genomeA
Journals.ASM.org

Genome Sequence of a *Xylella fastidiosa* Strain Causing Mulberry Leaf Scorch Disease in Maryland

Wei Guan,[a,b] Jonathan Shao,[c] Tingchang Zhao,[a] Qi Huang[b]

Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China[a]; Floral and Nursery Plants Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, USA[b]; Molecular Plant Pathology Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, USA[c]

***Xylella fastidiosa* causes bacterial leaf scorch in landscape trees, including mulberry. We determined the draft genome of the mulberry strain Mul-MD in order to gain a better understanding of the molecular basis of strain divergence, host specificity, nutrient requirements, and pathogenicity, as well as to develop genome-based specific detection methods.**

Received 30 September 2013 **Accepted** 13 February 2014 **Published** 6 March 2014

Citation Guan W, Shao J, Zhao T, Huang Q. 2014. Genome sequence of a *Xylella fastidiosa* strain causing mulberry leaf scorch disease in Maryland. Genome Announc. 2(2): e00916-13. doi:10.1128/genomeA.00916-13.

Address correspondence to Qi Huang, qi.huang@ars.usda.gov.

Xylella fastidiosa is a Gram-negative, nutritionally fastidious, insect-transmitted, and xylem-inhabiting bacterium that causes a wide range of plant diseases, including Pierce's disease of grapevine and bacterial leaf scorch, in landscape trees such as mulberry. So far, eight *X. fastidiosa* genomes are available, including five complete genomes for the citrus variegated chlorosis strain 9a5c (1), Pierce's disease strains Temecula 1 (2) and GB514 (3), and almond strains M12 and M23 (4), as well as three draft genomes for the oleander strain Ann1, the almond strain Dixon (5), and the *X. fastidiosa* biocontrol strain EB92-1 from elderberry (6). However, no genomes from landscape trees had been reported. We therefore sequenced the mulberry strain of *X. fastidiosa*, Mul-MD, which was isolated in 2011 from a mulberry tree displaying leaf scorch symptoms in Beltsville, MD.

Genomic DNA of *X. fastidiosa* strain Mul-MD was extracted from a triply cloned pure culture in periwinkle wilt medium (7) using a Blood and Tissue kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions. Random shotgun and 3-kb mate-pair libraries of Mul-MD were generated and sequenced using Roche 454 GS (FLX titanium) pyrosequencing, resulting in 137,284 shotgun reads and 426,457 mate-pair reads totaling 254,742,009 bases, with a read-length average of about 450 bases. The total number of reads after processing by the Newbler Assembler from all libraries was 852,805 aligned reads, with 133,080,992 bases aligned. Using Newbler gsAssembler v 2.6, we assembled the genome into 188 contigs, of which 101 were >500 bases in size, and 27 scaffolds. The largest contig was 395,385 bases. Among the large contigs, the N_{50} contig size was 134,146 bases. The contigs have an average length of 13,528 bases and were run through the annotation pipeline, which uses GeneMark to predict coding regions based on prior *Xylella fastidiosa* gene models and runs BLASTX against a protein set that includes UniProt and all known *Xylella* proteins to determine edges of genes. The translated protein sequences were processed using Interproscan v. 4.8 for functional annotation and UniProt for additional descriptive information. Open reading frames shorter than 150 bases were eliminated. Selected open reading frames from *in silico* analysis that were not consistent with annotated *Xylella* genes were manually annotated. tRNA and rRNA predictions were made using the latest tRNAscan-SE and RNAmmer servers, respectively.

The 5× draft genome of the *X. fastidiosa* strain Mul-MD contains 2,543,372 bp and has a GC content of 51.65%. A total of 2,286 protein-encoding genes are predicted, 1,437 of which have tentatively been assigned a function. In addition, an ~25-kb plasmid sequence was found that is similar to the four plasmids, pXF-RIV11, pXF-RIV16, pXF-RIV19, and pXF-RIV25, present individually in four California mulberry strains of *X. fastidiosa* (8), as well as to the plasmid associated with the grapevine GB514 strain of *X. fastidiosa*.

Nucleotide sequence accession numbers. This whole-genome shotgun sequence has been deposited at DDBJ/EMBL/GenBank under the accession number AXDP00000000. The version described in this paper is version AXDP01000000.

ACKNOWLEDGMENTS

This research was supported by the U.S. Department of Agriculture.

This study was conducted in the Floral and Nursery Plants Research Unit, USDA-Agricultural Research Service, Beltsville, MD, and will be presented as part of the doctoral dissertation of the first author, Wei Guan, at the Chinese Academy of Agricultural Sciences, Beijing, China.

REFERENCES

1. **Simpson AJ, Relnac FC, Arruda P, Abreu FA, Acencio M, Alvarenga R, Alves LM, Araya JE, Baia GS, Baptista CS, Barros MH, Bonaccorsi ED, Bordin S, Bové JM, Briones MR, Bueno MR, Camargo AA, Camargo LE, Carraro DM, Carrer H, Colauto NB, Colombo C, Costa FF, Costa MC, Costa-Neto CM, Coutinho LL, Cristofani M, Dias-Neto E, Docena C, El-Dorry H, Facincani AP, Ferreira AJ, Ferreira VC, Ferro JA, Fraga JS, França SC, Franco MC, Frohme M, Furlan LR, Garnier M, Goldman GH, Goldman MH, Gomes SL, Gruber A, Ho PL, Hoheisel JD, Junqueira ML, Kemper EL, Kitajima JP, Krieger JE, Kuramae EE, Laigret F, Lambais MR, Leite LC, Lemos EG, Lemos MV, Lopes SA, Lopes CR, Machado JA, Machado MA, Madeira AM, Madeira HM, Marino CL, Marques MV, Martins EA, Martins EM, Matsukuma AY, Menck CF,**

Miracca EC, Miyaki CY, Monteriro-Vitorello CB, Moon DH, Nagai MA, Nascimento AL, Netto LE, Nhani A, Nobrega FG, Nunes LR, Oliveira MA, de Oliveira MC, de Oliveira RC, Palmieri DA, Paris A, Peixoto BR, Pereira GA, Pereira HA, Pesquero JB, Quaggio RB, Roberto PG, Rodrigues V, de M Rosa AJ, de Rosa VE, de Sá RG, Santelli RV, Sawasaki HE, da Silva AC, da Silva AM, da Silva FR, da Silva WA, da Silveira JF, Silvestri ML, Siqueira WJ, de Souza AA, de Souza AP, Terenzi MF, Truffi D, Tsai SM, Tsuhako MH, Vallada H, Van Sluys MA, Verjovski-Almeida S, Vettore AL, Zago MA, Zatz M, Meidanis J, Setubal JC. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. The *Xylella fastidiosa* Consortium of the Organization for Nucleotide Sequencing and Analysis. Nature **406:**151–159. http://dx.doi.org/10.1038/35018003.

2. **Van Sluys MA, De Oliveira MC, Monteiro-Vitorello CB, Miyaki CY, Furlan LR, Camargo LE, da Silva AC, Moon DH, Takita MA, Lemos EG, Machado MA, Ferro MI, da Silva FR, Goldman MH, Goldman GH, Lemos MV, El-Dorry H, Tsai SM, Carrer H, Carraro DM, de Oliveira RC, Nunes LR, Siqueira WJ, Coutinho LL, Kimura ET, Ferro ES, Harakava R, Kuramae EE, Marino CL, Giglioti E, Abreu IL, Alves LM, do Amaral AM, Baia GS, Blanco SR, Brito MS, Cannavan FS, Celestino AV, da Cunha AF, Fenille RC, Ferro JA, Formighieri EF, Kishi LT, Leoni SG, Oliveira AR, Rosa VE, Sassaki FT, Sena JA, de Souza AA, Truffi D, Tsukumo F, Yanai GM, Zaros LG, Civerolo EL, Simpson AJ, Almeida NF, Setubal JC, Kitajima JP.** 2003. Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. J. Bacteriol. **185:**1018–1026. http://dx.doi.org/10.1128/JB.185.3.1018-1026.2003.

3. **Schreiber IV, Koiral H, Lara M, Ojeda A, Dowd M, Bextin SE, Moran L.** 2010. Unraveling the first *Xylella fastidiosa* subsp. *Fastidiosa* genome from Texas. Southwest. Entomol. **35:**479–483. http://dx.doi.org/10.3958/059.035.0336.

4. **Chen J, Xie G, Han S, Chertkov O, Sims D, Civerolo EL.** 2010. Whole genome sequences of two *Xylella fastidiosa* strains (M12 and M23) causing almond leaf scorch disease in California. J. Bacteriol. **192:**4534. http://dx.doi.org/10.1128/JB.00651-10.

5. **Bhattacharyya A, Stilwagen S, Reznik G, Feil H, Feil WS, Anderson I, Bernal A, D'Souza M, Ivanova N, Kapatral V, Larsen N, Los T, Lykidis A, Selkov E, Walunas TL, Purcell A, Edwards RA, Hawkins T, Haselkorn R, Overbeek R, Kyrpides NC, Predki PF.** 2002. Draft sequencing and comparative genomics of *Xylella fastidiosa* strains reveal novel biological insights. Genome Res. **12:**1556–1563. http://dx.doi.org/10.1101/gr.370702.

6. **Zhang S, Flores-Cruz Z, Kumar D, Chakrabarty P, Hopkins DL, Gabriel DW.** 2011. The *Xylella fastidiosa* biocontrol strain EB92-1 genome is very similar and syntenic to Pierce's disease strains. J. Bacteriol. **193:**5576–5577. http://dx.doi.org/10.1128/JB.05430-11.

7. **Davis MJ, Raju BC, Brlansky RH, Lee RF, Timmer LW, Norris RC, McCoy RE.** 1983. Periwinkle wilt bacterium: axenic culture, pathogenicity, and relationships to other Gram-negative, xylem-inhabiting bacteria. Phytopathology **73:**1510–1515. http://dx.doi.org/10.1094/Phyto-73-1510.

8. **Stenger DC, Lee MW, Rogers EE, Chen J.** 2010. Plasmids of *Xylella fastidiosa* mulberry-infecting strains share extensive sequence identity and gene complement with pVEIS01 from the earthworm symbiont *Verminephrobacter eiseniae*. Physiol. Mol. Plant Pathol. **74:**238–245. http://dx.doi.org/10.1016/j.pmpp.2010.03.003.