INFLUENCE OF HOST XYLEM CHEMISTRY ON REGULATION OF *XYLELLA FASTIDIOSA* VIRULENCE GENES AND HOST SPECIFICITY

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ABSTRACT
Xylella fastidiosa (Xf), a xylem-limited plant pathogen, causes leaf scorch diseases in many plant hosts, but individual strains may exhibit considerable host specificity. In previous work, we began to look at the effect of different host xylem fluids on expression of virulence genes. In a Pierce's disease (PD) strain of *Xf*, several virulence genes were more highly expressed in xylem fluid of grapevine vs. xylem fluid of citrus, a non-host plant for the PD strain (Shi et al., 2010). This finding suggested that host range of *Xf* may be influenced by differential expression of virulence genes in response to different host xylem chemistry. This project is to further explore that hypothesis with several strain/host combinations and to investigate components of xylem fluid that are responsible for either inducing or repressing virulence in *Xf*. We have inoculated strains of *Xf* from grapevine, almond, mulberry (Temecula-1, Ann1, M12 and Mul024) into xylem fluid extracted from each of those hosts and citrus to detect differential growth patterns and compare the gene expression using DNA macroarray and microarray techniques. Our progress in this work is described here.

LAYPERSON SUMMARY
We have shown that genes involved in disease induction by a Pierce's disease strain of *Xylella fastidiosa (Xf)* were expressed differently in sap of a susceptible plant (grape) vs. a resistant plant (citrus). This raises the possibility that the host range of different strains of *Xf* is in part due to differential regulation of bacterial genes in response to differences in chemical components of plant sap. We are further testing this idea by examining gene expression in *Xf* grown in sap with several different strain/host combinations for which we have already defined whether the particular combinations result in susceptibility, resistance, or tolerance. We will also examine specific chemical components of plant sap that influence bacterial gene expression, with the goal of discovering components that could be used for practical disease control by reducing expression of genes necessary for disease induction.

INTRODUCTION
Xylella fastidiosa (Xf) is a gram-negative gamma-proteobacterium limited to the xylem system of plants (Wells et al. 1987) and is transmitted by xylem-feeding insects (Purcell, 1990). It has been known to cause disease in a wide range of economically important plants in America, such as grapevine, citrus, mulberry, almond, peach, plum, coffee, oleander, and etc (Hopkins, 1989). *Xf* has been divided into four different subspecies (Schaad et al. 2004; Schuenzel. Et al. 2005): i) subsp. *fastidiosa*, ii) subsp. *sandyi*, iii) subsp. *multiplex*, and iv) subsp. *pauca*. The subspecies of *Xf* differ in host range, and strains within some of the subspecies can also differ widely in their host specificity. We are interested in the possible contribution of differences in host xylem fluid chemistry in determining the host specificity of specific strains.

Xf not only causes diseases at variety of host plants, but it can grow in symptomless hosts that can serve as sources of inoculum (Costa et al., 2004). Our previous study reported differential growth and expression profiles of a Pierce's disease (PD) strain inoculated into pure sap from grapevine (a symptomatic host for PD) and citrus (symptomless with PD). A number of virulence-related genes were expressed at a greater level in grapevine sap compared with citrus sap. However, some genes had greater expression in citrus sap (Shi et al. 2010). We have also shown differential growth and expression patterns in sap from different genotypes of grapevines (PD-tolerant vs. PD-susceptible) with a PD strain (Shi et al., Unpublished data). Understanding which specific chemical components of plant sap influence virulence gene expression could lead to strategies for practical disease control.

OBJECTIVES
1. Assess virulence gene expression of several different host-range strains of *Xf* in the xylem fluid of a common set of plant hosts.
2. Assess the influence of specific components of plant xylem fluids on the expression of virulence genes of *Xf*.

RESULTS AND DISCUSSION
Preparation of xylem fluid
Xylem fluid of grape, citrus, almond and mulberry was collected in August and September, 2010 in Riverside using a pressure chamber apparatus as previously described (Anderson *et al*., 1992; Bi *et al*., 2007). After sterilized using 0.22 um filters, all the xylem fluids were stored at −80°C until use.

In total, at least 40ml fluid was needed from each plant host: four (isolates) x three (replicates) x three ml (minimum volume for *Xf* growth in vitro). So far, we have collected the following amounts: grapevine (42ml), almond (12ml), mulberry (21ml) and citrus (50ml) and are continuing to collect xylem fluid this fall.

DNA macroarray preparation

DNA macroarray membranes were prepared with 110 selected genes with putative roles in *Xf* virulence, as well as others involved in the metabolism of nucleic acids and proteins, and cellular transport and stress tolerance, based on the genome sequences of *Xf* 9a5c (a CVC strain) (Simpson et al., 2000) and *Xf* Temecula-1(a PD strain) (Van Sluys et al., 2003). DNA fragments (average 600 bp) of the ORFs of the 110 genes were individually amplified by specific PCR from the genomic DNA of *Xf* Temecula-1, purified, and spotted onto nylon membranes (Hybond, Amersham Pharmacia Biotech Inc., NJ) using a manual 384-pin replicator (V&P Scientific Inc., CA). Spotted DNA was denatured with 0.4M NaOH, neutralized with standard saline phosphate EDTA, UV cross-linked, and boiled in 0.1% sodium dodecyl sulfate (Hernandez-Martinez, 2005). The primers for specific regions of genes XF0077, XF0889 and XF1968 were modified, because the original primers gave no amplification. The updated primer sequences are XF0077 (for-CGGCCTAGTGTGATAGCTT-, rev-CCAAGTTGAACTGATCAAGAC-); XF0889 (for-GGCAAGAAACATCACCATC-, rev-CCGATTTGAAAGGTGCTC-); and XF1968 (for-GCAAATATTGGGGAATCG-, rev-AAACTCAACGCCGAAGAT-). In total, 74 membranes were prepared for macroarray hybridization. Total RNA extracted for Temecula-1 cells grown on PD3 was used to verify that the hybridization protocol as described previously (Shi et al., 2010) was working well, and we are now growing *Xf* strains in the different xylem fluids for extraction of RNA to conduct our macroarray studies.

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