PROGRESS REPORT
California Department of Food and Agriculture

A. PROJECT TITLE
Evolution of *Xylella fastidiosa* avirulence

B. CDFA CONTRACT NUMBER
07-0324

C. TIME PERIOD COVERED BY THE PROGRESS REPORT
March 01 to July 31, 2008

D. PRINCIPAL INVESTIGATOR AND COOPERATOR
Rodrigo Almeida, Principal Investigator
Department of Environmental Science, Policy and Management
University of California, Berkeley, CA 94720, e-mail – rodrigo@nature.berkeley.edu
Steve Lindow, Cooperator
Department of Plant and Microbial Biology
University of California, Berkeley, CA 94720, e-mail – icelab@socrates.berkeley.edu

E. LIST OF OBJECTIVES AND DESCRIPTION OF ACTIVITIES CONDUCTED TO ACCOMPLISH EACH OBJECTIVE
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.

This project was funded for one year. Funds for this project were available in November 2007. We were only able to hire a person to conduct the research in February 2008, thus this will be a brief report on our activities. A no-cost extension for this project was requested and granted so we can accomplish the objectives proposed using funds provided.

F. RESEARCH ACCOMPLISHMENTS AND RESULTS FOR EACH OBJECTIVE

We have started to work on Objectives 2-4, Objective 1 has been finished. For every 10 passages of populations in medium 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 8 frozen populations per lineage. We are using 4 randomly selected lineages and passages 0, 10, 20, 40 and 80 for our characterization studies.

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.

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 (results pending). Once phenotypic characterization in plants has been finished we will test potential avirulent isolates in relation to their biological control potential with pathogenic isolates of *X. fastidiosa*.

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 *X. fastidiosa*, 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.

G. PUBLICATIONS, REPORTS, AND PRESENTATIONS WHERE THE INFORMATION GENERATED FROM THE RESEARCH WAS PRESENTED
None.

H. RESEARCH RELEVANCE STATEMENT
Hopkins (2005) demonstrated the potential of avirulent *X. fastidiosa* as a tool to control 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 PD 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 isolate. Being able to retrospectively compare these isolates using high resolution tools and biological assays will allow us to determine when, and how, *X. fastidiosa* looses avirulence. We will also shed light on the mechanism how avirulent isolates suppress pathogenic ones. This proposal seeks to understand the evolution of avirulent *X. fastidiosa* 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 *X. fastidiosa*.

I. SUMMARY IN LAY TERMS OF THE SPECIFIC ACCOMPLISHMENTS OF THE RESEARCH PROJECT
We have different *X. fastidiosa* 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 4 out of 10 lineages we have created. We are comparing the evolved populations with the original and intermediate clones to determine if they have different phenotypes. The goal is to identify avirulent isolates with potential as biological control agents against pathogenic *X. fastidiosa*.

J. SUMMARY AND STATUS OF INTELLECTUAL PROPERTY PRODUCED DURING THIS RESEARCH PROJECT
No intellectual property produced.