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 enhancing *X. fastidiosa* utilization of chitin by using strong selective pressures through the availability of chitin as the sole carbon source in media; previous reports discussed other aspects of the project.

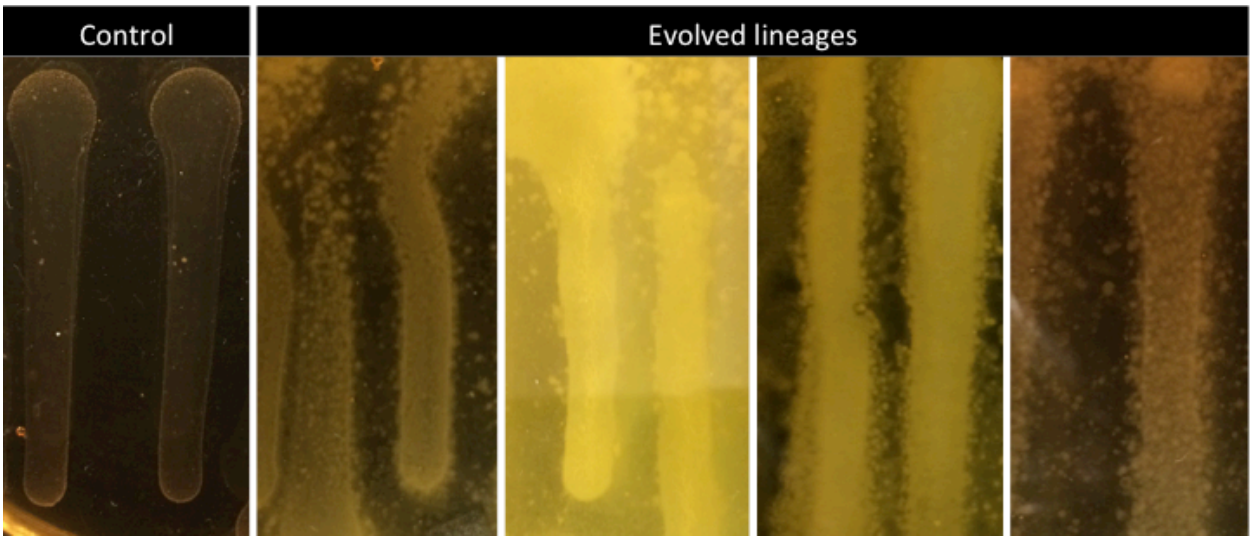
Lay Summary
The previously identified *X. fastidiosa* chitinase (ChiA) represents a unique opportunity to try to disrupt *X. fastidiosa* interactions with both insect and plant hosts, as well as sharpshooter transmission, because all of these processes are affected in the mutant strain that does not 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 enhancing the ability of *X. fastidiosa* to utilize chitin; we hypothesize that by forcing *X. fastidiosa* to use only chitin as a carbon source, proteins involved in chitin processing/binding will be under selection and detectable after NGS sequencing.

Results and Discussion
The basis of this project was an earlier finding that *X. fastidiosa* has a functional ChiA (Killiny et al. 2010), and that ChiA is required for insect and plant colonization, as well as transmission to plants (Labroussaa et al. 2017). In addition, we have shown that other *X. fastidiosa* proteins that bind to chitin substrates can be used to block *X. fastidiosa* transmission (Labroussaa et al. 2016) – transgenic plants are now being generated based on those proteins. Therefore, there is evidence indicating that ChiA is of major importance in determining *X. fastidiosa*-host interactions, so understanding how it interacts with plant and insect substrates is of importance. Previous reports have described efforts towards achieving this goal. However, the identification of additional chitin-binding proteins has remained elusive. Here we report on an alternative approach based on natural selection to reach this objective.

Bacterial populations grown in vitro with high population sizes (i.e. no bottlenecks) have been demonstrated to adapt fairly quickly to new environmental conditions. We proposed that growing *X. fastidiosa* on media with chitin as its carbon source would select for populations that can more efficiently utilize this substrate. Following this rationale, the entire chitin-utilization machinery would be under selective pressure, leading to substitutions that can be identified with whole-genome sequencing of lineages. This ongoing experiment now has over 6 months of sequential passages of an ancestral wild type strain of *X. fastidiosa*.

Large phenotypic differences can be observed among control and evolved lineages (see figure below). Because of the extreme differences in phenotype, we have routinely tested populations using diagnostic *X. fastidiosa* PCR primer sets; all results so far have been positive. However, fringe formation of colonies is rampant, as well as what appears to be exolpolysaccharide production (glossy aspect of colonies). In addition, colonies are growing substantially faster on plates than *X. fastidiosa* would normally do. Due to these large phenotypic changes already observed, we have submitted all lineages for NGS sequencing to confirm the absence of contaminants. NGS data will also allow us to preliminarily identify lineages that should be studied in more detail, in relation to phenotypes observed. It is our expectation that genes involved in chitin utilization will have a molecular signature with accumulated substitutions, allowing us to better understand the role of ChiA in insect and plant colonization.



Control *X. fastidiosa* on the left shows standard colony phenotype on PWG medium, commonly used to grow *X. fastidiosa* in vitro. The other four images are *X. fastidiosa* lineages that evolved on chitin as a carbon source for ~6 months, also plated on PWG for comparison purposes. PCR-based detection confirmed the presence of *X. fastidiosa* in all lineages, and these extreme phenotypes have emerged in some but not all of those. Light microscopy also indicates these are pure *X. fastidiosa* lineages. NGS sequencing is being used to identify substitutions, as well as to confirm that there is no contamination.

Conclusions

Extreme phenotypes of *X. fastidiosa* were observed on plates, including faster growth, production of exopolysaccharides as well as colony fringe formation. Samples of populations

from a dozen lineages (~6 months after experiment began) have been submitted to a sequencing facility on campus. We hope to soon be able to identify substitutions involved in these emerging phenotypes. The strong selection for chitin utilization in these lineages should allow us to identify genes important for chitin processing, facilitating the identification of targets useful for PD control that have not been successfully found following other protocols.

Literature Cited

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.

Labroussaa, F., Ionescu, M., Zeilinger, A.R., Lindow, S.E. and Almeida, R.P.P. 2017. A chitinase is required for *Xylella fastidiosa* colonization of its insect and plant hosts. Microbiology, accepted.

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Funding Agencies

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