

DNA MICROARRAY AND MUTATIONAL ANALYSIS TO IDENTIFY VIRULENCE GENES IN *XYLELLA FASTIDIOSA*

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Reporting Period: The results reported here are from work conducted from July 1, 2003 to September 30, 2003.

ABSTRACT

The identification of genetic factors that enable *Xylella fastidiosa* to express Pierce's disease symptoms is essential to the further development of several disease control strategies. One such control strategy is to use non-pathogenic derivatives of the Pierce's disease pathogen itself to competitively exclude pathogenic strains in grapevines. We have made progress in the development of genetic tools for this purpose and in identifying several putative virulence genes through comparative genome and mutational analyses. However, with over 50% of the *X. fastidiosa* genome consisting of genes with no known function, a more comprehensive approach is needed to identify genes that are important for virulence of the Pierce's disease strain of *X. fastidiosa*. This new project is using a DNA microarray approach for this purpose.

INTRODUCTION

The DNA microarray approach to identifying bacterial virulence genes follows the hypothesis that many virulence genes are differentially expressed (up-regulated or down-regulated) during infection of the host. This hypothesis has been proven over and over for numerous virulence genes in both plant and animal pathogens (Handfield and Levesque, 1999). With the full genome sequence now available (Bhattacharyya, A., et al. 2002; Simpson et al., 2000; Van Sluys et al., 2003), a complete DNA microarray of the genome of the Pierce's disease strain of *Xylella fastidiosa* is feasible to produce. The major advantage of this approach is that, unlike conventional reporter gene or hybridization strategies, microarrays can simultaneously produce relative expression data for thousands of genes in the target organism, and no prior knowledge of the genes and their function or regulation is required. Microarrays therefore represent an appealing approach to identifying bacterial gene sets that are up-regulated or down-regulated by growth in specific environments, and are attractive for studying gene expression by pathogenic bacteria in their hosts because of the large number of genes involved.

We previously conducted a preliminary study with a DNA macroarray of about 100 genes from the *Xyella fastidiosa* genome arrayed on a filter (Hernandez-Martinez et al., 2002). This study showed strong evidence of differential expression of several genes that were identified as possible virulence factors from the original genome sequence annotation. Using techniques similar to our work on the functional genomics of another plant pathogen, *Erwinia chrysanthemi* (Okinaka et al., 2002; Yang et al., 2003), we have just begun conducting a comprehensive DNA microarray/mutational approach to identify genes that are important for virulence of the Pierce's disease strain of *Xylella fastidiosa*.

OBJECTIVES

1. Conduct DNA microarray analysis of gene expression patterns in *Xylella fastidiosa* during infection of plants vs. growth in other conditions.
2. Mutate putative virulence genes and characterize virulence defects.

RESULTS AND CONCLUSIONS

Results

Research during the first three months of this project have been directed toward the development of effective RNA extraction methods for infected grapevines and in designing a full-genome DNA microarray.

Conclusions

This work will contribute significantly to fundamental information on the genetics and pathogenicity of *Xylella fastidiosa* and will benefit researchers pursuing various strategies of management of Pierce's disease. As mentioned above, this information is essential for our continuation of the strategy to use non-pathogenic strains for biological control of Pierce's disease.

Identification of virulence genes can also lead to recognition of new unforeseen targets for management strategies. In addition, the construction of a DNA microarray for this pathogen, and identification of genes differentially expressed during infection, will complement work by others on differential expression of grapevine genes during infection. This will open the door to "interactive genomic" studies that will enhance our understanding of the bacterial-plant interaction that leads to Pierce's disease, and in the future, studies of interactions with its insect vectors.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, and the University of California Agricultural Experiment Station.