PROGRESS REPORT – July 2011

I. Project title

Blocking *Xylella fastidiosa* transmission

II. Principal Investigators and Cooperators

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III. List of objectives and description of activities

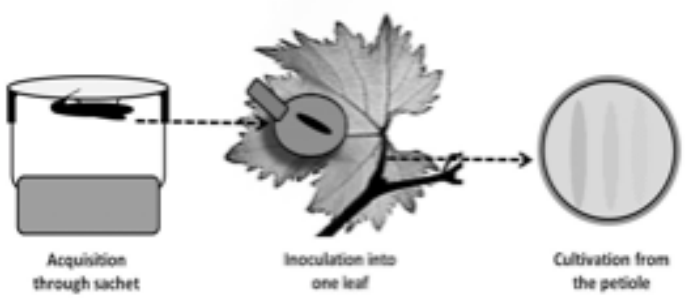
1. Molecular characterization of the *X. fastidiosa*-vector interface
2. Identification of new transmission-blocking chitin-binding proteins

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 March 2011 to July 2011 period.

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

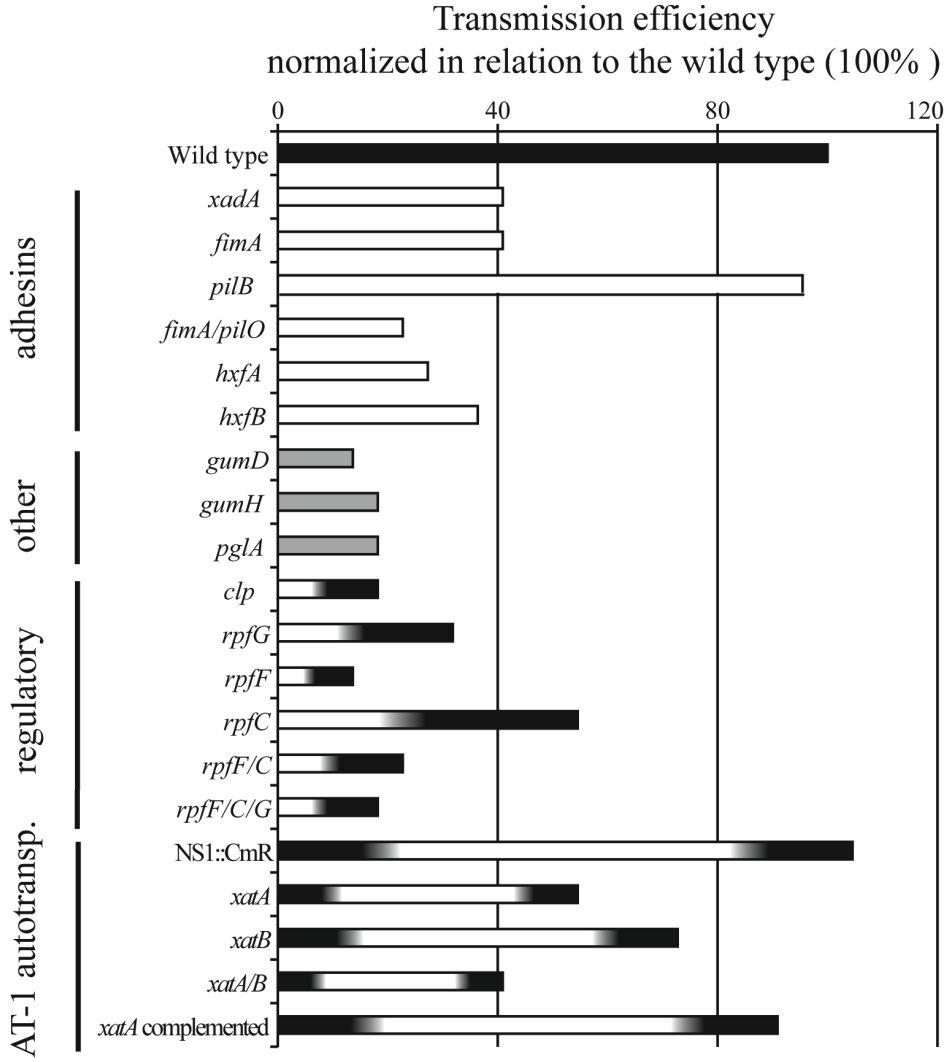
In our previous report we presented results on the discovery that *X. fastidiosa* has a fully functional chitin-processing machinery (Killiny et al. 2010) and how chitin influences *X. fastidiosa* phenotype and gene expression patterns. This report provides a summary of research testing the transmissibility of several *X. fastidiosa* mutants. As one of our goals in this project, we have tested the transmission efficiency of 20 *X. fastidiosa* mutants to better characterize the *X. fastidiosa*-vector interface. We used a protocol developed by our group based on an artificial diet system that eliminates the need of using infected plants as a pathogen source to insects (Killiny and Almeida 2009), which prohibits adequate analyses of vector-pathogen interactions because many mutants are deficient in plant colonization. We note that these mutants were generated by other research groups and kindly provided to us for this study.

Briefly, our experimental design is aimed at determining if strains are transmitted by vectors without considering plant-pathogen interactions. Insects acquire cells from an artificial diet system for a few hours, then are transferred to a leaf on a healthy plant for inoculation. This leaf is tested two weeks after inoculation for *X. fastidiosa*. We use plants for inoculation, but those are tested two weeks after insect access to reduce potential problems with mutants that cannot colonize plants. This approach has allowed us to show



that mutants that are deficient in plant colonization are transmissible by vectors. We were successful in obtaining positive inoculation events into artificial diets as well (Rashed et al. 2011), but we believe that vector probing behavior in plants and diets are reasonably different and could affect inoculation efficiency. Thus, the approach used looks at initial cell adhesion and colonization of vectors, followed by inoculation into plants, requiring short term survival/multiplication of cells, which is expected even from mutants that do not colonize grapevines. We have also quantified bacterial populations within sharpshooters with qPCR (Killiny and Almeida 2009) after acquisition and one week later to gain insights on retention of the various strains. Data have been collected but are still being analyzed.

Because of the large number of mutants tested, different experiments (n=8) were performed and each one had its own wild type control. Transmission rates for the wild type ranged from 80 to 92%. The figure below summarizes our results. For comparative purposes, transmission rates of the wild type control in independent experiments were all normalized (to 100%), allowing a comparison among the various mutants. Statistical analyses need to be performed to compare the treatments tested, but for the purpose of discussion we may consider mutants with less than ~40% transmission as being deficient in this essential trait to *X. fastidiosa*'s biology.



Mutants tested so far can be arbitrarily divided into four categories: adhesins, gene regulation, AT-1 transporters and gum- and pectinase-deficient mutants. We will briefly discuss our results, a more complete discussion depends on the retention data we are yet to analyze. Among adhesins, all those tested appear to affect transmission, except for *pilB*, which is part of type IV pilus involved in *X. fastidiosa* twitching motility. Gum-deficient mutants were expected to be unable of biofilm formation within vectors, thus their lower transmission rates is not unexpected. We have previously shown that pectin degradation by the polygalacturonase (PglA) is required for vector transmission of *X. fastidiosa* cells grown on the XFM-pectin medium used to induce vector transmissibility (Killiny and Almeida 2009). Thus, we did not expect much if any transmission of the *pglA* mutant. Regulatory genes involved in cell-cell signaling and within-cell signaling have been demonstrated to be deficient in transmission, results obtained here were similar to those with whole plants, for example, *rpfC* is affected in transmission, but less than *rpfF* (Newman et al. 2004, Chatterjee et al. 2008, 2010). Our results were within expectations, as much of *X. fastidiosa*'s biology is affected by cell-cell signaling and within cell gene regulation. The AT-1 autotransporters tested were not affected as much, although the double mutant *xatA/B* was transmitted only 40% of the time. However, results with the complemented mutant *xatA* show that this protein has some role in transmission, as complementation revert transmission back to wild type level.

A few general points need to be addressed. First, it is now evident that the *in vitro* system we developed to study transmission independently of plants mimics experiments with plants adequately. For example, general trends obtained with transmission of the following mutants with plants were reproduced with the artificial diet system: *hxfA*, *hxfB*, *rpfF*, *rpfC*. This is important as a majority of mutants, such as *gumD* and *pilB*, cannot be tested using plants, but this protocol allows them to be studied in relation to vector transmission. Second, all mutants were transmissible to some degree in this study. This indicates that transmission is complex and not dependent on a single factor, such as only one adhesin. The fact that multiple factors are important for vector colonization is not surprising, but the results identified novel targets to block transmission. In exciting results recently obtained (future report), we have found that blocking several cell surface proteins with specific antibodies (e.g. Hxfs, PilA, PilC, FimA) resulted in reduced transmission efficiency, similarly to the results observed with mutants – antibodies against Hxfs reduced transmission, those against Pil proteins did not. These data clearly show that blocking of *X. fastidiosa* transmission is not only possible, but that it can also be very specific. Targeting multiple proteins may be required to absolutely eliminate transmission, and although zero transmission may not be required to severely reduce disease spread, it may lower the chances of *X. fastidiosa* becoming resistant to management practices taking advantage of this approach.

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.

'Regulation of host switching and transmission in *Xylella fastidiosa*' Almeida. Hemiptera-plant Interactions International Symposium. Piracicaba, Brazil. July 10-14, 2011.

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 generated new tools used by the community at large. The main new findings from this project so far are that i) *X. fastidiosa* uses chitin as a carbon source, ii) that several mutants are affected in vector transmission, suggesting that this is a complex interaction, and iii) that transmission can be blocked by disrupting specific proteins on the surface of *X. fastidiosa*. These findings have dramatically modified our view of *X. fastidiosa*-vector interactions and on *X. fastidiosa* biology in general, and opened several new research venues.

VIII. Lay summary of current year's results

The main finding in this specific report is that *X. fastidiosa*-vector interactions are complex and do not depend on single proteins. Results suggest that disruption of multiple targets would be necessary to completely abolish transmission, but under field conditions the disruption of one target alone, out of multiple options, may be sufficient to significantly impair disease spread. Importantly, we have recently found that *X. fastidiosa* transmission can be blocked in a very specific manner by affecting individual proteins on the surface of the bacterium (details in next report). Immediate consequences of our findings are that a completely new array of targets to block disease spread has been discovered, and that we are now beyond the proof-of-concept phase of this project – i.e. vector transmission can be significantly reduced in a very specific manner. Future work will result lead to further characterization and testing of an array of molecules that block *X. fastidiosa* transmission.

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.