GENETIC DIVERSITY ANALYSIS OF *XYLELLA FASTIDIOSA* STRAINS USING MULTIPLE *TONB* GENES AND THE *ZOT* GENE

Principal Investigator:
Blake Bextine
Department of Biology
University of Texas
Tyler, TX 75799
bbextine@uttyler.edu

Co-Principal Investigator:
Lisa Morano
Dept. of Natural Sciences
Univ. of Houston-Downtown
Houston, TX 77002
MoranoL@uhd.edu

Researchers:
Stanley Gunawan
Department of Biology
University of Texas
Tyler, TX 75799
st_lie1282@yahoo.com

Henry Schreiber Jr.
Department of Biology
University of Texas
Tyler, TX 75799
hlschreiber@gmail.com

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ABSTRACT

Multiple subspecies of *Xylella fastidiosa* (*Xf*) exist which are differentially pathogenic. Previously, DNA sequence analysis of the *mopB* and *gyrB* gene has been used to separate *Xf* strains into their subspecies groups. The *TonB* gene family can be used to confirm this genetic diversity between *Xf* strains and regions within these genes can be used to separate strains beyond subspecies due to increased variability. *TonB* protein is a cytoplasmic outer membrane protein that can be found on gram negative bacteria, such as *Xf*. The protein functions as an energy transducer to support a variety of transport events across the outer membrane and interacts with outer membrane receptor proteins which carry out high-affinity binding and energy-dependant uptake of specific substrates into periplasmic space. In this study, five different *TonB* genes (*TonB*-a through *TonB*-e) were used to differentiate between three *Xf* strains (grape, ragweed, and oleander). The results of this study were consistent with genotype differentiation using conserved *mopB* and *gyrB* genes. Additionally, sequencing of another gene, analogous to the zonula occludens toxin (ZOT) gene, was used to separate groups below the strain level. The discovery of new variable genes provides another genomic location to be exploited for the improvement of diagnostics to aid in the management of Pierce's disease.

INTRODUCTION

Xylella fastidiosa (Xf) is a fastidious, xylem-limited, Gram negative bacterium that resides in the xylem tissue of many plants. This bacterium, which contains multiple subspecies can disease in multiple plants taxa, such as Pierce's disease (PD) on grapevine, oleander leaf scorch (OLS), and citrus leaf chlorosis (CVC) (Schaad 2004). One unique structure to Gram-negative bacteria is the outer membrane. This membrane has distinctive permeabiality process and possesses active transport system called *TonB*-dependent transport system. This system has high affinity and specificity for binding and transporting scarce nutrient across the outer membrane (Cadieux and Kadner 2003). *TonB* is an outer membrane protein that localized in the cytoplasmic membrane by its uncleaved amino-terminal signal sequence, with the bulk of the protein extending into the periplasmic membrane (Skare and Postle 1991). The function of this protein is as an energy transducer to couple cytoplasmic protonmotive force to active transport of nutrients and metabolic products across the outer membrane. TonB-dependent transport system consists of high affinity membrane receptor, a periplasmic binding protein, and a cytoplasmic membrane transporter homologous to other traffic ATPases (Skare 1993). *TonB* genes, which encodes for *TonB,* can be used to differentiate different *Xf* strains due to multiple singe base pair alterations. A search of the *Xf* genome determined that multiple *TonB* genes were present in the genome.

The zonula occludens toxin has been suggested as a new potential virulence factor in the CVC system (da Silva 2004). This protein is similar to one found in *Vibrio cholerae* which has been linked to disruption of tight junctions (Johnson 1993). DNA sequence variation in this gene may be useful in separating strains from one another and potentially separating populations with in a strain group.

MATERIALS AND METHODS

Extracted DNA from grape and ragweed strains of *Xf* were received from Lisa Morano. DNA from oleander strain was cultured Hercules, CA), 6.4-µl auctoclaved nanopure water, 0.8 µl forward and reverse primers, and 2-µl of DNA template. PCR was conducted using initial denaturing step of 3 min at 94°C, the reaction was cycled 35 times under the following parameters: 94°C for 60 s, 65°C for 90 sec, 72°C for 150 s and followed with another extension at 72°C for 7 min. A non-template control (NTC) was also run with each assay as a negative control. The presence of the desired amplicon was determined by agarose DNA gel electrophoresis that was run at 75V for 60 minutes.

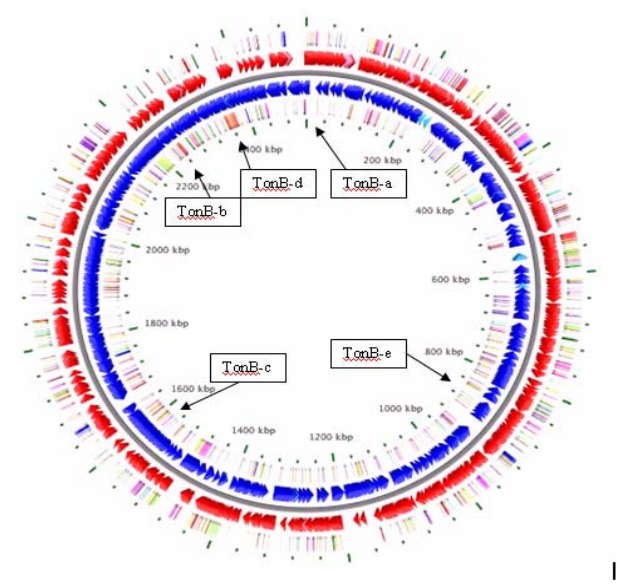

Figure 1. Location of multiple *TonB* genes in the *X. fastidiosa* genome.

The positive samples PCR products were purified using QIAquick PCR purification kit (Qiagen, Valencia, CA), using the manufacturer's protocol. DNA sequencing reaction was performed in a I CyclerTM Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). The reaction was carried out in a10-µL reaction contained 4 µL DTCS Quick Start Master Mix (Beckman and Coulter, Fullerton, CA), 2 µL of either forward or reverse primer, 2 µL autoclaved nanopure water, and 2 µL DNA template. The reaction was cycled 30 times under the following parameters: 95°C for 20 s, 50°C for 20 sec, and 60°C for 4 min, followed by holding at 4°C. DNA samples were precipitated using ethanol precipitation process according to Beckman and Coulter's protocol. DNA pellets were resuspended with 40 µL of sample loading solution (Beckman and Coulter, Fullerton, CA) and transferred to the appropriate wells of a sample plate and loaded into a CEQTM 8000 Genetic Analysis System (Beckman and Coulter, Fullerton, CA) for DNA sequencing. Sequences were retrieved and analyzed in BioEdit and compared to GenBank database (http://www.ncbi.nlm.nih.gov/).

Sequence data is currently being collected for nearly 2,000bp of the ZOT gene for these strains. The same procedures apply.

RESULTS AND DISCUSSION

BLAST search shows alignment of grape, ragweed, and oleander strains with *Xf* Temecula and *Xf* 9a5c. Alignment of grape, ragweed, and oleander strains using BioEdit software shows multiple single base pair alterations between grape, ragweed, and oleander strains. From these multiple base pair alterations, oleander strain shows partial alignment to both grape and oleander strain **(Figure 2 and 3).**

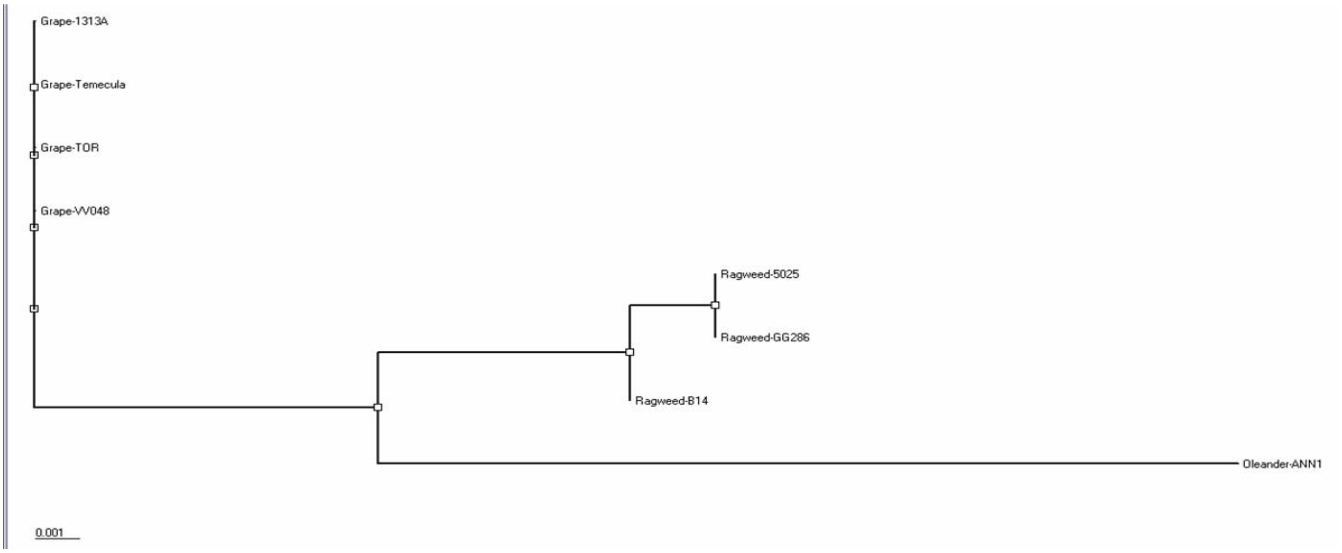

Figure 2. Alignment of *TonB* sequences from multiple *Xf* strains from Texas.

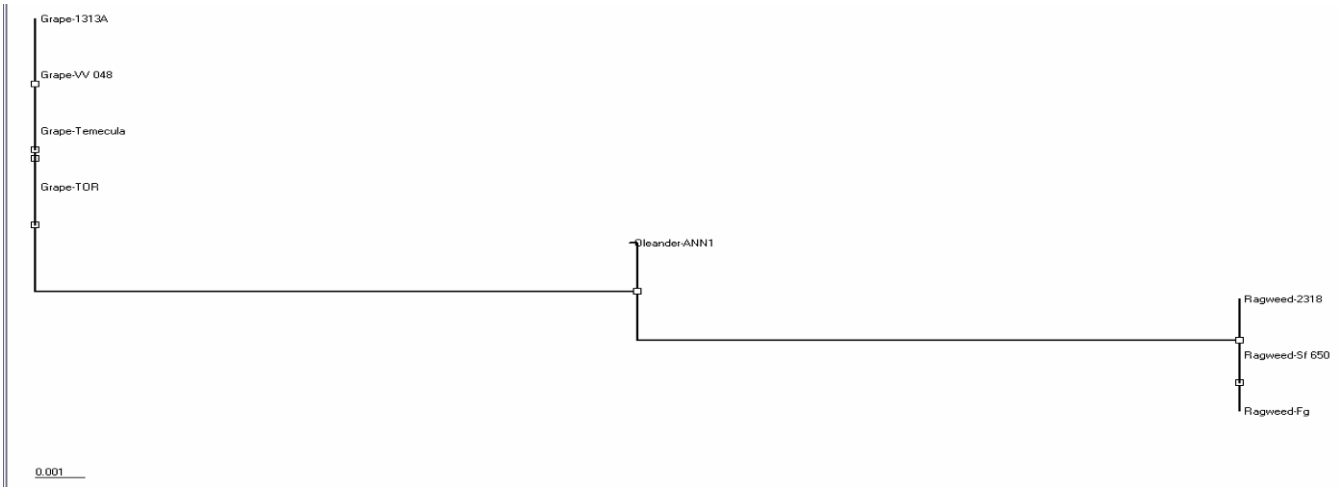

Figure 3. Alignment of *gyrB* sequences from multiple *Xf* strains from Texas (also analogous to *mopB*).

The multiple single-base alterations in oleander strain and single base alteration in ragweed strain result in different polarity and acid-base properties of amino acids to be translated. For instance, single base alteration in ragweed strain causes neutral-polar amino acid (serine) to be translated instead of neutral-non-polar amino acid (proline). The multiple single-base alterations in oleander strain cause basic-polar (histidine), neutral-polar (serine and alanine), and neutral-non-polar (proline) amino acids to be translated instead of neutral-polar (serine and threonine), and basic-polar (arginine) amino acids. These will have an effect on absorption properties, different reactions with other amino groups, and different functionality since different proteins will be produced.

We are currently working through the ZOT gene for all of our strains. Within this gene, we have found a conserved domain (most likely a beta barrel associated with attachment to the *Xf* membrane) and another domain that appears to be hypervariable. Analysis of this preliminary sequence data indicates that the conserved domain follows the same separation as *gyrB*, *mopB*, and *tonB* (i.e. separation by strain). However, the hypervariable region may be useful for separation beyond strain.

CONCLUSIONS

Sequencing using *TonB* genes and the ZOT gene highlights genetic variability between three different strains of *Xf* (grape, ragweed, and oleander strains). The results of this study are consistent with genotype differentiation using conserved *mopB* and *gyrB* genes. The discovery of this variable gene provides another genomic location to be exploited for the improvement of diagnostics to aid in the management of PD.

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