

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

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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

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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.