

NEW *XYLELLA FASTIDIOSA* GENOMES FROM TEXAS

Principal Investigator:

Lisa Morano
Dept of Natural Sciences
Univ. of Houston-Downtown
Houston, TX 77002-1001
moranol@uhd.edu

Co-Principal Investigator:

Blake Bextine
Dept. of Biology
University of Texas-Tyler
Tyler, TX 75799
bbextine@uttyler.edu

Cooperators:

Scot Dowd
Research & Testing Lab.
Lubbock, TX 79407
sdowd@pathogenresearch.org

Leonard Nunney
Dept. of Biology
University of California
Riverside, CA 92521
leonard.nunney@ucr.edu

Researchers:

Henry Schreiber IV
Dept. of Biology
University of Texas-Tyler
Tyler, TX 75799
henrylschreiber@gmail.com

Adolfo Lara
Dept of Natural Sciences
Univ. of Houston-Downtown
Houston, TX 77002-1001

Mitesh Koirala
Dept of Natural Sciences
Univ. of Houston-Downtown
Houston, TX 77002-1001

Matthew Ojeda
Dept of Natural Sciences
Univ. of Houston-Downtown
Houston, TX 77002-1001

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ABSTRACT

In this report we present the final completion of the first *Xylella fastidiosa* (*Xf*) genome from Texas, *Xf* grape strain GB514. Preliminary report of the genome was presented at the Southwestern Entomology meetings in spring 2010, but the genome has now been uploaded to NCBI. We present the accession numbers and initial findings as we begin to compare this genome to two California *Xf* grape strains. Not surprisingly, GB514 shows strong homology to the California grape strain of *Xf* (M23 and Temecula). GB514 also includes a plasmid which contains genes for a completely functional Type IV secretion system and shows 94% query coverage with a plasmid recently published from a *Xf* mulberry strain. The mulberry strain plasmid (and ours) shows alignment to a plasmid from the earthworm symbiont *Verminephrobacter eiseniae*. We report differences between the California *Xf* grape genomes and this Texas grape strain for the *zonula occludens toxin* (*zot*) gene (a possible virulence gene). In addition to analyzing this genome, we report on our planned sequence of a second Texas *Xf* isolate called MM067-2 from Pecan. According to preliminary genome comparison (MLST) this isolate is from an “oak” type of the *Xf multiplex subsp.* This “oak” type has been isolated from multiple states and no oak isolates of *Xf* have yet been sequenced.

LAYPERSON SUMMARY

Comparison of *Xylella fastidiosa* (*Xf*) genomes is a powerful tool to search for virulence genes and to map the evolutionary history of an organism. Instead of comparing strains one gene at a time, genome comparisons allow scientists to quickly identify where strains are different. These differences likely hold the key as to why some isolates are pathogenic in some plants and not others. Genome studies also allow us to search for better sequences to use in detection (making detection more efficient or strain specific). We have sequenced a *Xf* grape strain from Texas and uploaded the genome (and plasmid) to the NCBI (national genomic database) for all to use. This is the first *Xf* genome from outside California (with the exception of the first *Xf* genome which was done in Brazil). We have uncovered a few distinctions between this Texas grape strain and those in California. We have also begun sequencing a second genome from Texas. This second genome is from pecan and preliminarily looks like an ‘oak’ type. This will be the first ‘oak’ type of *Xf* sequenced and will likely present useful information about the natural history of *Xf* in North America.

INTRODUCTION

Xylella fastidiosa (*Xf*) Wells *et al.* is the gram-negative plant pathogen which causes Pierce’s disease in grapevines, as well as diseases in other plants such as almonds, citrus and oleander. Over the last decade a total of six *Xf* genomes have been sequenced including the citrus variegated chlorosis strain 9a5c from Brazil (Simpson *et al.* 2000), Temecula grape strain (Van Sluys *et al.* 2003), the California oleander strain Ann-1 and almond leaf scorch strain Dixon (Copeland *et al.* 2002a, b) and two additional *Xf* isolates from California, a grape strain M23 and an almond strain M12 (Chen *et al.* 2007). The analysis of genomes of *Xf* outside of California is essential for us to address questions about the natural history of *Xf* in North America. Preliminary analysis suggested that the grape strains in Texas and California were highly similar (Yuan *et al.* 2007), but the whole genome comparison is required to search for small but potentially significant differences in the genomes. We have also begun sequencing an additional *Xf* isolate from pecan.

OBJECTIVES

1. Sequence and upload the genome from a Texas grape strain of *Xf* (*Xylella fastidiosa subsp. fastidiosa*).
2. Compare the Texas grape strains to the two grape *Xf* genomes which have been done in California.
3. Report on the new Texas *Xf* genome sequencing project underway.

RESULTS AND DISCUSSION

We have sequenced a Texas grape strain of *Xf* (GB514) using whole genome shotgun titanium pyrosequencing with 25X coverage. The project revealed two primary contigs, a main chromosome of 2.49 megabases and a possible low copy plasmid. Accession numbers for this project are **CP002165** for the main chromosome and **CP002166** for the plasmid. Not surprisingly, the main chromosome of GB514 shows strong homology to the California grape strain of *Xf* (M23 and Temecula). However, comparison of grape *Xf* genomes in Texas and California has and will likely continue to uncover small differences which can help us address questions about the natural history of *Xf* in North America. For example, the California grape strains each have two versions of a potential virulence factor called the *zonula occludens toxin* (*zot*) gene (da Silva et al. 2007). There are two copies of *zot1* and one copy of *zot2* in California grape strains whereas GB514 contains only one version (one copy *zot2* and no *zot1*). Did GB514 lose its copy of *zot1* or did *zot1* evolve only among the California *Xf* grape strains?

The plasmid of *Xf* GB514 codes for 39 functional genes of which 11 are for proteins used in the Type IV secretion system of gram negative bacteria, virulence factors involved in the injection of DNA or substrates into host plants (Cascales and Christie 2003). According to Cascales and Chirsite (2003) and Christie (personal communication) these genes are sufficient for a completely functional Type IV secretion system. What role might this plasmid play in virulence of this particular isolate? Of additional interest, the plasmid from grape strain of *Xf* GB514 shows 94% query coverage with a plasmid recently published from a *Xf* mulberry strain (Stenger et al. 2010). This plasmid (and our plasmid) shows strong homology with a plasmid from a bacterial symbiont *Verminephrobacter eiseniae* of earthworms. We thought this alignment unusual (and perhaps unlikely) until we read about the published finding of Stenger and colleagues (2010). Does this plasmid may have some broad host range within environmental bacteria? Is this plasmid frequent in *Xf* isolates regardless of strain or host?

In addition to analyzing the GB514 genome, we are currently underway with our second Texas *Xf* genome sequencing project. We plan to sequence the *Xf* isolated called MM067-2. This isolate was isolated from the host of pecan in Texas. This “oak” type has been isolated from multiple states and no oak isolates of *Xf* have yet been sequenced. Preliminary analysis of this isolate using MLST (Schuenzel et al. 2005), suggests it is a multiplex isolate (*Xf subs. multiplex*) of the “oak” type.

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