PROJECT REPORT 3-1-2010

University of California Pierce's Disease Research Grant Program

I. PROJECT TITLE:
CONTINUED ASSESSMENT OF *XYLELLA FASTIDIOSA* FIMBRIAL ADHESINS AS IMPORTANT VIRULENCE FACTORS IN PIERCE'S DISEASE: INFLUENCE OF XYLEM SAP

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Period Covered for this report is January 2010 to March 2010 (funds were not transferred and available until mid-December 2009)

III. OBJECTIVES AND ACTIVITIES CONDUCTED TO ACCOMPLISH EACH OBJECTIVE
This project continues efforts toward understanding better the biological relationship between *Xylella fastidiosa* (*Xf*) cells and the xylem environment, and specifically the roles of fimbrial adhesins (type I and type IV pili, and associated proteins) in *Xf* virulence, motility, aggregation and autoaggregation, and biofilm development. The research targets the functional biology of *Xf* in xylem sap and along xylem vessel walls. It tests and explores traits of sap and xylem vessels from resistant and susceptible grapevines, as well as that of citrus, that inhibit or promote *Xf* cell activities associated with *pil* and *fim* gene products.

Specific objectives of the project are to:

- *Establish a baseline of Xf activity in vitro for grapevine sap and xylem tissue. This will include temporal and spatial activities for pili-associated functions—motility, cell aggregation, and biofilm formation.*
- *Assess pili-associated functions in grapevine sap and on xylem walls from Vitis vinifera cultivars and Vitis species expressing distinct PD resistance and susceptibly.*
- *Assess pil and fim gene expression for conditions in Objective 2 that exhibit significant differences in functional Xf activities.*
- *Compare pili-associated functions in grapevine vs. citrus sap.*
- *Assess pil and fim-related gene expression by microarray analyses to determine significant differences in functional Xf activities that occur between grapevine and citrus saps.*

Thus far, in the short time this project has been underway activities have concentrated on the first two objectives—i) establishment of baseline activities of *Xf in vitro* for grapevine sap and xylem tissue, and ii) evaluation of *Xf* in grapevine sap from *Vitis vinifera* cultivars and *Vitis species* expressing distinct PD resistance and susceptibly. This included development and fabrication of a new microfluidic chamber (Figure 1) with the ability to test and observe four experimental parameters concurrently, viz., different saps, different *Xf* strains/mutants, etc. In addition, observations have been made of *Xf* activities in saps from several grapevine species/cultivars, including those expressing resistance and susceptibility to Pierce's disease.

IV. Summary of Major Research Accomplishments and Results

Objective 1). *Establish a baseline of Xf activity in vitro for grapevine sap and xylem tissue. This will include temporal and spatial activities for pili-associated functions—motility, cell aggregation, and biofilm formation.*

As noted above, a new four-channeled chamber was developed and is currently being used to facilitate experimental observations of several parameters at the same time. The width and depth of the individual channels was designed so that in subsequent experiments thin sections of grapevine xylem wood can be placed in the channels for direct observation of *Xf* on xylem vessel walls—in xylem fluid.

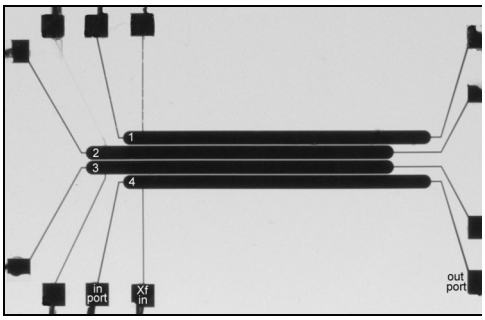

Fig. 1. Four-channeled microfluidic device with each channel having media in and out ports, and one access port for introduction of *Xf*. Channels filled with dye for photographic purposes.

Under continuous flow conditions over seven days in microfluidic chambers, sap from susceptible grapevine hosts supported growth and also promoted the development of thick biofilms. While PD2 medium also allowed biofilm development, these biofilms were poorly attached to the chamber surface, frequently detached, and were often passed from the chamber as we have noted in the past. Aspects of this objective are still underway.

Objective 2). *Assess pili-associated functions in grapevine sap and on xylem walls from Vitis vinifera cultivars and Vitis species expressing distinct PD resistance and susceptibly.*

A significant increase in *Xf* cell attachment was observed in wells (96-well plate assay) containing 100% grapevine sap, relative to PD2 medium which until recently has been the primary medium used by us and other researchers. Sap from both PD susceptible and resistant varieties induced surface attachment, with the only exception being *V. champinii* (PD resistant), which inhibited attachment when compared to PD2 and *V. vinifera*.

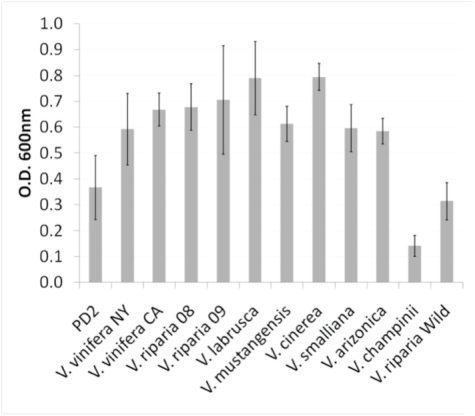

Fig. 2. *Xf* biofilm development in 96-well plate assay for PD2 medium and various grapevine saps.

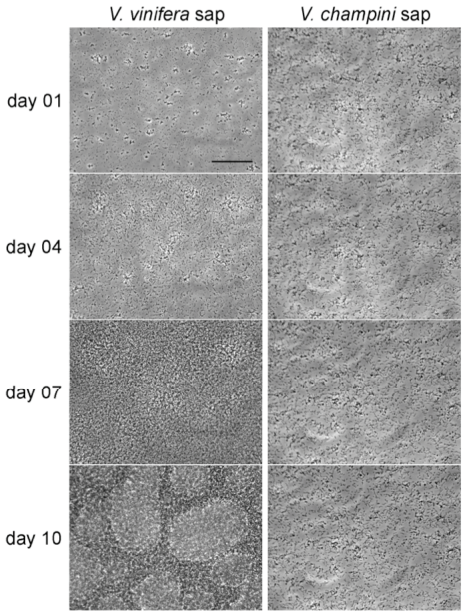

Fig. 3. *Xf* biofilm development in *Vitis vinifera* and *V. chanpini* saps observed over 10 days in microfluidic chambers.

Data obtained with microfluidic chambers provided a clear distinction between responses to sap from susceptible and resistant grapevine hosts, in which thick biofilms only formed in sap from susceptible hosts (Fig. 3, 4). Significantly reduced biofilm formation as well as *Xf* cell activity (e.g., twitching motility) was reflected in cell viability assays, which showed a possible bactericidal effect of sap from *V. smalliana* and *V. champinii*.

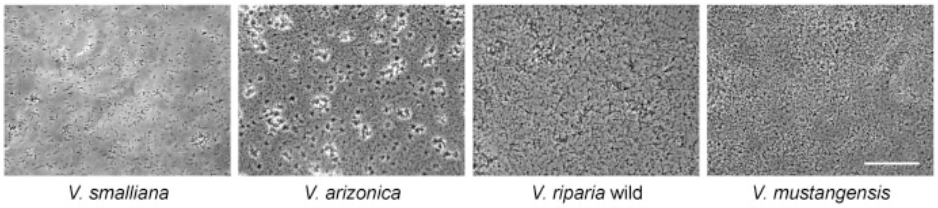

Fig. 4. *Xf* biofilm development in various *Vitis* species at 5 days growth in microfluidic chambers. No biofilm developed in sap from *V. smalliana* (in fact, these cells did not remain viable in this sap); star-shaped aggregates (normally, a precursor stage to a more complete biofilm) formed in *V. arizonica* sap; long cells (commonly over 20 μm in length) of wild *V. riparia* are indicative of vigorous growth; and a slower developing biofilm in *V. mustangensis* sap.

V. Publications and Reports

None for this period on this project

VI. Presentations on Research

None for this period on this project

VII. Research Relevance

Determining how the *Xf* bacterium is able to spread readily in the xylem environment and block the transpiration stream through the production of biofilms and bacterial cell masses will facilitate development of novel, effective control measures. In particular, the research examines more keenly the relationship of *Xf* cells in a significantly more natural environment, as best as can be done *in vitro*, using xylem sap, xylem vessel tissue, and nanofabricated fluidic chambers. A number of *Vitis* species have been identified over the years that exhibit resistance to Pierce's

disease, yet it is not know the mechanisms by which these plants confer resistance. In the short time we have been researching this project, we have observed that in sap from some of those known resistant species, *Xf* does not appear to grow well, and in some instances does not remain viable. It will be important that we follow up with these observations with a second year's sap collection as well as saps from similar grapevine species collected from different geographical locations.

VIII. Lay Summary of Current Year's Results

Biofilm formed by *Xylella* cells develop in grapevine xylem vessels and plug the sap transportation pathways. Small fluidic devices have been microfabricated that allow direct microscopic observation of *Xf* cell growth, motility, and colonization (biofilm development) in grapevine sap. Such observations cannot be made with intact grapevines. Thus far, we observed that sap from some grapevine species known to express some resistance to Pierce's disease, e.g., *V. smalliana* (also known as Blue Lake), *V. champinii* (also known as Ramsey or Salt Creek) has either killed the bacteria or has prevented them from developing complete biofilms.

IX. Status of Funds

Dr. Dusit Athinuwat the primary postdoc working on this project. Partial support is also for Dr. Xiangyang Shi (a postdoc). Transfer of funds for this project to Cornell OPS were not completed until 11/20/2009 and we did not start the project until January 2010; at present we have remaining approximately $41,000 uncommitted funds.

X. Summary and Status of Intellectual Property Produced During this Research Project

No intellectual property to date has resulted from research done under this grant.