Interim Progress Report for CDFA Agreement Number 10-0275

Title of Project
Blocking *Xylella fastidiosa* transmission

Principal Investigator
Rodrigo Almeida, Associate Professor
Dept. Environmental Science, Policy and Management
University of California, Berkeley
Berkeley, CA, 94720

Time Period Covered by the Report
December 2011-March 2012

Introduction
The goal of this project is to develop a technology to block the vector transmission of *Xylella fastidiosa* to plants. Since the beginning of this project, we have focused on the characterization of the *X. fastidiosa*-sharpshooter interface to better understand how interactions between these organisms can be disrupted, leading to reduce pathogen transmission. The work was followed by proof-of-concept experiments that clearly demonstrated the feasibility of blocking transmission. Essentially, we used knowledge obtained during initial studies to design experiments with treatments that should block transmission and others that should not, obtaining successful results. This report focuses on the identification of new transmission-blocking chitin-binding proteins, which is ahead of expectations given that the current award finishes in June 2012.

List of Objectives – as in the approved research proposal

Objective 1. Molecular characterization of the X. fastidiosa-vector interface
Objective 2. Identification of new transmission-blocking chitin-binding proteins

Objective 1 was completed in a timely manner, some of the results have been published and a few papers are now being drafted. Essentially, we showed that:

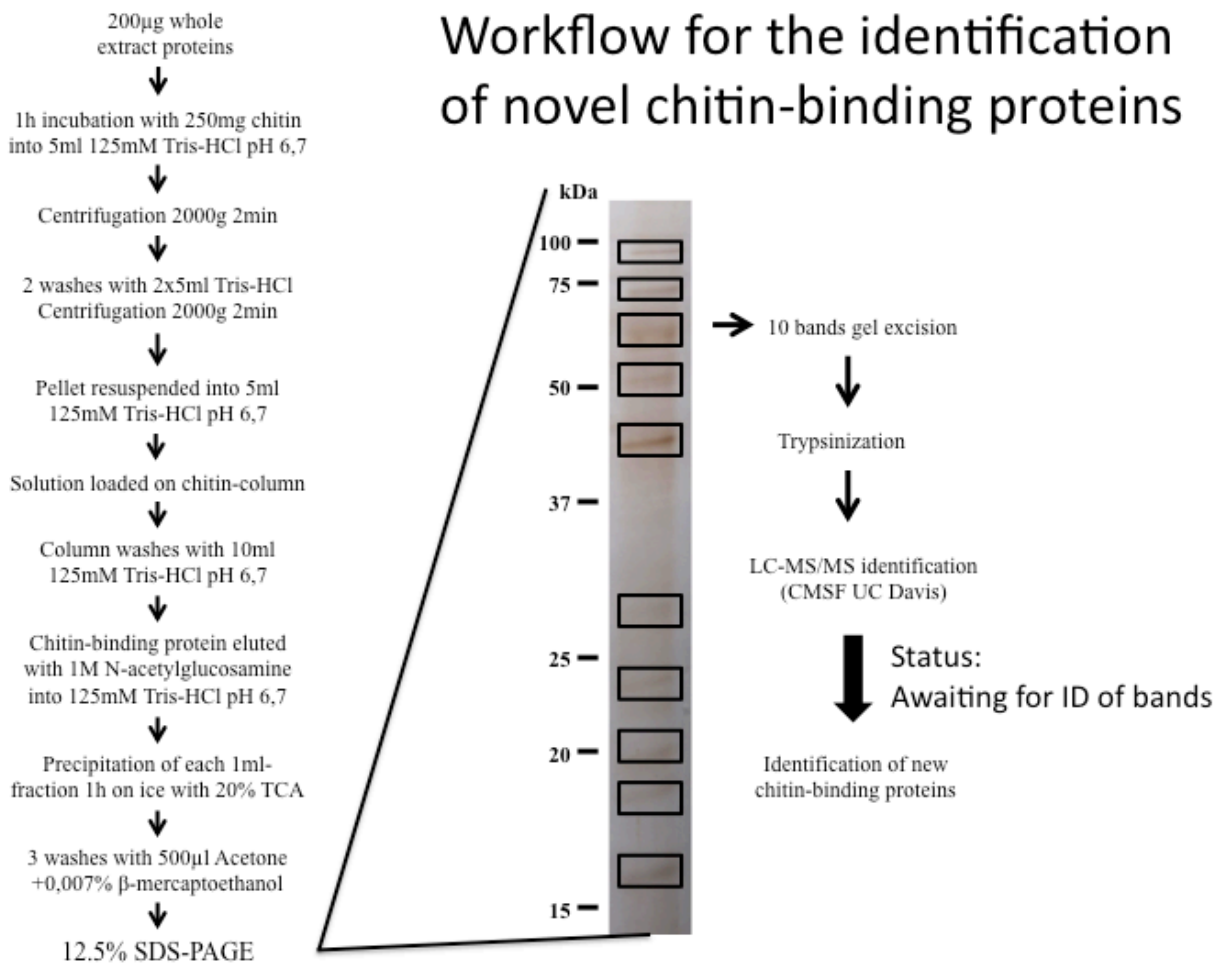
i) *X. fastidiosa* utilizes chitin as a carbon source,
ii) tested 20 mutants in relation to their transmissibility - greenhouse assays,
iii) performed mRNA-seq studies to analyze gene expression patterns to better understand *X. fastidiosa* colonization of vectors and to identify new targets to block transmission,
iv) showed that protein-carbohydrate interactions are very important for *X. fastidiosa* colonization of vectors and that disrupting these interactions blocks transmission of the pathogen to grapevines under greenhouse conditions.

Efforts focusing on Objective 2 are described in this report. We are very glad that this component is going well and its proposed goal is estimated to be finished by April/June. This will allow us to pursue the testing of candidate peptides under greenhouse conditions this season, moving the project ahead of schedule and hopefully identifying a definitive list of transmission blocking peptides by the end of 2013.

Description of activities

Objective 1. Molecular characterization of the X. fastidiosa-vector interface
As mentioned above, activities associated with Objective 1 have been previously reported, and some of that work has been published in peer-reviewed journals. We refer the reader to those sources. However, if the reader would like to have a detailed description about those efforts the Principal Investigator (Almeida) would be happy to provide that information via phone or email.

Objective 2. Identification of new transmission-blocking chitin-binding proteins
The specific goal of this Objective was to identify *X. fastidiosa* chitin-binding proteins using a proteomics approach. Briefly, we proposed to grow cells under conditions that allow *X. fastidiosa* to colonize vectors, extract total or cell membrane proteins, purify those adhering to chitin, and identifying those proteins. We currently have ten samples obtained following this protocol for total proteins being processed by a facility at UC Davis (results pending, expected by the end of March). Once results are back we expect to quickly identify these proteins. Thus, we expect our proposed work to be complete soon. However, we are taking advantage of the fact that protocols are working well and results are very promising to move to the next step of this research. We already have started making constructs to express proteins/peptides we already know to be involved in transmission so that testing of candidates can be initiated this year. Here we report on work to obtain these novel ten chitin-binding proteins, as well as additional work to test candidates as soon as possible.



We will briefly discuss our results, as a more complete discussion depends on the proteins identified. Elution of these ten proteins bands extracted from *X. fastidiosa* grown on medium that induces vector transmissibility (XFM-pectin; Killiny and Almeida 2009b) was performed using total proteins preparations. Utilization of whole cell extract proteins has the advantage of potentially identified most of the chitin-binding proteins. However, additional washings steps were required to obtain a specific elution profile. The protocol described above allowed us to specifically eluted ten bands of *X. fastidiosa* proteins using N-acetylglucosamine (GlcNAc) as a competitor. GlnNAc is the main subunit of chitin polymers, the principal component of insect cuticle found in the insect foregut region colonized by *X. fastidiosa* (Almeida and Purcell 2006). Previous results showed that *X.fastidiosa* binding to vector was reduced in the presence of GlcNAc (Killiny and Almeida 2009a).

We also repeated these assays for conditions that do not induce *X. fastidiosa* transmission (Killiny et al. 2009b). Good correlation was found between proteins specifically bound to chitin and the medium inducing transmissibility. In other words, no proteins specifically bound to chitin were found for samples from a medium (XFM) poorly capable of inducing *X. fastidiosa* transmission. On the other hand, faint bands of proteins specifically bound to chitin (molecular weight (MW) identical of those identified for medium enhancing transmissibility were identified

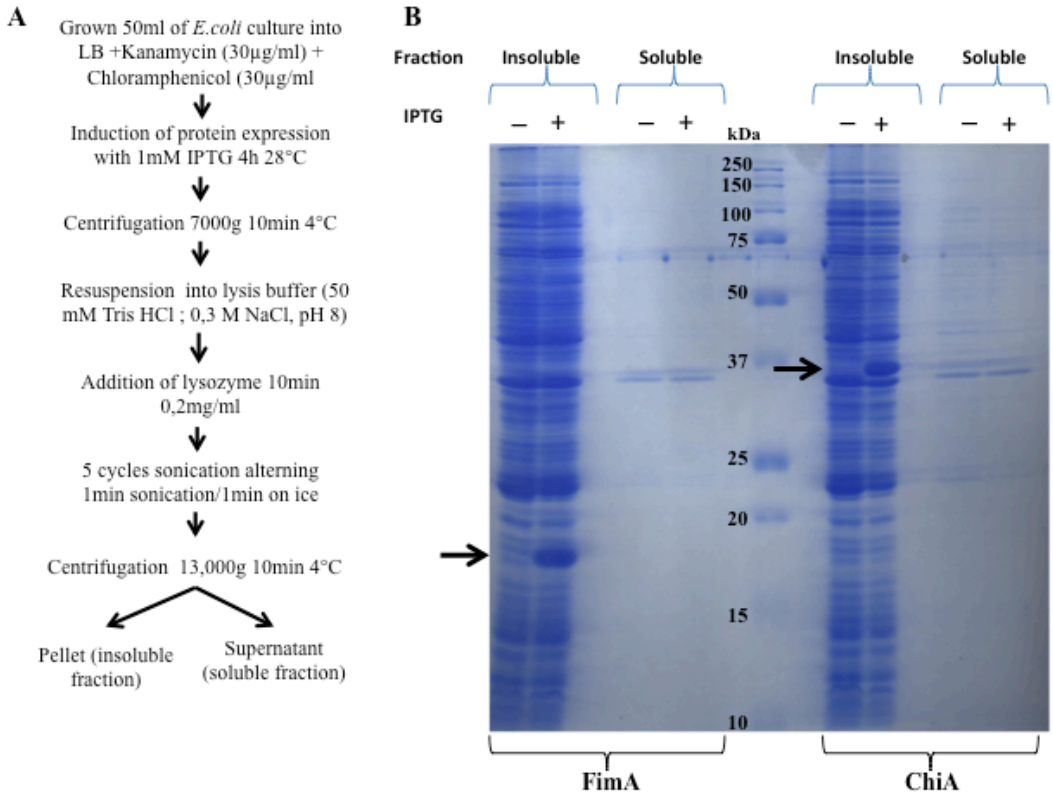
when we used proteins extracted from *X. fastidiosa* grown on XFM supplemented with chitin (Killiny et al. 2010). Together, these experiments support previous results and indicate that *X. fastidiosa* colonization of vectors involves complex regulation of protein expression and response to environmental cues.

Because of the initial centrifugation at low-speed for initial binding of proteins to chitin, we potentially lost high-molecular weight proteins such as Hxfs (hemagglutinin-like proteins) (Voegel et al. 2010). Although some of these proteins could be important for *X.fastidiosa* initial adhesion on the insect foregut, we would prefer focus our efforts on proteins at or below 100kDa. Our final goal is to identify and use as transmission-blocking molecules, peptides or domains on proteins implicated in the binding with chitin. Such identification will be much easier if proteins have a low molecular weight. As shown in Figure 1, bands ranged from 16kDa to 95kDa. Interestingly, some bands match the molecular weight of proteins already implicated in *X. fastidiosa* transmission. Briefly, these proteins include type I fimbriae (short pili, FimA), afimbrial adhesin XadA and the chitinase we identified earlier in this project (ChiA). Indeed, XadA (expected MW 97kDa), ChiA (expected MW 40kDa) and FimA (expected MW 19 kDa) could match with elution bands of the corresponding molecular weight. Identification of these proteins in corresponding bands could give us additional information concerning their role in the initial adhesion process.

In addition to the proteomic approach described above, we are also pursuing a targeted alternative based on the assumption that *X. fastidiosa* proteins we have identified as important for sharpshooter colonization could be used to block transmission to plants (i.e. Hxfs, XadA, ChiA and FimA). Based on our work characterizing the *X. fastidiosa*-vector interface we expect these to have high affinity to the foregut of sharpshooters, which is the site colonized by *X. fastidiosa*. Constructs have already been built to express all these proteins (or shorter peptides with putative chitin-binding domains; Hxf already provided by Bruce Kirkpatrick, UC Davis) with histidine tags in *E. coli*. ChiA and FimA have already been shown (see below) to be expressed in *E. coli* and will be purified for *in vitro* trials soon, while expression of the other proteins in *E. coli* is currently being tested. The novel chitin-binding proteins (ten) currently being identified will also be expressed following this protocol.

Induction test for expression of ChiA and FimA

A: General protocol. B: SDS-PAGE showing expression of FimA and ChiA proteins. Arrows show expression of FimA at 18kDa and ChiA at 35kDa in the insoluble fraction.



Differences in molecular weight for both proteins (ChiA: around 35kDa vs 40kDa for predicted MW; FimA: around 18kDa vs 19kDa for predicted MW) are due to absence of the signal peptide in the construct. Indeed, ChiA and FimA are both secreted proteins and have a signal peptide on their N-terminal region. Deletion of this signal peptide avoids issues with recombinant protein preparation and purification steps using nickel-affinity chromatography. Both proteins were recovered in the insoluble fraction, which could require extra work for the final purification step. Trials to purify these proteins will soon determine if we need to optimize the induction step to recover recombinant proteins in the soluble fraction. To do so, we could induce protein expression overnight at lower temperatures (instead of 4h at 28°C) or using denaturing agents. Elimination of histidine tags will be the last step before using these proteins as transmission-blocking molecules in *in vivo* using artificial diet systems (Killiny and Almeida 2009b). The effect of recombinant proteins and peptides on transmission will be tested this summer when sharpshooter colonies are well established in our greenhouse.

Publications and presentations related to the funded project

Peer-reviewed publications

Almeida, R.P.P., Killiny, N., Newman, K.L., Chatterjee, S., Ionescu, M. and Lindow, S.E. 2012. Contribution of *rpfB* to cell-cell signal synthesis, virulence, and vector transmission of *Xylella fastidiosa*. Molecular Plant-Microbe Interactions 25: 453-462.

Killiny, N., Rashed, A. and Almeida, R.P.P. 2012. Disrupting the transmission of a vector-borne plant pathogen. Applied and Environmental Microbiology 78: 638-643.

Chatterjee, S., Killiny, N., Almeida, R.P.P. and Lindow, S.E. 2010. Role of cyclic di-GMP in *Xylella fastidiosa* biofilm formation, plant virulence and insect transmission. Molecular Plant-Microbe Interactions 23: 1356-1363.

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.

We also expect to publish a few more manuscripts based on work already done testing several *X. fastidiosa* mutants for their transmissibility. Transcriptomics work (mRNA-seq) yielded high quality data that are now being analyzed, a couple of manuscripts are expected from that work as well. We are not including reports at the PD/GWSS symposium on this list as those are required by this funding program and archived by CDFA.

Presentations

Talk: Host switching in a vector-borne plant pathogen. Essig Museum talk series, University of California Berkeley. March 2012.

Talk: Ecology of *Xylella fastidiosa* diseases. International Congress for Bacterial Diseases of Stone Fruits and Nuts. Joint Meeting European Cost 873 and ISHS. Zurich, Switzerland. February 2012.

Talk: New insights on the vector transmission of *Xylella fastidiosa*. Pierce's Disease Research Symposium, Sacramento, CA, sponsored by CDFA. December 2011.

Talk: Regulatory networks and the leafhopper transmission of the bacterium *Xylella fastidiosa*. Annual Meeting of the Entomological Society of America, Reno, NV. November 2011

Talk: Regulation of host switching and transmission in *Xylella fastidiosa*. Hemipteran-Plant Interactions Symposium, Piracicaba, Brazil. July 2011.

Talk: Vector transmission of *Xylella fastidiosa*. Departmental seminar, Department of Entomology, sponsored by University of California, Riverside. March 2011.

Talk: Blocking the transmission of a leafhopper-borne bacterial pathogen. Facing the Challenges of Vector-Borne Diseases in the 21st Century Symposium, Center for Disease Vector Research, UC Riverside. Riverside, CA. March 2010.

Research Relevance Statement

Current efforts to control Pierce's disease focus on either reducing pathogen populations after plant infection or reducing vector populations. This project focuses on vector-pathogen interactions and how to disrupt them, effectively leading to reduced *X. fastidiosa* spread in vineyards. Initial work characterizing these interactions lead to the hypothesis that *X. fastidiosa* carbohydrate-binding adhesins were responsible for adhesion to vectors. This was tested using lectins and carbohydrates, when we showed that transmission can be blocked *in vivo*. Current efforts are aimed at identifying and testing chitin-binding proteins/peptides for their *in vivo* activities. This research will contribute towards finding solutions to Pierce's disease by

exploiting an essential component of this disease system, i.e. vector transmission, which represents an untapped source of novel control strategies.

Layperson Summary of Project Accomplishments

This project had two goals. First, to characterize *X. fastidiosa*-vector interactions so that knowledge about how the pathogen colonizes sharpshooters was improved. This work was successfully performed, resulting in proof-of-concept experiments under greenhouse conditions that clearly demonstrate the feasibility of blocking sharpshooter transmission of *X. fastidiosa* to grapevines. The second goal, to identify specific transmission-blocking candidate proteins is now is its final phase (i.e. sequencing and identification itself) and we have started to express and purify already available candidates for *in vivo* testing this summer. Altogether, we expect a final list of candidates that have been already tested *in vitro* and *in vivo* in the greenhouse to be available by the end of 2013, when planning for larger experiments, including field trials, would be initiated.

Status of Funds

Funds are being used as planned, a small surplus is expected by the end of the contract (June 2012) and we have requested a no-cost extension to finish ongoing experiments with those funds.

Summary and Status of Intellectual Property

None expected so far, but PIPRA has been contacted in this regard and will be consulted if IP issues become relevant.

Literature Cited

Almeida, R. P. P., and Purcell, A. H. 2006. Patterns of *Xylella fastidiosa* colonization on the precibarium of sharpshooter vectors relative to transmission to plants. Ann. Entomol. Soc. Am. 99:884-890.

Killiny, N., and R. P. P. Almeida. 2009a. *Xylella fastidiosa* afimbrial adhesins mediate cell transmission to plants by leafhopper vectors. Appl. Environ. Microbiol. 75:521-528.

Killiny, N., and R. P. P. Almeida. 2009b. Host structural carbohydrate induces vector transmission of a bacterial plant pathogen. Proc. Natl. Acad. Sci. U. S. A. 106:22416-22420.

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

Voegel, T. M., Warren, J. G., Matsumoto, A., Igo, M. M., and Kirkpatrick, B. C. 2010. Localization and characterization of *Xylella fastidiosa* haemagglutinin adhesins. Microbiol. 156:2172-2179.