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*. In the reporting period, we cultured Temecula-1 (a PD strain) into grapevine, mulberry and citrus in vitro, with inoculated PD3 medium and non-inoculated xylem fluid as controls, to detect differential growth and expression patterns. Temecula-1 grew similarly in pure xylem fluid of grapevine, mulberry and citrus. Transcriptional profiles based on macroarray analysis of 110 pathogeneticity-related genes showed that 27 genes had higher expression, and three lower, in grape xylem fluid compared with that of mulberry and citrus. Expression of these genes in mulberry vs. citrus was not significantly different. Although the PD strain grew similarly in xylem fluid from all three hosts, the increased expression of pathogenicity genes likely contributes to disease development in grape by Temecula-1, whereas there were no symptoms are produced by this strain in mulberry and citrus.

LAYPERSON SUMMARY
We have previously shown different gene expression profiles of Pierce's disease (PD) strain A05 in the xylem fluid of grapevine (PD-susceptible) vs. citrus (PD-tolerant). This raised the possibility that the differential host range of *Xylella fastidiosa (Xf)* strains may to some extent be related to their genetic response to the chemical composition of xylem fluid from the different hosts. In this report we used grape, mulberry and citrus fluid to compare the expression patterns of another PD strain, Temecula-1. With its fully annotated genomic information, we hope to more accurately explain the influence of host xylem chemistry on the growth and pathogenesis of *Xf*, and to use this information to develop strategies to interfere with disease development in susceptible plants.

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 in a 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 xylem fluid 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 xylem fluid compared with citrus xylem fluid. However, some genes had greater expression in citrus xylem fluid (Shi et al. 2010). We have also shown differential growth and expression patterns in xylem fluid 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 xylem fluid 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 from February to August 2011 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.

Bacteria Growth *in Vitro*

Cells of *Xf* strain Temecula-1 were collected from seven-day-cultured PD3 agar and adjusted to an optical density of 0.05 at 600 nm in xylem fluid from grapevine, mulberry and citrus in borosilicate glass culture tubes, which were placed on a rotary shaker under constant agitation at 28°C for 20 days as previously described (Shi *et al*., 2010). Xylem fluid without the bacteria, and bacteria inoculated in PD3 broth were used as controls. Bacterial cells were dispersed by repeated pipetting and vortexing. Bacterial cell concentration in the tubes was determined by measuring the OD_{600} at 10 and 20 days after culture. Cells from the tubes were cultured on plates containing PD3 medium , and then confirmed to be *Xf* using specific primer pair RST31/RST33 (Minsavage *et al.* 1994).

The Temecula-1 strain of *Xf* could grow in pure xylem fluid of grapevine, mulberry and citrus with low densities compared to those in PD3 medium. *Xf* cell densities in grape xylem fluid were not significantly different than in mulberry and citrus fluid, and the cell densities increased by 20 days in all xylem fluids (**Figure 1**). *Xf* cells have been reported to grow in xylem fluid of grape (Andersen et al, 2007; Zaini et al, 2009, Shi et al, 2010) and citrus (Shi et al, 2010). In this study, mulberry was used as another symptomless host for the PD strain, and its xylem fluid could also support the growth of *Xf* stain Temecula-1.

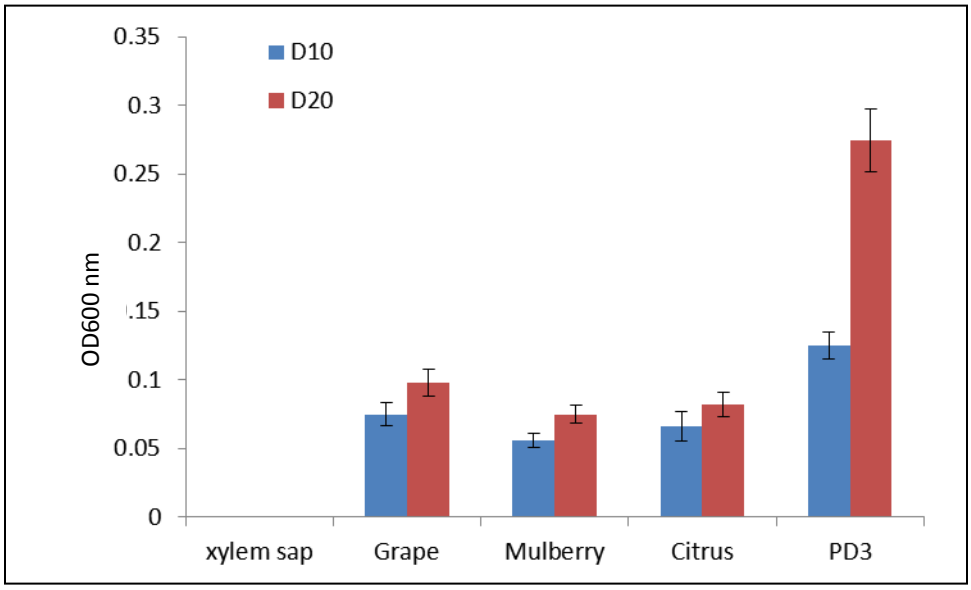

Figure 1. One *Xf* stain Temecula-1 was inoculated into xylem fluid of grape, mulberry, and citrus at OD600 nm of 0.05 in borosilicate glass culture tubes, which were placed on a rotary shaker at 28°C. PD3 medium and non-inoculated xylem fluids were used as controls. The cell concentrations in xylem fluid of grape, mulberry and citrus were measured at OD600 nm at 10 and 20 days after inoculation. All tubes were covered with a black cardboard box to protect the culture from light.

RNA Isolation and Macroarray Analysis

The extraction of total RNA from the cultures of *Xf* isolated grown in xylem fluid, the determination of qualities of isolated prokaryotic RNA, the synthesis of cDNA, and cDNA digoxigenin (DIG)-labeled by reverse transcription (RT), were all done using previously described methods (Shi et al., 2010). DNA macroarray nylon membranes were hybridized with DIG-labeled cDNA following the manufacturer's instructions (Roche Applied Science, IN, USA). Signal intensities of spots on the membranes were analyzed using Quantity one® software (Bio-Rad, CA) per the methods of Shi et al. (2010). Briefly, one-way analysis of variance of the expression values was used to select differentially expressed genes among mRNA samples. Genes with expression levels significantly different among combinations were identified using the student's t-test (α=0.05).

Of 110 selected genes (Hernandez-Martinez, 2005) from *Xf* tested in the DNA macroarray, 30 genes were differentially expressed in grape xylem fluid vs. mulberry and citrus fluid, but there is no significant difference between the expression of these genes in mulberry and citrus fluids (**Table 1**). Three genes had lower expression in grape fluid compared with citrus and mulberry, and 27 genes had higher expression in grape fluid, which included some virulence factors and virulence

regulatory genes, such as *rsmA*, *algU* and *gacA*, In addition, some genes involved in the biogenesis of pili and twitching motility were also highly expressed in grape xylem fluid compared with xylem fluid from other the non-host plants.

Table 1. Genes of *Xf* strain Temecula-1 differentially expressed in cells growing in xylem fluid of grape, mulberry, and citrus.

Gene ID [1]	Name	Hypothetical function	Index [2,3]		P value [4]	
			G/M	G/C	G/M	G/C
XF2228	*algH*	Transcriptional regulator	3.73/3.17		2.3E-04/1.9E-03	
XF2466	*pglA*	polygalacruronase precursor	2.93/2.65		6.0E-03/7.0E-03	
XF0125	*rsmA*	Carbon storage regulator	2.87/2.60		1.1E-03/1.0E-04	
XF2625	*htpX*	Heat shock protein	2.86/2.62		1.1E-04/9.9E-03	
XF2538	*pilC*	Fimbrial assembly protein	2.86/2.50		6.5E-03/5.9E-03	
XF2239	*algU or algT*	RNA polymerase sigma-H factor	2.85/2.37		6.1E-03/5.5E-03	
XF0478	*pilY1*	Fimbrial assembly protein	2.80/2.56		1.6E-03/1.0E-04	
XF1940	*msrA or pms*	Peptide methionine sulfoxide reductase	2.77/2.50		7.7E-03/6.9E-03	
XF2420	*mviN*	Virulence factor	2.68/2.18		5.2E-03/4.6E-03	
XF2608	*gacA*	Transcriptional regulator (luxr/uhpa family)	2.65/2.25		2.0E-04/1.7E-04	
XF2397	*hlyB*	Toxin secretion ABC transporter ATP-binding protein	2.62/2.34		1.7E-03/2.8E-03	
XF0432	*brk*	Brkb protein	2.61/2.43		8.0E-03/9.3E-03	
XF0028	*fimT*	Pre-pilin like leader sequence	2.58/2.19		1.7E-04/1.6E-04	
XF1804	*sphIM*	Site-specific DNA-methyltransferase	2.52/2.16		6.6E-03/5.6E-03	
XF0619	*cutA, cycY*	periplasmic divalent cation tolerance protein	2.45/2.53		1.3E-04/1.0E-04	
XF0132	*copA*	Copper resistance protein A precursor	2.42/2.22		6.8E-03/5.9E-03	
XF0506	*vapE*	Virulence-associated protein E	2.14/1.96		1.1E-04/2.9E-03	
XF0285	*hrA*	Heat shock protein	2.13/1.95		2.6E-03/3.3E-03	
XF1954	*pilIalgH*	pilus biogenesis protein	2.08/1.88		4.2E-03/3.7E-03	
XF1858	*exsb*	Transcriptional factor	2.07/1.93		7.1E-03/6.8E-03	
XF0962	*gcvR*	Transcriptional regulator	2.06/1.79		3.0E-04/2.4E-03	
XF1379	*HI1201*	Luciferase	2.05/1.84		1.0E-04/7.7E-03	
XF0591	-	Virulence factor	2.04/1.86		5.1E-03/4.4E-03	
XF1182	*act*	lipase modulator	1.98/1.66		4.2E-03/3.2E-03	
XF2539	-	Fimbrial protein	1.98/1.61		1.0E-02/1.1E-02	
XF0677	*pilZ*	Type 4 fimbriae assembly protein	1.93/1.77		5.2E-03/4.7E-03	
XF2545	*pilR*	Two-component system, regulatory protein	1.83/1.58		1.3E-04/1.0E-04	
XF0122	*lexa*	Lexa repressor	0.50/0.45		2.0E-04/1.0E-04	
XF0081	*fimD*	outer membrane usher protein	0.10/0.08		1.0E-04/8.7E-03	
XF0858	*surE*	Survival protein	0.07/0.06		3.2E-04/1.0E-04	

[1]Genes ID were determined on the basis of 9a5c genomic sequences at the NCBI website

[2]Hybridization signal intensity (mean of three hybridization replicates) obtained with grape was divided by that obtained with mulberry(G/M) and citrus (G/C) respectively to obtain the ratio

[3]Normalized hybridization signals for grape, mulberry and citrus for indicated genes were significantly different based on ANOVA and t- test ($P<0.05$)

[4]Genes having > 1.5 or < 0.6 ratios were designated as having a higher or a lower expression in grape respectively. There is no transcriptional difference of all loci between Temecula-1 inoculated into mulberry and citrus.

CONCLUSIONS

Xylem fluid is nutrient-poor compared with culture media, but it is still feasible for culturing *Xf* in vitro (Andersen et al. 2007; Zaini et al. 2009; Shi et al.2010). In this study, we compared the influence of grape, mulberry and citrus xylem fluids on one PD strain Temecula-1 in vitro. Results showed that the PD strain grew similarly in the xylem fluid from host and non-host plants, but different gene expression profiles were observed with Temecula-1 inoculated into grape xylem fluid vs. that from mulberry and citrus. We will also examine specific chemical components of xylem fluid that influence virulence gene expression, with the goal of discovering components that could be used to reduce virulence gene expression for practical disease control.

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