Interim Progress Report for CDFA Agreement Number 14-0142-SA

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

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Time Period Covered by the Report
February – July 2015

Introduction
The goal of this new project is to determine which protein(s) interact with a chitinase (ChiA) we identified in *X. fastidiosa*. A *chiA* mutant strain is deficient in chitin degradation, insect and plant colonization, as well as plant-to-plant vector transmission. The gamut of biological activities associated with ChiA make it a desirable target to limit *X. fastidiosa* colonization of plants and vectors, as well as Pierce's disease spread. However, although active, we did not identify a chitin-binding domain in the sequence of *chia* using bioinformatics tools, and we showed experimentally that ChiA is not active without the presence of other *X. fastidiosa* proteins. The goal of this project is to identify these partners and the role of ChiA in plants so that disease control strategies can be developed exploiting ChiA by suppressing its biological roles.

List of Objectives – as in the approved research proposal
i) to identify *X. fastidiosa* proteins or protein complexes that bind to ChiA and are required for its chitinolytic activity.
ii) to screen potential substrates cleaved by ChiA.
iii) to functionally demonstrate the role of ChiA partners during insect and plant colonization.

Description of activities
We have subdivided this report on three sections, highlighting research performed during the period covered by this report. Research has move all three objectives forward. We address methodological problems and how we are addressing them, primarily with the first objective aimed at identifying ChiA partners. For objective two, after preliminary results, we are now optimizing protocols so that Biolog plates can be used to identify substrates utilized by *X. fastidiosa* and those that a *chiA* mutant strain cannot degrade. The power of this approach is that we are screening for 190 substrates at once, rather than doing it individually. Optimization of protocols was necessary due to the fastidious nature of *X. fastidiosa* growth *in vitro*. Although ChiA partners are still being identified, we are also moving forward with the last aim by testing the potential role of ChiA in plant immune system evasion. Plants have no chitin, yet the *chiA* mutant strain is deficient in plant colonization. We are now trying to explain why that is the case.

Chitinase partners :
One method proposed for determining binding partners for ChiA was to incubate purified ChiA with cell lysate of Temecula grown on XFM supplemented with either colloidal chitin or galacturonic acid. This mixture would then be run on a native PAGE gel, which would preserve protein folding and therefore enzymatic activity ; make an overlay gel containing 4-Methylumbelliferyl β-D-N,N′,N″-triacetylchitotrioside, which fluoresces when cleaved would then be poured onto the native PAGE and imaged under UV light. Areas of fluorescence would indicate enzymatic activity. We found, however, that the isoelectric point of our chitinase was too close to the pH of the gel, and therefore our protein did not run.

The current method we are investigating is to reversibly bind the purified ChiA to its partners using DTSSP. We plan to incubate purified ChiA with cell lysate of Temecula grown on XFM supplemented with either colloidal chitin or galacturonic acid. This mixture will then be bound to a Ni++ ion exchange column, and eluted with imidazole. The fractions will be run on SDS PAGE, with 5% 2-mercaptoethanol acting to cleave the DTSSP and release the ChiA and its binding partners, resulting in multiple bands, which will then be excised and processed via mass spectroscopy. Initial tests provided multiple bands, but we are now working on reproducing the results prior to protein identification.

Chitinase substrates :
Two different biolog plates (PM1 and PM2) containing 190 different carbon sources are currently being tested to determine what carbon sources can be degraded and used by *X. fastidiosa*. By comparing the activity of WT cells with the *chiA* mutant cells, we might be able to (i) determine what are the carbon sources which could be used by *X. fastidiosa* and (ii) which ones are directly degraded by the chitinase. As mentioned above, protocol optimization has been necessary but we hope that is now finished.

In parallel, different arabinogalactan proteins extracted from grapes will be tested as potential targets of *X. fastidiosa* ChiA; this will be done through a collaboration with the Joint BioEnergy Institute. Arabinogalactan proteins are indeed good candidates since (i) they could be one of the only plant cell wall molecules containing GlcNAc and GlcN residues and (ii) they seem to contain cleavage sites for endochitinases (Van Hengel et al 2001). We are currently finishing expression/purification of enough ChiA for these assays.

Exploring a potential role of ChiA :
Another potential function of the chitinase -not previously evoked in the project- could be to degrade a bacterial defense elicitor, like peptidoglycan, 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 will be tested by measuring by qPCR the expression of some defense-associated genes -such as the genes encoding for PR1 and PR5- on plants infecting by the WT strain, the *chiA* mutant strain, the *chiA* complemented strain or mock-inoculated (Figure 1A). Besides, an exogenous application of the elicitor (the example of peptidoglycan is here used) either pre-treated or not by a functional chitinase (a complex of the chitinase with its partners) to the plant should led to a colonization of the plant and a virulence respectively similar to the ones observed in plants infected by WT or *chiA* mutant cells (Figure 1B).

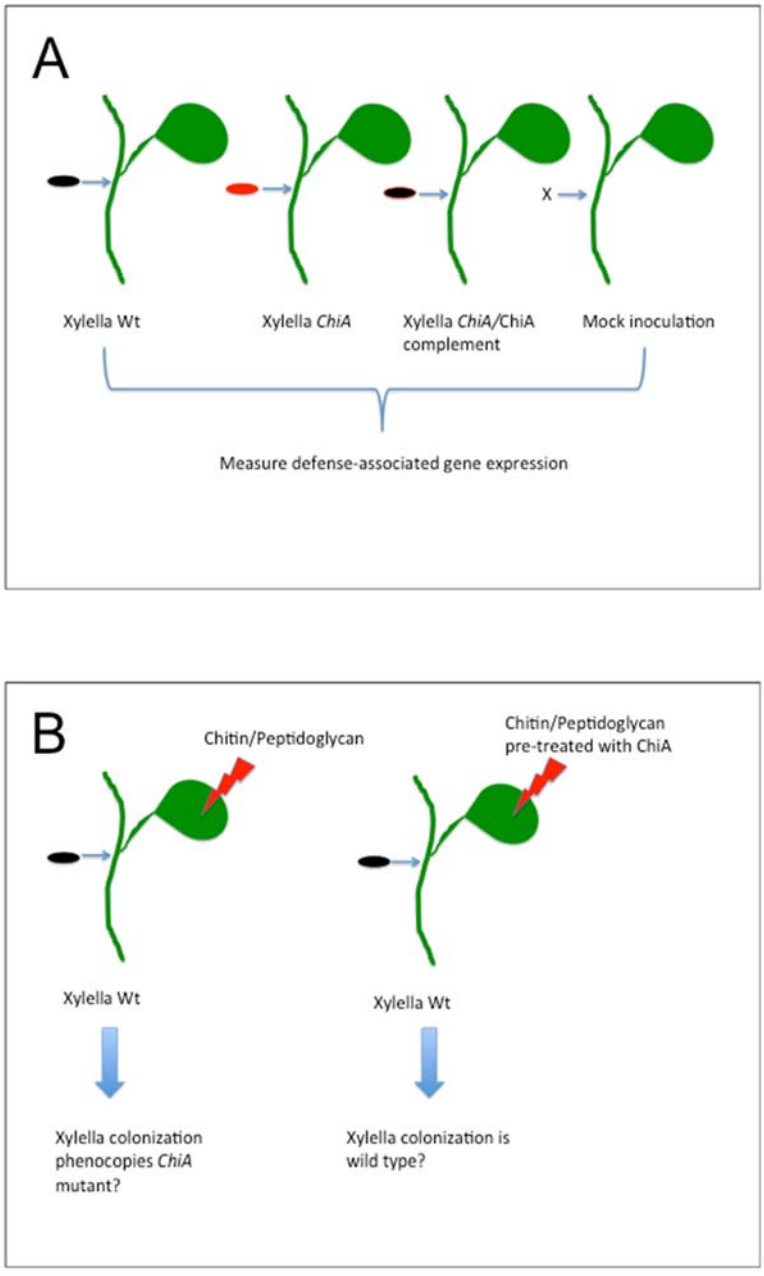

Figure 1: Schemes to test the role of *X. fastidiosa* chitinase in bypassing plant defense.

Publications produced and pending, and presentations made that relate to the funded project

Publications: a manuscript describing the role of ChiA on the biology of *X. fastidiosa* is currently being finalized and will be submitted for publication soon.

Presentations:

. Exploiting a chitinase to suppress *Xylella fastidiosa* colonization of plants and insects. Pierce's Disease Research Symposium, Sacramento, CA.

. Pierce's disease in winegrapes and olive. 3rd Annual Vineyards & Wineries Continuing Education Class Series, Nov 4, 2014, Napa, CA. Sponsored by UCCE, Farm Bureau and the Napa Ag Commissioner's Office.

. Emerging Vector-borne Diseases; Forum on Microbial Threats, Institute of Medicine, National Academy of Sciences, Washington DC.

Research relevance statement

We have shown that ChiA plays a central role in the *X. fastidiosa* transmission cycle. It is also essential for the successful colonization of both plant and insect hosts of *X. fastidiosa*. However, there are several important questions related to the activity of this enzyme. First, it is not clear what substrates it cleaves, especially in plants. Second, ChiA is not active by itself, apparently requiring a partnership with substrate-binding proteins. We propose to characterize ChiA so that we can suppress its activity in both plants and insects. ChiA represents a unique target to control Pierce's disease because it is required for the colonization of both plants and insects, in addition to being important for vector transmission and spread. Therefore, disruption of its activity should lead to control of *X. fastidiosa* colonization of plants and movement from plant-to-plant.

Layperson summary of project accomplishments

Initial personnel issues previously described delayed the generation of data for this project. We are now moving forward on all three objectives and expect that novel information will be generated soon. We are also testing a hypothesis that ChiA may have a role in plant immune system evasion. This new project is advancing forward with success, but at a slower pace than proposed.

Status of funds

Funds were being used slower than originally proposed, but are now being used as proposed.

Summary and status of intellectual property associated with the project

Not applicable at this stage.

Literature cited

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.