BIOLOGICAL CONTROL OF PIERCE'S DISEASE WITH NON-PATHOGENIC STRAINS OF *XYLELLA FASTIDIOSA*

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INTRODUCTION

Competitive exclusion of plant pathogenic bacteria with nonpathogenic strains has been demonstrated in other systems where pathogens were excluded from either plant surfaces or vascular tissues. The ideal situation is where the nonpathogenic bacteria are stable derivatives of the pathogen itself, so that nutritional and other growth requirements are identical and thus facilitate successful competition for colonization. Our project is to construct such mutants from the Pierce's disease (PD) pathogen, *Xylella fastidiosa*, through a systematic process of identifying which virulence genes are important for disease expression but not essential for colonization of plants in a nonpathogenic state. We are utilizing knowledge from comparative genomic sequence analysis with mutational studies to identify important virulence genes. This year, we have begun to employ an additional strategy (DNA macro/microarrays) based on analysis of differential gene expression between the bacterium grown in culture vs. during infection of plants.

OBJECTIVES

1. Construct deletion mutations in putative virulence genes of *Xylella fastidiosa*.
2. Test mutant strains for virulence in grapevines.
3. Test mutant strains for biological control of pathogenic strains in grapevines.

RESULTS AND CONCLUSIONS

Macro/microarray analysis of the expression profile of select candidate pathogenicity genes in Xf:

A procedure for macroarray analysis of about 100 genes selected as possible virulence genes based on comparative sequence analysis was developed, and this was reported at the Annual Meeting of the American Phytopathological Society in July 2002 (Hernandez-Martinez et al., 2002). To identify genes involved in pathogenicity in the PD strain, the sequence of the CVC strain was used to select open reading frames specifying putative pathogenicity and virulence factors. DNA fragments of these genes were obtained by PCR amplification from the genome of the PD strain I03. In this preliminary study, we constructed macroarrays for the analysis of the expression profile of select candidate pathogenicity genes of *Xf* and to study their expression in PD3 medium. We have shown that these genes are expressed to varying degrees ranging from none to very high. These arrays are being used to analyze the gene expression profile of different *Xylella* strains *in planta* and *in vitro*. This work is following the hypothesis that genes important in virulence and symptom expression are up-regulated in the plant. This will help us to refine the potential target genes for construction of non-pathogenic derivatives for biological control.

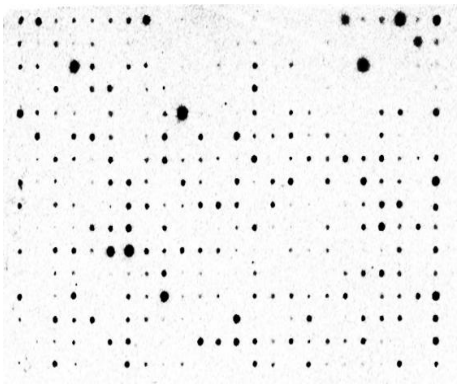

Figure 1. Autoradiograph of nylon filter macroarray probed with label cDNA from *Xylella fastidiosa*.

Mutational analysis of virulence genes:

Construction of several virulence gene mutants of *X. fastidiosa* has been done using the EZTN transposon or by insertional cloning of antibiotic resistance cassettes to create disruptive insertions into cloned genes that were amplified by PCR based on genomic sequence. The mutated clones have been subcloned into pUC129 for gene knockout experiments. Among

virulence genes included in mutational studies are those of the *gum* operon, for which we have recently constructed successful knockout mutations in *Xylella*. Other genes we are manipulating include a number of regulatory genes that likely control other virulence factors, such as RsmA.

We are also working to develop a more efficient transposon delivery system for *Xylella* especially for the analysis of the genes of unknown functions. Because of the high price of the EZTN system, we have cloned the 16S rRNA promoter of *Xf* to drive the transposase gene in a self-cloning modular transposon, pTnModOKm (Dennis and Zylstra, 1998). We expect this to significantly increase the transposition efficiency, since the low level of transposase expression has often been given as the reason for the low efficiency. If this improves the efficiency of the transposon, we will clone a promoter-less reporter within the transposon to be used as a measure of gene expression level of the genes to be inactivated.

Tissue culture of grape to develop in vitro inoculation system for Xylella pathogenesis:
We are working with grape tissue cultures, as well as other potential host plants that would show rapid symptoms, such as mustard, to develop rapid assays for analysis of virulence. The tissue culture system should also be useful in our macro/microarray work, where gene expression profiles of the bacterium with and without contact with grape cell cultures can be accomplished in a more controlled and sterile environment than whole plants.

REFERENCES

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