BIOLOGICAL, CULTURAL, GENETIC, AND CHEMICAL CONTROL OF PIERCE’S DISEASE: PRODUCTION AND SCREENING OF XYLELLA FASTIDIOSA TRANSPONSON PATHOGENICITY AND ATTACHMENT MUTANTS

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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 KanR 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, 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 rpfF is required, together with the rpfB 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 rpfF 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 rpfF gene was accomplished by a double crossing over event. The disrupted rpfF 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 rpfF Xf mutant compared to the plants inoculated with the parental Temecula wild strain. The presence of the rpfF Xf mutant in the symptomatic tissues was confirmed by immunocapture PCR (Smart et al. 1997) using the oligonucleotide primers used to amplify the rpfF gene. The population, systemic colonization, as well as the insect transmissibility of the rpfF 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 10⁸ 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**

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