

THE CONTRIBUTION OF THE PECTIN-DEGRADING ENZYME POLYGALACTURONASE (PG) IN TRANSMISSION OF *XYLELLA FASTIDIOSA* TO GRAPE AND THE USE OF PG-INHIBITING PROTEINS FOR TRANSGENIC RESISTANCE TO PIERCE'S DISEASE

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ABSTRACT

Work this year has shown that the *Xylella fastidiosa* (*Xf*) polygalacturonase (PG) is an endo-acting enzyme. This provides us a reference that we can now use to biochemically characterize the interaction between the pathogen's PG and PG inhibiting proteins (IPs) that is implied by the fact that transgenic expression of PGIPs protects vines against PD. We also report that PG, in combination with a bacterial β -1,4-glucanase enzyme (BGase) that has also been discussed in previous reports can act to cause tears in the pit membranes that are likely to be barriers slowing the systemic spread of the *Xf* population. We also report on a few set-backs that have slowed our examination of the roles of insect plant cell wall-degrading enzymes in PD development. Fortunately, the set-backs are now being corrected so that important questions can be addressed in work supported by a no-cost extension.

INTRODUCTION

In a companion report we have discussed continuing work that has been done to test the model we have proposed to describe the development of Pierce's disease (PD) in grapevines. That model proposes a key role for a pectin-degrading enzyme, polygalacturonase (PG). Over the past few years we have demonstrated that *Xylella fastidiosa* (*Xf*) has a PG encoding gene and that the *Xf* PG is important for disease development. This project is a spin-off from our "model" project, based on the observation that, when expressed in transgenic grapevines, plant PG-inhibiting proteins (PGIPs) provide some protection against PD.

OBJECTIVES

1. Determine whether the pectin-degrading enzyme of *Xf* contributes to the systemic spread of the bacterial population in inoculated grapevines (first priority).
2. Determine whether the pectin-degrading enzyme(s) in the salivary secretions of the glassy-winged sharpshooter (GWSS) contributes to inoculation success of *Xf* into grapevines (second priority).

RESULTS

Objective 1

Determine whether the pectin-degrading enzyme of *Xf* contributes to the systemic spread of the bacterial population in inoculated grapevines

This question is related to a major component of our model for PD development and prior results in the project focused on testing that model led to this project. We had previously shown that the *Xf* PG gene coded for some sort of PG activity. This conclusion was based on isolation of the protein from *E. coli* transformed to express the putative PG gene. This year's work involved the development of a PG assay based on identification of the products of enzyme action. If the products included a series of oligosaccharides, rather than only monosaccharide galacturonic acid, the PG would be an *endo*-acting PG (Figure 1).

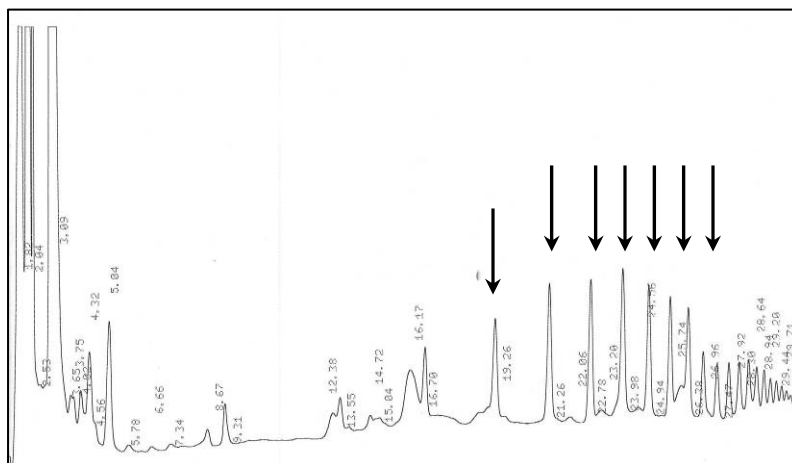


Figure 1. Protein was isolated from *E. coli* transformed to express the *Xf* DNA sequence thought to encode PG. When the protein was incubated with the PG substrate polygalacturonic acid, several oligosaccharide digestion products (arrows) were produced. Shown is an HPLC analysis of these products; oligomer size of peaks increases as they emerge later from the column (i.e., further to the right). There were no digestion products present when HPLC was done with the substrate incubated with no protein or with protein from untransformed *E. coli*. (Data from Roper, 2006; Roper et al., 2006)

When PGs are incubated with polygalacturonic acid substrate in the presence of PGIP a complex forms between the PGIP and the PG. This slows the rate of substrate digestion by PG and shifts the oligomeric digestion products to larger sizes; i.e., further to the right in the chromatogram. The next step is to isolate more PG protein from the transformed *E. coli* and test the ability of the pear PGIP that has been expressed in grapevines (Aguero et al., 2005) and PGIPs from several other plant species to see if they inhibit *Xf* PG. These tests may identify PGIPs that are more active than the pear PGIP in blocking the pathogen PG's action.

In work reported last year (Labavitch et al., 2005) we indicated that the combined action of two *Xf* cell wall-degrading enzymes, PG and BGase was sufficient to increase the porosity of grapevine pit membranes, allowing the passage of 20 nm gold particles through explanted stem segments. Our colleagues Tom Rost and Mark Matthews and their postdoctoral researcher, Qiang Sun have been carrying out studies involving electron microscope examinations of the grapevine xylem system and have obtained detailed images of pit membranes. We flushed solutions containing fungal PG and the *Xf* BGase into the xylem system of grapevines and then looked at the impact of the enzyme treatment on pit membrane integrity (Figure 2).

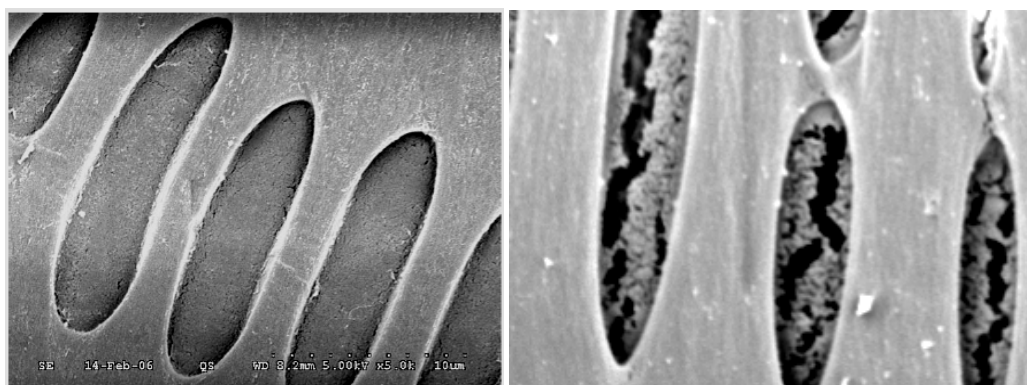


Figure 2. In the left-hand image pit membranes are seen as somewhat grainy-looking, oval surfaces framed by the lignified secondary wall of a xylem water conduits. The right-hand image is a similar view of pit membranes after the vessels have been flushed with PG and BGase. The magnification is approximately 5,000X for the two images.

It is clear that the combined actions of the two enzymes has caused a substantial erosion of the pit membranes. Although the images have been cropped to make this side-by-side comparison, all of the pit membranes in the image of enzyme-treated stem tissue were extensively damaged.

Objective 2. Determine whether the pectin-degrading enzyme(s) in the salivary secretions of GWSS contributes to inoculation success of *Xf* into grapevines.

In the past project year we were not able to obtain a sufficient supply of GWSS for use in isolating salivary glands and their enzyme complements. Thus, little progress was possible. However, it appears that a more reliable insect supply may be developing and we will be continuing the insect side of the project in the 2006-2007 project year. We requested a no-cost extension to support that work beyond the June 30, 2006 end date of this project.

CONCLUSIONS

PG is an important *Xf* virulence factor supporting the bacterium's colonization of grapevine xylem and the development of PD. Work in the past year will be useful in determining the specific nature of the protection against PD symptom development that has been reported to result from expressing PGIPs in grapevines. The assay used to demonstrate the endo-nature of *Xf*'s PG will be of value when testing the relative action of several PGIPs against *Xf* PG. Once a reliable GWSS supply is available, similar tests of the GWSS PG can be made and studies can be carried out to determine whether PGIP-expressing transgenic grapevines are useful in suppressing disease development resulting from *Xf* introduced during GWSS feeding.

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MICROARRAY GENE EXPRESSION ANALYSIS OF GRAPE PLANTS IN RESPONSE TO *XYLELLA FASTIDIOSA* INFECTION

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ABSTRACT

Transcriptional profiling using a custom high-density microarray chip of 20,020 *Vitis* transcripts showed significant variations in responses between the susceptible and resistant genotypes to *Xylella fastidiosa* (*Xf*) infection. Differentially expressed transcripts reflecting spatial and temporal responses to Pierce's disease (PD) involved in metabolic processes such as diseases resistance, water stress, photosynthesis and cell wall synthesis were identified. The results suggest that *Vitis* responses to *Xf* are genotype and tissue dependent, and are stage specific. VitisExpDB is an online MySQL-php driven relational database that houses annotated EST data. The database will provide genomic resource to grape community for functional analysis for both vinifera and non-vinifera grape varieties and aid in the grape genome annotation.

INTRODUCTION

The impact of Pierce's disease (PD) on the California grape industry has been significant since the introduction and establishment of a more effective vector, *Homalodisca vitripennis*, the glassy-winged sharpshooter (GWSS) (Almeida and Purcell 2003). Development of resistance in grape is stymied by the relatively limited amount of genetic and molecular information regarding genotype specific resistance to PD infection (Davis et al. 1978). From genotypic screening and genetic mapping studies, it was concluded that a dominant allele controls PD resistance and recently, Krivanek et al. (2006) identified a major quantitative trait locus that controls PD resistance and denoted it as 'Pierce's disease resistance 1' (*PdR1*). The above studies confirm that the genetic basis of PD resistance in grapes varies from tolerance to resistance and suggest that host responses to the pathogen are genotype dependent. Further, in the PD resistant genotypes, differential responses between stem and leaf tissues were also noted (Krivanek and Walker, 2005). The results from these studies prompted study of molecular basis of this host / pathogen interaction.

Plants respond to pathogen attack through a variety of signaling pathways consisting of a large number of regulatory as well as effector genes. Microarrays facilitate automated analysis of transcriptional profiling data to enable complete understanding of such gene function and interactions. The goal of this study was to identify and characterize the molecular events in the grape / *Xylella fastidiosa* (*Xf*) interaction using genome wide transcriptome profiling between resistant and susceptible genotypes and among the different tissue types.

OBJECTIVES

1. Microarray gene expression analysis.
2. Develop of a grape transcriptional relational database.

RESULTS

Objective 1 - Microarray gene expression analysis.

Custom microarray chip design: Previously, we have characterized transcriptomes (5,794 ESTs) from 12 tissue specific (stem, leaf and shoot) subtractive suppression hybridization (SSH) libraries. All the sequenced ESTs that are at least 100 bp in length (5421 ESTs) were submitted to the NCBI's ESTdb under the accession numbers DN942225 to DN947645. These ESTs and all the other EST sequences publicly available till July of 2005 were analyzed to deduce a non-redundant set of 20,020 ESTs with 1,947 from the SSH libraries, including 40 from the cDNA-AFLP experiments, 10,014 from *V. vinifera*, 5,470 from *V. shuttleworthii*, 1,219 from *V. aestivalis*, 780 from *V. rupestris* x *V. sp* and 588 from *V. riparia*. Nine individual 60-mer probes were designed for each EST. A total of 191,450 probes were selected for the entire set and there were two spots for each probe on the slide totaling 382,900 spots per slide.