

UNIVERSIDADE ESTADUAL DE CAMPINAS
SISTEMA DE BIBLIOTECAS DA UNICAMP
REPOSITÓRIO DA PRODUÇÃO CIENTÍFICA E INTELECTUAL DA UNICAMP

Versão do arquivo anexado / Version of attached file:

Versão do Editor / Published Version

Mais informações no site da editora / Further information on publisher's website:

<https://mra.asm.org/content/4/5/e01124-16>

DOI: 10.1128/genomeA.01124-16

Direitos autorais / Publisher's copyright statement:

© by American Society for Microbiology. All rights reserved.

DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo

CEP 13083-970 – Campinas SP

Fone: (19) 3521-6493

<http://www.repositorio.unicamp.br>

Draft Genome Sequence of 11399, a Transformable Citrus-Pathogenic Strain of *Xylella fastidiosa*

Bárbara Niza,^{a,b} Marcus V. Merfa,^{a,b} Valquíria C. Alencar,^c Fabiano B. Menegidio,^c Luiz R. Nunes,^d Marcos A. Machado,^a Marco A. Takita,^a Alessandra A. de Souza^a

Instituto Agronômico, Centro de Citricultura Sylvio Moreira, Cordeirópolis, São Paulo, Brazil^a; Universidade Estadual de Campinas, Departamento de Genética, Evolução e Bioagentes, Campinas, São Paulo, Brazil^b; Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes, Mogi das Cruzes, São Paulo, Brazil^c; Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, São Paulo, Brazil^d

B.N. and M.V.M. contributed equally to this work.

The draft genome of *Xylella fastidiosa* subsp. *pauca* strain 11399, a transformable citrus-pathogenic strain, is reported here. The 11399 genome size is 2,690,704 bp and has a G+C content of 52.7%. The draft genome of 11399 reveals the absence of four type I restriction-modification system genes.

Received 18 August 2016 Accepted 20 August 2016 Published 13 October 2016

Citation Niza B, Merfa MV, Alencar VC, Menegidio FB, Nunes LR, Machado MA, Takita MA, de Souza AA. 2016. Draft genome sequence of 11399, a transformable citrus-pathogenic strain of *Xylella fastidiosa*. *Genome Announc* 4(5):e01124-16. doi:10.1128/genomeA.01124-16.

Copyright © 2016 Niza et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Alessandra A. de Souza, alessandra@centrodecitricultura.br.

Xylella fastidiosa is a Gram-negative bacterium restricted to the xylem of infected plants and to the foregut of insect vectors (1). Different isolates of *X. fastidiosa* cause diseases in a wide range of economically important crops worldwide (2). In Brazil, it is responsible for causing citrus variegated chlorosis (CVC), a disease that has significantly damaged the Brazilian citrus industry (3). Due to its importance, the CVC-associated strain 9a5c of *X. fastidiosa* subsp. *pauca* was the first plant pathogenic bacterium to have its genome entirely sequenced, providing a framework for subsequent functional genomics studies (4). However, genetic manipulation of strain 9a5c is difficult and, as a consequence, experiments aimed at characterizing pathogenicity/virulence genes by gene knockout/overexpression approaches could not be performed with this strain.

Nonetheless, another citrus-pathogenic strain of *X. fastidiosa*, strain 11399 (5), has been recently reported to be transformable, opening new possibilities for studies on the biology and host interactions of this bacterium (6–9). This finding provides the opportunity to understand the genetic mechanisms that determine *X. fastidiosa* pathogenicity during the development of CVC, using transformants derived from direct genetic manipulation of strain 11399. Thus, we hereby report the draft genome of *X. fastidiosa* subsp. *pauca* strain 11399, to be used as a reference for such experiments.

For sequencing, *X. fastidiosa* strain 11399 was grown in periwinkle wilt medium (10) for 7 days at 28°C, and total genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Inc., Valencia, CA, USA). The genomic library construction and whole-genome sequencing was performed by Macrogen (Seoul, Republic of Korea) in an Illumina HiSeq 2000 platform (Illumina, Inc., San Diego, CA, USA), generating 40,407,960 paired-end reads (101 bp each). The 2,690,704 bp (70× coverage; G+C content, 52.7%) draft genome of strain 11399 was assembled into 35 contigs (ranging from 479 bp to 331,284 bp) by the referenced assembly method, using the Burrows-Wheeler aligner “MEM” al-

gorithm (BWA-MEM) version 0.7.9 (11) and *X. fastidiosa* strain 9a5c as the reference genome. In addition, a plasmid sequence (45,356 bp in 1 contig) that is most similar to the 9a5c plasmid pXF51 (4) was also found in 11399. The genome of strain 11399 is likely nearly (approx. 98.5%) complete, as the genomes of *X. fastidiosa* strains range from 2.39 to 2.73 Mbp (12). Annotation was performed through submission to the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP), which identified 2,248 open reading frames and 57 RNA genes.

Comparative analysis between the genomes of strains 11399 and 9a5c were performed using Mauve version 2.4.0 (13) and revealed that strain 11399 lacks four genes of the type I restriction-modification system (two specificity determinants [XF2722 and XF2726] and two DNA methylases [XF2723 and XF2724]; <http://www.lbi.ic.unicamp.br/xf>), which protect bacterial cells against foreign DNAs (14). The lack of these genes could thus contribute to enhance transformation efficiency in strain 11399, when compared to strain 9a5c.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number JNBT00000000. The version described in this paper is the first version, JNBT01000000.

FUNDING INFORMATION

This work, including the efforts of Alessandra Alves De Souza, was funded by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (2010/50712-9, 2013/17485-7, and 2013/10957-0). This work, including the efforts of Marcos Machado, was funded by INCT-Citros (08/57909-2 and 573848/08-4). This work, including the efforts of Marcus Vinicius Merfa, was funded by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (2013/02014-9). This work, including the efforts of Bárbara Niza, was funded by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (2011/14307-5).

V.C.A. and F.B.M. are fellow Ph.D. students from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). M.A.M., M.A.T., and A.A.D.S. are recipients of research fellowships from CNPq. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

- Almeida RPP, Coletta-Filho HD, Lopes JRS. 2014. *Xylella fastidiosa*, p. 841–850. In Liu D (ed), Manual of security Sensitive Microbes and Toxins, CRC Press, Boca Raton, FL. <http://dx.doi.org/10.1201/b16752-83>.
- Hopkins DL, Purcell AH. 2002. *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis 86:1056–1066. <http://dx.doi.org/10.1094/PDIS.2002.86.10.1056>.
- Bové JM, Ayres AJ. 2007. Etiology of three recent diseases of citrus in São Paulo State: sudden death, variegated chlorosis and Huanglongbing. IUBMB Life 59:346–354. <http://dx.doi.org/10.1080/15216540701299326>.
- Simpson AJ, Reinach 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-Dorri 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, Lam-Bais 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, Montero-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*. Nature 406:151–159. <http://dx.doi.org/10.1038/35018003>.
- Coletta-Filho HD, Takita MA, de Souza AA, Aguilar-Vildoso CI, Machado MA. 2001. Differentiation of strains of *Xylella fastidiosa* by a variable number of tandem repeat analysis. Appl Environ Microbiol 67: 4091–4095. <http://dx.doi.org/10.1128/AEM.67.9.4091-4095.2001>.
- Janissen R, Murillo DM, Niza B, Sahoo PK, Nobrega MM, Cesar CL, Temperini ML, Carvalho HF, de Souza AA, Cotta MA. 2015. Spatio-temporal distribution of different extracellular polymeric substances and filamentation mediate *Xylella fastidiosa* adhesion and biofilm formation. Sci Rep 5:9856. <http://dx.doi.org/10.1038/srep09856>.
- Niza B, Coletta-Filho HD, Merfa MV, Takita MA, de Souza AA. 2015. Differential colonization patterns of *Xylella fastidiosa* infecting citrus genotypes. Plant Pathol 64:1259–1269. <http://dx.doi.org/10.1111/ppa.12381>.
- Merfa MV, Niza B, Takita MA, de Souza AA. 2016. The MqsRA toxin-antitoxin system from *Xylella fastidiosa* plays a key role in bacterial fitness, pathogenicity, and persister cell formation. Front Microbiol 7:904. <http://dx.doi.org/10.3389/fmicb.2016.00904>.
- Sahoo PK, Janissen R, Monteiro MP, Cavalli A, Murillo DM, Merfa MV, Cesar CL, Carvalho HF, de Souza AA, Bakkers EP, Cotta MA. 2016. Nanowire arrays as cell force sensors to investigate adhesion-enhanced holdfast of single cell bacteria and biofilm stability. Nano Lett 16:4656–4664. <http://dx.doi.org/10.1021/acs.nanolett.6b01998>.
- Davis MJ, French JW, Schaad NW. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum scald. Curr Microbiol 5:309–314. <http://dx.doi.org/10.1007/BF01566883>.
- Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:13033997. <http://arxiv.org/abs/1303.3997>.
- Varani AM, Monteiro-Vitorello CB, de Almeida LG, Souza RC, Cunha OL, Lima WC, Civerolo E, Van Sluys MA, Vasconcelos AT. 2012. *Xylella fastidiosa* comparative genomic database is an information resource to explore the annotation, genomic features, and biology of different strains. Genet Mol Biol 35:149–152. <http://dx.doi.org/10.1590/S1415-47572012005000019>.
- Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14:1394–1403. <http://dx.doi.org/10.1101/gr.2289704>.
- Kobayashi I. 2001. Behavior of restriction-modification systems as selfish mobile elements and their impact on genome evolution. Nucleic Acids Res 29:3742–3756. <http://dx.doi.org/10.1093/nar/29.18.3742>.