PROGRESS REPORT – February 2011

I. Project title

Blocking *Xylella fastidiosa* transmission

II. Principal Investigators and Cooperators

Rodrigo Almeida, Principal Investigator
Nabil Killiny, Cooperator
Steve Lindow, Cooperator
Department of Environmental Science, Policy and Management
University of California, Berkeley, CA 94720

III. List of objectives and description of activities

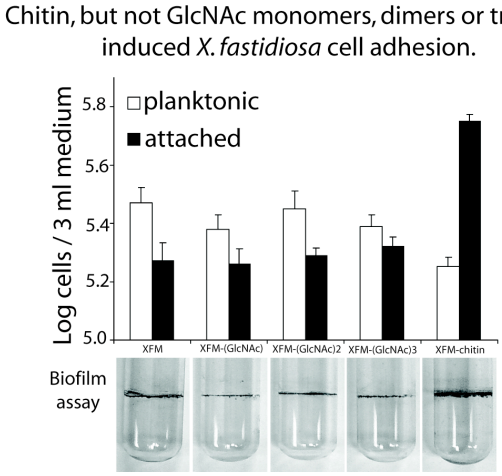
1. Molecular characterization of the *X. fastidiosa*-vector interface
2. Identification of new transmission-blocking chitin-binding proteins

Research activities to accomplish each objective are described together with a summary of major accomplishments, so that the rationale and outcome of studies are directly connected. This report covers the November 2010 to February 2011 period.

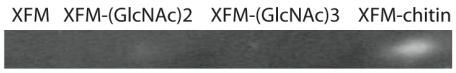
IV. Summary of major research accomplishments and results for each objective

In our previous report (July to October 2010) we presented results on the discovery that *X. fastidiosa* has a fully functional chitin-processing machinery. That work has now been published (Killiny, Prado and Almeida. AEM 2010). This finding opened several new research venues on *X. fastidiosa* physiology, gene regulation and our knowledge of how this bacterium interacts with its sharpshooter vectors. Furthermore, because blocking *X. fastidiosa* chitinolytic activity may affect its colonization of vectors and subsequently transmission efficiency, we were keen to explore it in more detail. This report focuses on additional work done to characterize this interactions. We also provide a summary of research testing the transmissibility of several *X. fastidiosa* mutants, which is ongoing and will be reported in detail in our next report.

In our previous report, our work focused on chitin, which is an *N*-acetyl glucosamine polymer. However, work with other organisms suggested that bacterial responses are not the same when polymers and monomers are used. Thus, giving the large role chitin has on *X. fastidiosa*'s biology and, consequently, vector colonization, we addressed this question in more detail. We found that chitin polymers [(GlcNAc)4 or larger] induce chitinase activity and are responsible for phenotypic changes in *X. fastidiosa*. More specifically, we found that cells grown in medium supplemented with chitin adhered significantly more to tissue culture tubes than cells grown in chitobiose, chitotriose or the basal medium XFM. While smaller oligomers often induce chitinases in some bacteria, in species such as *Thermococcus chitonophagus* require larger oligomers such as we have found in *X. fastidiosa* (Andronopoulou and Constantinos 2004).



Although further work is necessary, we hypothesize that GlcNAc polymers function as environmental cue molecules that are sensed by receptors on the cell's outer membrane, similarly to plant cell contact-dependent receptors in *Ralstonia solanacearum*, which are responsible for signal transfer and control of *hrp* genes in that pathogen (Brito et al. 2002). Although it is likely that other hexosamines are present on the outermost layer of the insect surface where *X. fastidiosa* attaches, the glycosyl hydrolase in *X. fastidiosa* may have little specificity, as similar hydrolases in other species have little specificity and may be able to catalyze reactions with other substrates. Supporting this hypothesis, we found that chitinase activity was detected only with cells grown in XFM-chitin. XFM is a simple defined medium for *X. fastidiosa* (Killiny and Almeida. PNAS 2009). When chitobiose or chitotriose were incorporated into XFM, no chitinase activity was tested. This is expected, given that the substrate for the chitinase, chitin, was not present in the medium. Band in gel represents *X. fastidiosa* endochitinase activity when cells are grown in XFM media supplemented with chitobiose, chitotriose or chitin.

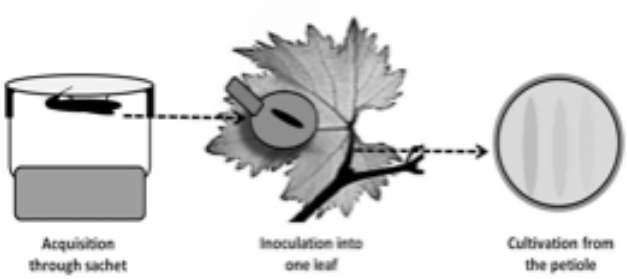


Altogether these results show that chitin polymers function as environmental cues for *X. fastidiosa*, even though cells probably use *N*-acetyl glucosamine monomers as a carbon source. We are pursuing this interesting observation in more detail, as it is certainly relevant to how *X. fastidiosa* colonizes insects and could lead to new ideas on how to block transmission.

We have also performed transmission experiments for several mutants using the artificial diet system we previously developed (Killiny and Almeida 2009 PNAS). We have finished testing the majority of mutants listed in our proposal. Furthermore, some of those were tested twice as we slightly modified our experimental design. Because finished work includes some but not all the mutants being studied, and some of the data are still being analyzed, we will report on these efforts in our next report so that all results are presented at the same time with appropriate context. Nevertheless, we present results from studies with cell-cell signaling mutants to highlight ongoing work.

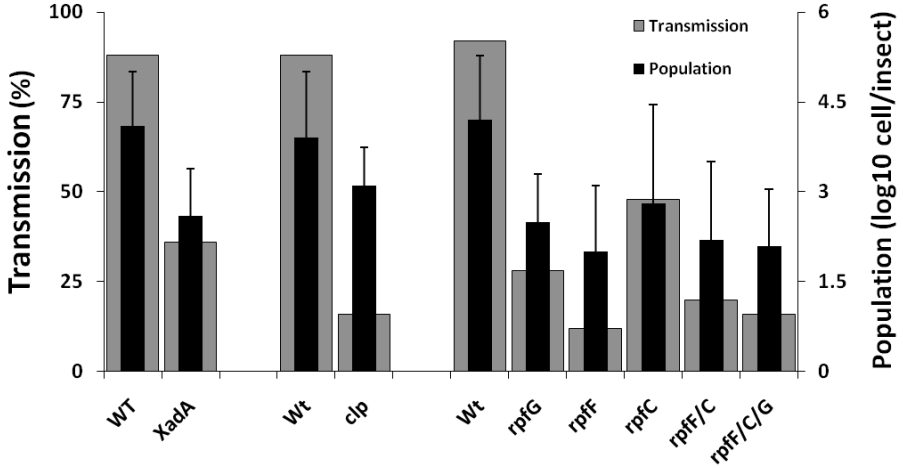
Our experimental design is aimed at determining if strains are transmitted by vectors without considering plant-pathogen interactions. Using artificial diets for acquisition we have eliminated the need for source plants. However, we still use plants for inoculation, but test plants 2 weeks

after insect access to reduce the potential impact that mutants that cannot colonize plants may have on the outcome of trials. Only the inoculated leaf is tested. We were successful in obtaining positive inoculation events into artificial diets as well (Rashed et al. 2011 API, in press), but we believe that vector probing behavior in plants and diets are reasonable different and could affect inoculation efficiency. Thus, the approach used looks at initial cell adhesion and colonization of vectors, followed by inoculation into plants.



The figure below summarizes work in collaboration with Steve Lindow's group at UC Berkeley. Three sets of mutants were studied in relation to their transmission efficiency and bacterial populations in insects. All studies have their respective wild type controls. Mutants and their respective functions/systems are: *xadA* (adhesin), *clp* (intracellular signaling), and *rpf* operon genes (cell-cell signaling). First, it can be seen that all mutants were affected in their transmission, by at least 40% (*rpfC*). Importantly, however, all were transmitted at least at a basal level, thus we cannot consider any of them as non-transmissible. Interestingly, so far we have not found any mutant that is not transmissible by vectors, although most are significantly affected. A different, and incorrect, picture was being formed based on prior studies that used source plants infected with mutants. That is due to extreme variation in patterns of mutant colonization of plants and the bacterial populations reached by those, as reported by us and other groups. The latter has been shown to directly affect transmission efficiency (Hill and Purcell 1997).

The number of cells within insects after 7 days was estimated with quantitative PCR. This is a powerful tool because it provides insights into patterns of vector colonization without the need for extremely laborious microscopy studies, which would eventually be necessary to carefully dissect the role of specific genes/proteins on transmission. These results are only for positive individuals, but show that all mutants had populations on average one order of magnitude smaller than the wild type.



V. Publications or reports resulting from the project

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.

Almeida, R.P.P. and Killiny, N. 2010. Blocking *Xylella fastidiosa* transmission. In: Proceedings of the 2010 Pierce's Disease Research Symposium, San Diego, CA, Dec. 15-17. p 55-59.

VI. Presentations on research

'*Xylella fastidiosa* transmission'. Almeida. Cornell University, New York State Agricultural Experiment Station. June 2010.

'Ecology of insect-transmitted plant diseases'. Almeida. Institut National Agronomique de Tunisie. Tunis, Tunisia, Oct 14, 2010.

'Colonization of insect mouthparts by a vector-borne pathogen; *Xylella fastidiosa*-leafhopper vector interactions' Killiny. Pierce's Disease Research Symposium, San Diego, CA. Dec 15-17, 2010.

VII. Research relevance statement

We are using tools and approaches recently developed to study how *X. fastidiosa* colonizes its insect vectors. This aspect of *X. fastidiosa*'s biology is the most poorly understood component of this system; yet, it is essential for bacterial dissemination and disease spread. Understanding how colonization occurs would lead to novel concepts on how to limit disease spread. In addition, several findings from this research has already contributed to a better understanding of how it causes disease in plants and the new tools used by the community at large. The main new finding from this project so far is that *X. fastidiosa* uses chitin as a carbon source. This has dramatically modified our view of *X. fastidiosa*-vector interactions and opened new research venues.

VIII. Lay summary of current year's results

The main finding of this project so far is that chitin is a carbon source that induces transcriptional and phenotypic changes in *X. fastidiosa*. Interestingly, however, the monomer/dimer/trimer of chitin do not appear to induce the same responses. Although chitin can be exploited as a carbon source, we have not shown that that occurs *in situ*; work with a chitinase mutant is ongoing to test this hypothesis. Altogether, these observations resulted in a paradigm shift on *X. fastidiosa*-vector interactions. We always thought that sharpshooters were only carriers of *X. fastidiosa* and that multiplication of this bacterium within vectors was supported by nutrients in xylem sap ingested from plants by sharpshooters. Results from this project strongly suggest that insects also serve as a 'food' source for *X. fastidiosa*. One of the applied consequences of these findings is that a completely new array of targets to block disease spread has been discovered. We have also tested several mutants in relation to their transmissibility by sharpshooters; those results will

be made presented in our next report. That work will result in a better understanding of how *X. fastidiosa* colonizes vectors and permit a more targeted approach to identify candidate molecules that could block its transmission to plants.

IX. Status of funds

No present funding problems for this project.

X. Summary and status of intellectual property produced during this research project

None expected.