INHIBITION *OF XYLELLA FASTIDIOSA* POLYGALACTURONASE TO PRODUCE PIERCE'S DISEASE RESISTANT GRAPEVINES

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ABSTRACT
Polygalacturonases (PG) (EC 3.2.1.15), catalyze the random hydrolysis of 1, 4-alpha-D-galactosiduronic linkages in pectate and other galacturonans. *Xylella fastidiosa* (*Xf*) possesses a single PG gene, *pglA* (PD1485) and *Xf* mutants deficient in the production of PG result in lost pathogenicity and a compromised ability to systemically infect grapevines. We have cloned the *pglA* gene into a number of protein expression vectors and a small amount of active recombinant PG has been recovered, unfortunately most of the protein expressed is found in inclusion bodies in an inactive form. The goal of this project is to use phage panning to identify peptides that can bind to and inhibit *Xf* PG. Once peptides are discovered that can inhibit PG activity in vitro these peptides will be expressed in grapevine root stock to determine if the peptides can provide protection to the plant from Pierce's disease.

INTRODUCTION
Polygalacturonases (PG) have been shown to be virulence factors of a number of plant pathogenic bacteria including *Ralstonia solanacearum*, *Xanthomonas campestris*, and *Erwinia carotova* (Huang and Allen 2000; Dow et al. 1989; Lei et al. 1985). *Xylella fastidiosa (Xf)* possesses a single PG gene *pglA* (PD1485), and mutation of this gene results in lost pathogenicity and reduced ability to systemically infect grapevines (Roper et al. 2007). In order for *Xf* to systemically infect a grapevine it must break down the pit membranes that separate individual xylem elements. Pectic polymers determine the porosity of the pit membrane (Baron-Epel, et al. 1988; Buchanan et al. 2000) and *Xf* PG allows the bacterium to breakdown the pectin in these membranes. The premise of this research is to identify a peptide that can be expressed in the xylem of a grapevine that can suppress *Xf* PG activity thus limiting the ability of *Xf* to spread systemically through grapevines and cause Pierce's disease (PD).

To accomplish this we will use phage display of a random dodecapeptide library attached to the coat protein gp38 of M13 phage in a phage panning experiment using active recombinant *Xf* PG as the target. After three rounds of panning, phage that show a high binding affinity for *Xf* PG will be screened for their ability to inactivate PG activity in vitro in reducing sugar assays. Once a suitable inhibitory peptide is discovered it will be cloned into an *Agrobacterium* binary vector and used to transform tobacco and grapevines by the UCD Plant Transformation Facility. These transgenic plants will then be inoculated with *Xf* and compared to non transgenic plants in PD symptom progression. If significant disease inhibition is shown we will use these transgenic grapevines as rootstock and see if they can also provide resistance to grafted scions.

OBJECTIVES
1. Isolate a sufficient amount of biologically active *Xf* polygalacturonase enzyme to conduct phage panning and PG-inhibition assays.
2. Isolate M13 phage that possess high binding affinities to *Xf* PG from a M13 random peptide library.
3. Determine if selected M13 phage and the gp38 M13 protein which mediates phage binding to *Xf* PG can inactivate PG activity in vitro.
4. Clone anti-*Xf* PG gp38 protein into an *Agrobacterium* binary vector and provide this construct to the UCD Plant Transformation facility to produce transgenic SR1 tobacco and Thompson Seedless grapevine.
5. Determine if anti-*Xf* PG gp38 protein is present in xylem sap of transgenic plants.
6. Mechanically inoculate transgenic plants with *Xf* and compare PD development with inoculated, non-transgenic control plants.

RESULTS
Objective 1. Currently we have obtained a small amount of active recombinant *Xf* PG, however, most of the expressed protein is in the form of insoluble and inactive inclusion bodies. Fortunately it is possible to measure the activity of the small amount of active PG that we have obtained by using visible spectrum reducing sugar assays such as the dinitrosalicylic acid (Figure 1) (Wang et al. 1997, Sumner 1921) and 3-Methyl-2-benzothiazolinonehydrazone methods (Anthon and Barrett 2002, Honda et al. 1981). This is a significant improvement over the tedious High-performance liquid chromatography (HPLC) assays that were previously used to demonstrate *Xf* PG activity (Roper et al. 2007). These methods should also be appropriate for the PG-inhibition assays once we have determined a suitable candidate peptide, however, we have yet to

produce enough active PG to use as a target for panning experiments. We are currently exploring different expression systems and refolding conditions in order to obtain enough active protein for the panning experiments.

Objectives 1-6. It will be necessary to obtain a significant amount of active *Xf* PG in order to carry out the panning experiments before the rest of the objectives can be completed. Fortunately the panning procedure will benefit from the experiences of Prof. George Bruening and Project Scientist Paul Feldstein who have used the phage display system extensively in their own research.

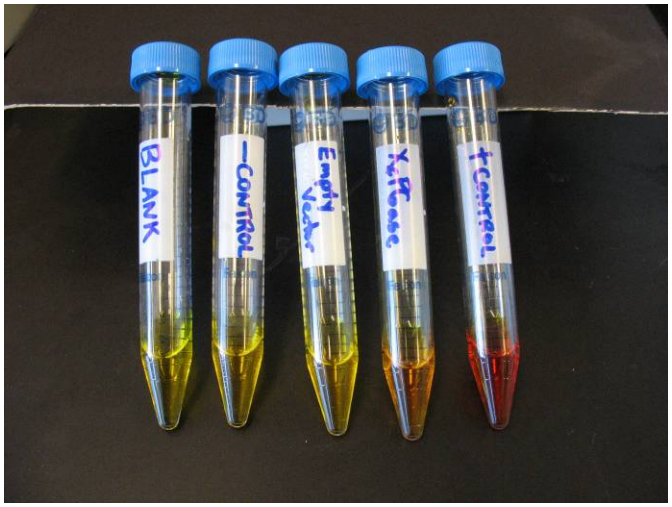

Figure 1. Dinitrosalicylic acid reducing sugar assay showing activity of *Xylella fastidiosa* Polygalacturonase (PG) versus positive control (commercial PG from *Aspergillus niger*) and empty vector negative controls.

CONCLUSIONS

We have made good progress thus far in finding suitable PG activity assays to use in the PG-inhibition assays. We are currently exploring different expression systems and continue to test different refolding protocols with the hopes of generating enough active PG to begin the phage panning experiments. Once a peptide is found that inhibits PG activity in vitro we can then transform grapevines with the peptide and determine if they provide plants with resistance to PD.

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