

the utilization of these substrates between the wild type strain and the *chiA* mutant. Results identified 7 substrates (in replicates) that the *chiA* mutant strain could not utilize. We are now developing an in vitro protocol to conclusively test these preliminary results. In addition, we have procured a range of plant carbohydrates to test if they can be used by *X. fastidiosa* as a carbon source. We hope to be able to report on these interesting findings in a more conclusive manner in our next report.

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; importantly, some appear to no 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.

References Cited

Killiny, N. and Almeida, R.P.P. 2009. Host structural carbohydrate induces vector transmission of a bacterial plant pathogen. Proceedings of the National Academy of Sciences USA 106: 22416-22420.

Killiny, N., Prado, S.S. and Almeida, R.P.P. 2010. Chitin utilization by the insect-transmitted bacterium *Xylella fastidiosa*. Applied and Environmental Microbiology 76: 6134-6140.

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