REAL TIME PCR FOR CLINICAL DETECTION AND DIFFERENTIATION OF *XYLELLA FASTIDOSA* STRAINS

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ABSTRACT
The overall goal of this work is to develop reliable protocols for the clinical detection and identification of *Xylella fastidiosa* (*Xf*) strains. The objectives are to (1) apply PCR-based methods to detect low populations of *Xf* strains causing the Pierce's disease of grapevines infected grape tissue; and (2) to distinguish different *Xf* strains in naturally-occurring single or mixed infections in different hosts, as well as in insect vectors. A major problem is the presence of PCR inhibitors in the grape tissue extracts that result in false negative results. A simplified method for the isolation of grape tissue DNA, using a single tube for grinding and extraction, was developed. Two real time PCR systems were developed for the generic detection of *Xf* strains and the specific detection of the *Xf*-PD strain or pathotype based on the currently available genomic sequences of four *Xf* strains. One system, based on a set of primers, designated HL5/HL6, and a probe labeled with FAM (HL5/HL6-FAM) as a fluorescent dye, detected four *Xf* strains (PD, almond leaf scorch (ALS), oleander leaf scorch (OLS)) and citrus variegated chlorosis (CVC) DNA. The specificity of the primers was tested against several other plant pathogenic bacteria and endophytic bacteria isolated from grape, no amplification products were obtained using 103-104 cells/reaction. The *Xf*-specific amplification product was 221 bp. As few as 5 bacteria per reaction were detected using this system. Standard curves were obtained with intact bacteria in water and in preparations containing grape leaf petiole DNA from the equivalent of 1 mg of fresh grape tissue per reaction. The Ct values ranged from 20 cycles for 105 bacteria per reaction to 36 cycles for 5 bacteria per reaction ($r2 > 0.9$). A second system, based on a set of *Xf*-PD specific primers, designated HL7/HL8, and a probe labeled with TET (HL7/HL8-TET) as a fluorescent dye, was developed. The *Xf*-specific product in this case was 302bp. This set of primers specifically distinguished *Xf*-PD from *Xf*-ALS and *Xf*-OLS, as well as from *Xf*-CVC DNA. The use of these two systems permits the detection of the *Xf* strains and the specific detection of *Xf*-PD, and could be used to detect as few as 5 bacteria per reaction.

USE OF FLUORESCENT RECOMBINANT ANTIBODIES FOR IDENTIFICATION OF *XYLELLA FASTIDIOSA*

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ABSTRACT
A library of recombinant antibodies ScFv was produced by phage display following immunization of a chicken with *Xylella fastidiosa*. Using ELISA, a clone in the vector pComb3X was selected from this library. The clone produces soluble antibody also carries 6His and HA epitope tags. Currently we are trying to develop a fluorescent immunocytochemical method with this antibody that will be able to detect *Xylella* in a plant or insect.

Figure 1. An example of the rapid progress being made breeding *Xf* resistant table grapes. From left to right first generation progeny from *V. vinifera* B90-116 x *V. shuttleworthii* F902: F902, 0070-12, 0070-14, 0070-28 and Redglobe (for size comparison).

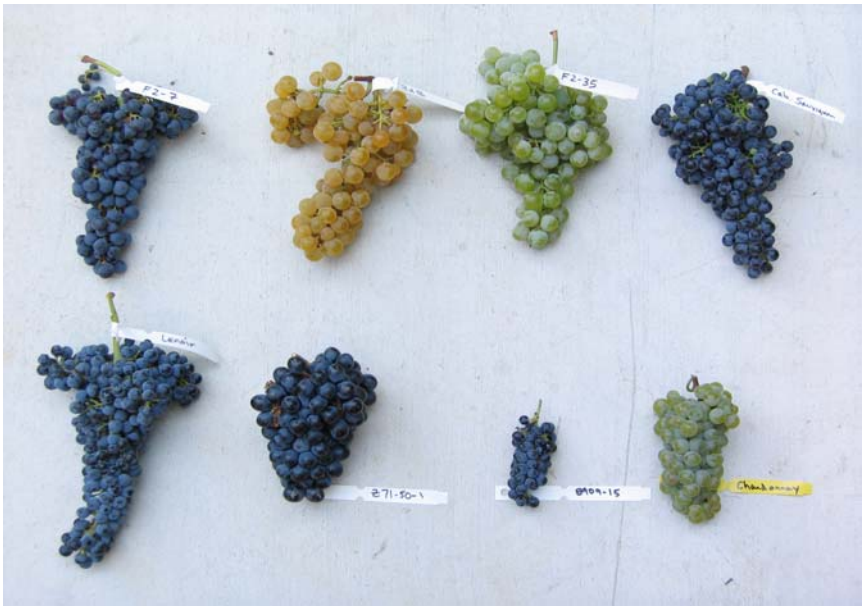

Figure 2. An example of wine grape types being used in crosses. Clockwise from the upper left: F2-7 (Carignane x Cabernet Sauvignon); Blanc du Bois; F2-36 (same cross as F2-7); Cabernet Sauvignon; Chardonnay; D8909-15; Zehnder 71-50-1; Lenoir (Jacquez or Black Spanish).