BIOLOGICAL, CULTURAL, GENETIC, AND CHEMICAL CONTROL OF PIERCE'S DISEASE: PRODUCTION AND SCREENING OF *XYLELLA FASTIDIOSA* TRANSPOSON PATHOGENICITY AND ATTACHMENT MUTANTS

Project Leaders:
Bruce Kirkpatrick and Magalie Guilhabert
Department of Plant Pathology
University of California
Davis, CA 95616

Cooperators:

A.H. Purcell
Division of Insect Biology
University of California
Berkeley, CA

Andy Walker
Department of Viticulture and Enology
University of California
Davis, CA

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INTRODUCTION

One of our projects involved the development of a transformation and transposon mutagenesis systems for the bacterium that causes Pierce's disease (PD), *Xylella fastidiosa* (*Xf*). We now have a random transposon mutagenesis system working for *Xf* (Guilhabert, et al. 2001) and recently we have developed an *E. coli/Xf* plasmid shuttle vector. We are currently assessing the stability of the *E.coli/Xf* plasmid shuttle vector in *Xf* without antibiotic selection. The results of these experiments will let us know whether this plasmid will be stably maintained in *Xf* cells that are inoculated in plants, something that will be essential for evaluating genes *in planta*.

Understanding the complex interactions between the plant, pathogen, and insect vector is imperative for the development of effective disease controls. Recently, the complete genome sequence of a citrus strain of *Xf* was determined (Simpson et al., 2000) and the complete sequence of a grape-infecting *Xf* strain (Temecula) is nearly complete. Earlier analysis of the CVC genome revealed important information on potential plant pathogenicity and insect transmission genes. However, more than half (53%) of the identified ORFs in *Xf* CVC encode proteins with no assignable function. In addition, some of the putative gene functions assigned on the basis of sequence homology with other prokaryotes may be incorrect. In order to identify and understand the function of *Xf* genes, it is imperative to develop techniques to knock out and complement putative pathogenicity or transmission genes.

OBJECTIVES

1. Development of transformation and marker-exchange systems for *Xylella fastidiosa.*
2. Screen *Xylella fastidiosa* Tn5 mutants for their ability to move and cause Pierce's disease in chardonnay grapevines

RESULTS AND CONCLUSIONS

Development of a transformation system for Xf with plasmid DNA:

The Tn903 kan-2 cassette, which we know is expressed in *Xf* (Guilhabert et al. 2001), was cloned in four broad host range plasmids, pUFR027, pLAFR3, RSF1010 and pMMB622 a derivative of RSF1010, forming the plasmids pXF002, pXF003, pXF004 and pXF005, respectively. Plasmids pXF002 and pXF003 failed to transform the Temecula strain of *Xf*. However, electroporation of *Xf* cells with 500 ng of pXF004 and pXF005 plasmid DNA produced an average of 131 and 208 *Xf* Kan^R clones respectively when selected on PD3 plates supplemented with 5 μg/mL of kanamycin. Plasmids pXF004 and pXF005 were found to be present as autonomous, structurally unchanged DNA molecules when propagated in *Xf*. However, neither pXF004 nor pXF005 were stably maintained in *Xf* after 5 passages without antibiotic selection. We are currently in the process of reproducing the same experiment with one, two and three passages without antibiotic pressure. When plasmid DNAs were isolated from *Xf* or plasmid DNAs isolated from *E. coli* were supplemented with a TypeOne™ Inhibitor, TRI, the frequency of transformation was increased by 13 or 5 fold, respectively. Plasmid pXF005 was also used to transform an additional grapevine strain of *Xf*.

Development of a marker exchange system for Xf:

The gene *rpf*F is required, together with the *rpf*B gene, for the production of a diffusible molecule, termed DSF, that may represent a novel cell density-dependent signaling factor in *Xanthomonas campestris* pv. *campestris* (Barber et al. 1997). The *rpf*F gene was PCR amplified from the Temecula strain, cloned and disrupted with the Tn903 Kan-2 cassette. Replacement of the wild-type gene in the genome of the Temecula strain by the disrupted *rpf*F gene was accomplished by a double crossing over event. The disrupted *rpf*F *Xf* mutant was inoculated into Chardonnay grapevines using a pinprick method (Hill and Purcell 1995; Purcell and Saunders 1999). The parental Temecula wild type strain served as a positive

control, and a water inoculation served as a negative control. The vines were observed for symptom development 16 weeks after inoculation. No difference was noted in the symptom development of vines inoculated with the *rpf*F *Xf* mutant compared to the plants inoculated with the parental Temecula wild strain. The presence of the *rpf*F *Xf* mutant in the symptomatic tissues was confirmed by immunocapture PCR (Smart et al. 1997) using the oligonucleotide primers used to amplify the *rpf*F gene. The population, systemic colonization, as well as the insect transmissibility of the *rpf*F *Xf* mutant, is currently being determined.

Testing of Tn5 mutant strain:

The bacterial cultures were inoculated into PD3 medium and adjusted to a concentration of 10^8 cells/mL. Approximately 1,000 random Tn5 mutants were inoculated each into two canes of Chardonnay grapevines using a standard pinprick method using 20 ul of the adjusted bacterial culture (Hill and Purcell 1995; Purcell and Saunders 1999). The parental wild type strain served as a positive control, and a buffer inoculation served as a negative control. The vines were observed for symptoms development for 16 weeks. After 16 weeks, each inoculated grapevine was sampled (0.5 g of cane tissue) 10 inches above the point of inoculation. *Xf-specific* IgG was purified from *Xf* antiserum and conjugated with peroxidase using procedures developed in the Walker lab. The same ELISA procedure used by the Walker laboratory has been used to analyze each inoculated grapevine. The tissue was ground in ELISA buffer and approximately 300 inoculated grapevine samples have been sampled and frozen to date. Any mutants that seems to show altered virulence, multiplication, survival or movement will be retested in a similar manner on 6 canes growing on 3 separate grapevines.

REFERENCES

Barber, C.E., J.L. Tang, J.X. Feng, M.Q. Pan, T.J.G. Wilson, H. Slater, J.M. Dow, P. Williams and M.J. Daniels. 1997. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. Mol. Microbiol. 24: 555-566.

Guilhabert, M.R., L.M. Hoffman, D.A. Mills and B.C. Kirkpatrick. 2001 .Transposon mutagenesis of *Xylella fastidiosa* by electroporation of Tn5 synaptic complexes. Mol. Plant-Microbe Interact. 14:701-706.

Hill, B.L. and A.H. Purcell. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathology 85: 1368-1372.

Purcell, A.H. and S.R. Saunders 1 999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. Plant Disease 83: 825-830.

Simpson, A. J. G. et al. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. Nature 406: 151-161.

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