EVOLUTION OF *XYLELLA FASTIDIOSA* AVIRULENCE

Principal Investigator:
Rodrigo Almeida
Dept. Environ. Sci., Policy, & Mgmt.
University of California
Berkeley, CA.
rodrigo@nature.berkeley.edu

Cooperators:
Steve Lindow
Dept. of Plant & Microbial Biology
University of California
Berkeley, CA 94720
icelab@socrates.berkeley.edu

Leonora Bittleston
Dept. Environ. Sci., Policy, & Mgmt.
University of California
Berkeley, CA 94720

Reporting Period: The results reported here are from work conducted March 2008 to September 2008.

ABSTRACT
The goals of this objective are to quantitatively and qualitatively determine how and when *Xylella fastidiosa* (*Xf)* loses pathogenicity and potentially vector transmissibility, after serial passages in vitro. We replicated *Xf* in vitro for one year (80 passages on solid rich medium), creating parallel populations that have phenotypes in vitro that differ from the ancestral isolate. We are now studying host plant colonization and insect transmission for selected populations (several passages were frozen at -80^{o}C for 10 lineages). Once phenotypes of interest are identified (reduced pathogenicity or transmissibility, results pending), we will compare these *Xf* populations with the original isolate and search for other phenotypic and molecular differences. We will also be able to quantify the rate of genetic change in these populations, providing a molecular calibration data for researchers interested in *Xf* evolution, diversity and ecology.

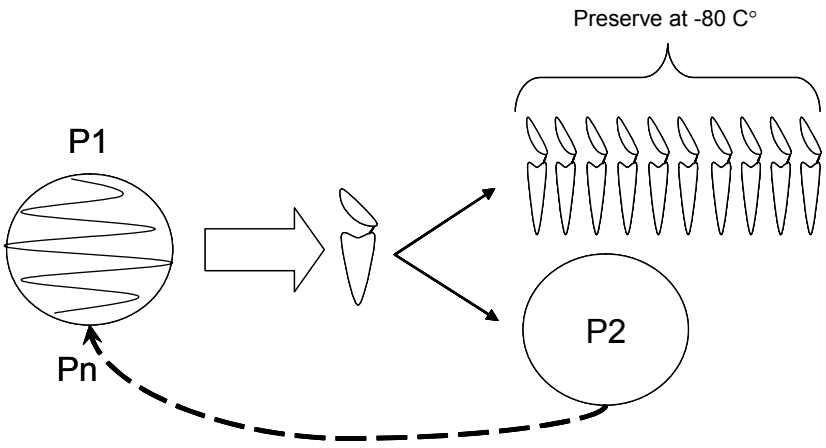
INTRODUCTION
Hopkins (2005) demonstrated the potential of avirulent *Xylella fastidiosa* (*Xf)* as a tool to control Pierce's disease (PD). He also illustrated the challenges of such an approach. For example, not all weakly virulent or avirulent isolates resulted in similar degree of control, and in most cases plants eventually become symptomatic. Understanding the biology of avirulent isolates and by which mechanisms they may reduce disease symptoms is of importance if this approach is to be widely adopted. This project tackles those questions by comparing evolved avirulent isolates with a parent pathogenic isolate. Being able to retrospectively compare these isolates using high resolution tools and biological assays will allow us to determine when, and how, *Xf* loses avirulence. We will also shed light on the mechanism how avirulent isolates suppress pathogenic ones. This proposal seeks to understand the evolution of avirulent *Xf* through serial passages in vitro. Such isolates have recently been shown to have potential to biologically control PD. We are studying how avirulent isolates evolve, biologically and genetically, and will test their potential as control agents of pathogenic *Xf*.

We have different *Xf* populations in the laboratory maintained under a selection protocol to obtain lineages that are avirulent in plants. This process is finished and now we are characterizing the phenotype of four out of 10 lineages we have created. We are comparing the evolved populations with the original one to determine if they have different phenotypes.

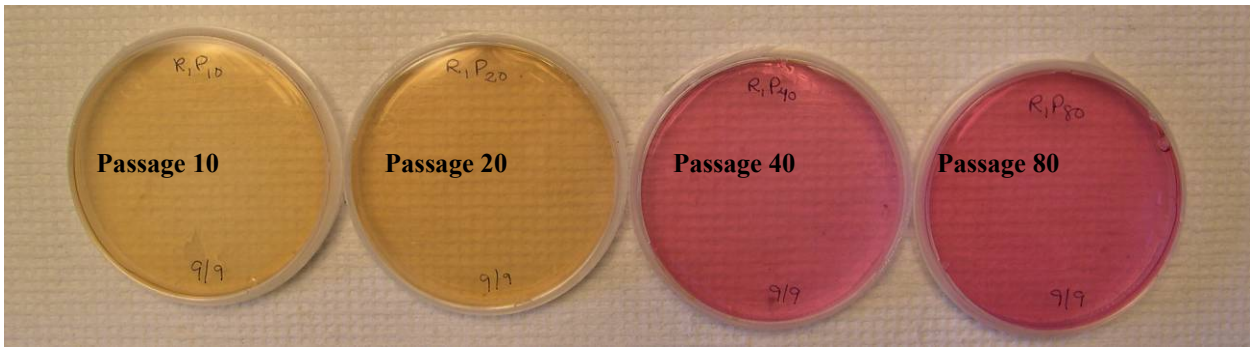
OBJECTIVES
Original objectives in the proposal submitted in 2007 were:
1. Generation of in vitro evolved populations.
2. Phenotypical characterization of populations.
3. Molecular characterization of populations.
4. To test avirulent populations as biological control agents.

Here we report on the first two objectives mentioned above. This project was funded for one year.

RESULTS AND DISCUSSION
We have started to work on Objectives 2-4. Objective 1 has been finished. For every 10 passages of the populations in rich medium (PWG), we stored a sample in a -80^{o}C freezer. We have recovered some of those for phenotypical and molecular characterizations. We have a total of 80 passages in this experiment, totaling eight frozen populations per lineage. We are using four randomly selected lineages and passages 0, 10, 20, 40 and 80 for our characterization studies. The general protocol is illustrated in the **Figure** below:



Although we do not have final data on the phenotype of these populations, we have noticed that on solid medium they are growing approximately twice as fast as the original population from which they derived, suggesting fast adaptation to new environmental conditions under selective pressure. One indirect measurement of growth on PWG medium is a change of pH to basic conditions, indicated by a pink color. Below, populations from selected passages were plated at the same time on PWG, illustrating the change in pH for later passages (40 and 80) that have been subject to the selection process longer than passages 10 and 20. Other factors may be increasing the pH on these plates, although we have not started to investigate this process at this point. To measure the growth rate of different passages we have tested different liquid medium-based approaches. However, we noted that cell attachment to surfaces and clumping varies significantly among these populations. Therefore, we are testing alternative protocols to determine the growth rate of representative populations. Ongoing experiments for phenotypic characterization of lineages include: growth rate, adhesion, biofilm formation, gum and protein production.



We have also inoculated these lineages/passages into plants (final results pending). Passages 0, 10, 20, 40 and 80 40d 80 from four parallel lineages were inoculated into almond plants. We will determine movement and multiplication of these populations by culturing from samples two and four months after inoculation (15 cm above inoculation site). From our two-month samples, we have determined that early passages were recovered in higher frequency than later passages. The proportion of infected plants for each passage (different lineages combined) 2 months after inoculation were: passage (P) 0 - 25%, P10 – 9.4%, P20 – 12.5%, P40- 0%, P80- 3%. We interpret these preliminary results as a gradual loss of pathogenicity by evolved populations, here interpreted as reduced movement and multiplication compared to ancestral population. We note that additional sampling will be performed on this experiment and we will repeat these assays next Spring.

Because we have noticed dramatic changes in growth rate of the lineages on rich solid media, we are also looking into potential protocols for identifying mutations in these clones if some of them are not pathogenic to plants. This would possibly identify spontaneous mutations and new pathogenicity factors in *Xf*, which could be used as targets for disease control. Lastly, we are conducting a multilocus sequence typing study to determine how fast these loci vary over time and to confirm the identity of the isolate we started our experiments with.

REFERENCES CITED

Hopkins, D.L. 2005. Biological control of Pierce's disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. Plant Disease 89: 1348-1352.

FUNDING AGENCIES
Funding for this project was provided by the University of California Pierce's Disease Grant Program, and the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

ACKNOWLEDGMENTS
We thank Nabil Killiny and Mate Lopes for technical assistance.