DEVELOPING A MICROARRAY-PCR-BASED IDENTIFICATION AND DETECTION SYSTEM FOR *XYLELLA FASTIDIOSA* STRAINS IMPORTANT TO CALIFORNIA

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ABSTRACT
This project is to develop and evaluate a microarray-PCR-based system for accurate and quick identification of *Xylella fastidiosa* strains from *in vitro* culture. Attempts will also be made to use this system to detect *X. fastidiosa* strains from their hosts. A particular emphasis is on strains of grape Pierce's disease, almond leaf scorch disease and oleander leaf scorch disease, which are currently important in California.

INTRODUCTION
Traditionally, *Xylella fastidiosa* is identified by cultivation on complex media, serological tests and pathogenicity tests. From the genetic standpoint, these traditional methodologies target the expressions and regulations of multiple genes characteristic to the pathogen. However, there are many drawbacks associated with the traditional multi-locus test methodology. The nutritional fastidiousness of *X. fastidiosa* poses the major challenge for the use of media-based identification scheme. A direct implication from this challenge is the difficulty in establish a taxonomy system under the species level, leading to, in many cases, the uncertainty of a newly isolated *X. fastidiosa* strain. This further affects the control of *Xylella* diseases. Serological tests have an advantage of being simple and fast. They, however, suffer from cross antibody-antigen reactions from different pathotypes. Pathgenicity tests are of high economical value, but such tests are laborious and time-consuming. Current pathogen regulation policy also hinders the large scale usage of this method.

Theoretically, PCR allows the amplification of characteristic gene or DNA sequences from a single DNA molecule. For this reason, PCR has recently been the most common technique for *X. fastidiosa* identification. There are, however, technical problems limiting the application of PCR. Specific primers may fail to amplify DNA from a particular isolate if there is a spontaneous mutation(s) in the primer-binding site, leading to a false negative result. Non-specific amplicons may occur which complicate the interpretation of the electroporesis data and may result in a false positive conclusion. The sensitivity and specificity of PCR amplification tend to be inversely related.

A combination of PCR with DNA-DNA hybridization can improve the detection specificity and maintain high sensitivity. While traditional DNA-DNA hybridization assays are laborious and time-consuming, microarray technology provides a solution. DNA microarrays have been widely used for gene expression studies. This technology is also a powerful tool in pathogen identification and detection. Unlike other hybridization tools, such as microplates or dot blots for DNA-DNA hybridization with membrane-bound probes, miniature glass microarrays are capable of containing DNA probes specific to thousands of individual target DNAs. Potentially, microarray technology allows the rapid determination of thousands of genetic characters of a microorganism in one hybridization experiment. This mimics the multi-genic methodology, but microarray is superior in that the genes or DNA sequences in test can be manipulated, testing time is much shorter, and test accuracy and efficiency are much greater.

OBJECTIVES
The primary objective of this project is to develop and evaluate a microarray-PCR-based system for accurate and quick identification of *Xylella fastidiosa* strains from *in vitro* culture. Attempts will also be made to use this system to detect *X. fastidiosa* strains from their hosts. A particular emphasis is on strains of grape Pierce's disease, almond leaf scorch disease and oleander leaf scorch disease, which are currently important in California. Two specific objectives are: 1) using the complete and annotated genome sequence of *X. fastidiosa* Temecula strain as a guide, select appropriate DNA sequences and evaluate their potential for pathotype / genotype identification. Design and construct a DNA microarray with PCR amplicons; and 2) evaluate the effectiveness of the constructed microarray through hybridization experiment. Using the microarray as a reference, analyze genomic variation of different pathotypes with multiple strains collected from broad

geographical areas and hosts. Attempt will be made to develop a genovar system to substitute the pathotype or pathovar system which is by far un-standardized and difficult to test.

RESULTS AND CONCLUSIONS

We believe that the proposed project has a high probability of success. There have been two reports regarding the use of microarray analysis focused on the transcriptome of CVC strain of *X. fastidiosa* (Costa de Oliveira et al., 2002; Nune et al., 2003). This proposed project will explore the application perspective of microarray in *X. fastidiosa* identification. Objective 1 of this proposal addresses the utilization of genome DNA sequence information. Complete genome sequences of four *X. fastidiosa* strains are publicly available. Unitization of the bioinformation implied by these sequences is the goal of genome sequencing and we have just begun this effort. Objective 2 links the *X. fastidiosa* genome research from laboratory to the field. A large number of field isolates will be incorporated into genome comparisons, leading to a better and faster characterization of this nutritionally fastidious prokaryote. Efforts to extensively collect strains of *X. fastidiosa* are currently underway.

REFERENCES

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