

IDENTIFICATION OF TRAITS OF *XYLELLA FASTIDIOSA* CONFERRING VIRULENCE TO GRAPE AND INSECT TRANSMISSION BY ANALYSIS OF GLOBAL GENE EXPRESSION USING DNA MICROARRAYS

Project Leader:

Steven Lindow
Department of Plant and Microbial Biology
University of California
Berkeley, CA 94720

Cooperators:

Nian Wang and Bill Feil
Department of Plant and Microbial Biology
University of California
Berkeley, CA 94720

ABSTRACT

Xylella fastidiosa (*Xf*) regulates virulence factors important in both virulence to grape as well as colonization of sharpshooter vectors via its production of a fatty acid molecule (known as DSF) whose production is encoded by *rpfF*. The *rpfF* homologue of *Xf* strains that cause Pierce's disease (PD), synthesizes a fatty acid cell-cell signal (DSF) that is apparently similar to that produced by *Xanthomonas campestris* *pv. campestris*. *Xf rpfF* mutants exhibit increased virulence to plants; however, they are unable to be spread from plant to plant by their insect vectors. While we have identified a key regulator of virulence and insect transmission in *Xf* we lack an understanding of the traits that are regulated by this pathogen in response to the DSF signal molecule. We thus are initiating studies to determine the *rpfF*-regulation in *Xf*. The objectives of our study are: 1) determine those genes in *Xf* whose transcription is controlled by *rpfF*, the regulator of virulence and insect transmission, by assessing global gene expression using DNA microarrays, 2) determine the number and identity of genes in *Xf* that are expressed in grape plants but not in culture by assessing global gene expression using DNA microarrays, and 3) assess the contribution of individual genes of *Xf* whose transcription is dependent on *rpfF* to its virulence and insect transmissibility. We are exploiting a DNA microarray developed in another project that addresses host specificity genes in *Xf* to assess gene expression differences in isogenic *rpfF*⁺ and *rpfF*⁻ strains of *Xf* strain Temecula. The microarray contains 2,555 gene-specific 70 bp oligodeoxynucleotides including negative and positive controls. We have isolated RNA from *Xf* strains grown both in culture as well as isolated from plants. After differential labeling with the fluorescent cyanine dyes Cy3 and Cy5, cDNAs made from these RNAs have been hybridized to the microarray. Preliminary results reveal that at least 150 genes are up-regulated in response to *rpfF* in *Xf* while at least 40 genes are repressed. Clearly this regulator has a large effect on the physiological function of *Xf*. Microarray-based gene expression results are being verified using quantitative Reverse Transcriptase-PCR. Work is also underway to determine the subset of *Xf* genes that might be plant-inducible and the identity of those whose expression is dependent on DSF production.

EVALUATION OF GENETIC DIVERSITY WITHIN *XYLELLA FASTIDIOSA* STRAINS ACROSS TEXAS

Project Leaders:

Marlin Mathews, Borislava Tsanova, and Lisa Morano
Department of Natural Sciences
University of Houston - Downtown
Houston, TX 77002

ABSTRACT

Strains of *Xylella fastidiosa* have been isolated from infected grapevines and the vegetation surrounding vineyards. The *gyraseB* gene has been sequenced for approximately 20 strains and most of the strains fall into one of two categories, the grape group and the mulberry/ragweed group. Strains isolated from grape typically matched grape strains in the database and strains isolated from weeds and trees around vineyards closely matched the mulberry/ragweed sequences. However, one isolate from an infected grapevine was found to be a mulberry/ragweed strain suggesting that strains typically found in weeds can move into nearby grapevines. Due to the highly conserved nature of the *gyraseB* gene within strains we are also evaluating our cultures by PCR amplicon size for several small subunit repeats as suggested by Dr. Lin of USDA, ARS in California. This method creates a DNA fingerprint of each strain. Using this technique we are able to demonstrate that there are multiple mulberry/ragweed strains and multiple grape strains across Texas. We hope to combine these fingerprints with information about strain location to better understand the epidemiology of disease spread into newly infected vineyards. With fingerprint information on strains we also hope to create a phylogenetic tree of Texas strains to combine with similar data in other states allowing us to further understand the natural history and epidemiology of Pierce's disease.

THE INFLUENCE OF THE CELL SUSPENSION REDOX POTENTIAL ON THE CAPACITY OF *XYLELLA FASTIDIOSA* TO AGGREGATE

Project Leaders:

Breno Leite
CVSFR, Florida A & M University
Tallahassee, FL 32317

Peter C. Andersen
NFREC, IFAS, University of Florida
Quincy, FL 32351

ABSTRACT

The Calcium Bridging Hypothesis (CBH) implies that surface redox changes on cells of *Xylella fastidiosa* (*Xf*) may influence the capacity of these cells to aggregate. A series of experiments were designed to challenge the proposed CBH. In this hypothesis, thiols (SH) located at the outer membrane level or in adhesion related structures of *Xf* could increase or decrease the cells attraction to the xylem wall surface and/or other *Xf* cells. The focus of this investigation was to address the possibility to alter the surface status of SH groups by exposing cells to reduced and oxidized forms of the tripeptide glutathione (commonly found in xylem fluid). CBH also assumes that divalent ions would mediate the interaction between thiols and other negative charges. *Xf* aggregation was measured after the following treatments: deionized water (negative control), CaCl₂ 100 mg/L (positive control), reduced glutathione 10 mM (GSH), oxidized glutathione 10 mM (GSSG), GSH 10 μM for 20 min + CaCl₂ 50 mg/L and GSSG 10 mM for 20 min + 50 mg/L. Maximum aggregation was obtained with pre-treatment with GSH 10 mM for 20 min followed by exposure of cells to CaCl₂ 50 mg/L. Results indicate that a reducing environment is essential for cell aggregation. A reducing environment apparently modified the surface of *Xf* cells and predisposed them to interact with divalent ions.

XYLELLA FASTIDIOSA GROWTH ON CHARD2, 3G10R AND *Xf*-26 CHEMICALLY-DEFINED MEDIA

Project Leaders:

Breno Leite
CVSFR, Florida A&M University
Tallahassee, FL 32317

Lyriam Marques
MBEC Bioproducts, Inc.
Calgary, AB, Canada

Peter C. Andersen
NFREC, IFAS, University of Florida
Quincy, FL 32351

Merle Olson
MBEC Bioproducts, Inc.
Calgary, AB, Canada

ABSTRACT

Pierce's disease (PD) in grapevines is caused by the bacterium *Xylella fastidiosa* (*Xf*). *Xf* is injected into xylem vessels by leafhoppers. *Xf* can grow planktonic (free cells) or can form aggregates or biofilm (colonies). Growth and biofilm formation of UCLA and STL PD strains was compared in three chemically-defined media, *Xf*-26 (22 components), CHARD2 (10 components) and 3G10R (9 components). PW⁺, a rich non-defined medium, was used as a control. Both planktonic growth and biofilm formation were assessed during the incubation period. CHARD2, which has the amino acid cysteine as a component, was by far the best medium inducing biofilm formation. CHARD2 and *Xf*-26 differed in planktonic growth; CHARD2 exhibited no detectable planktonic growth, whereas *Xf*-26 cultures were predominantly planktonic. 3G10-R performance was below the expectations, since this medium has performed satisfactorily before as an aggregation inducer. 3G10-R has reduced glutathione (reducing agent), however it contains glucose, which is not present in CHARD2. We hypothesize that the redox environment, in each medium, induced the differences in biofilm architecture verified.