

Draft Genome Sequence of *Xylella fastidiosa* subsp. *multiplex* Strain Griffin-1 from *Quercus rubra* in Georgia

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The draft genome sequence of *Xylella fastidiosa* subsp. *multiplex* strain Griffin-1, isolated from a red oak tree (*Quercus rubra*) in Georgia, is reported here. The bacterium has a genome size of 2,387,314 bp, with a G+C content of 51.7%. The Griffin-1 strain genome contains 2,903 predicted open reading frames and 50 RNA genes.

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Xylella fastidiosa is a Gram-negative, xylem-limited plant-pathogenic bacterium (1) causing many economically important diseases, including oak leaf scorch disease in the eastern United States (2–4). Due to nutritional fastidiousness, characterization of *X. fastidiosa* has been difficult, and many biological issues related to *X. fastidiosa* strains remain to be investigated. Both complete genome (5–8) and whole-genome shotgun (9, 10) sequences of *X. fastidiosa* are currently available. Three subspecies of *X. fastidiosa* (subspecies *fastidiosa*, subspecies *multiplex*, and subspecies *pauca*) have been proposed (11). The taxonomic statuses of *X. fastidiosa* strains from oak trees have not been evaluated. An early study based on a random amplified polymorphic DNA analysis showed that *X. fastidiosa* strains from turkey oak (*Quercus laevis*) in Florida and red oak (*Quercus rubra*) in Georgia were highly similar (12).

In the summer of 2006, a strain of *X. fastidiosa* was isolated from a symptomatic red oak tree (*Q. rubra*) in Griffin, Georgia (33°15'38.07"N, 84°16'48.69"W), and it has been maintained in our laboratory in California. This bacterial strain was triple cloned and designated Griffin-1. To obtain genomic DNA, Griffin-1 was cultured in periwinkle wilt (PW) broth (13) at 28°C for 10 days. Bacterial cells were collected by centrifugation; the total genomic DNA was extracted by a standard procedure (14). Genome sequencing was carried out on a 454 GS-FLX system using Titanium chemistry (Roche) (15). Paired-end reads were assembled with the Newbler software (Roche Diagnostics). The Griffin-1 genome consists of 2,387,314 bp (~30× coverage, G+C content of 51.7%) assembled into 84 contigs ranging from 523 bp to 142,581 bp. Annotation was performed by the RAST server (<http://rast.nmpdr.org/>) (16), which utilizes the GeneMark, Glimmer, and tRNAscan-SE databases. The Griffin-1 genome was predicted to have a total of 2,903 open reading frames (ORFs) and 50 RNA genes.

Using BLAST analyses (17), the sequences of *ssr* (16S rRNA) and four housekeeping genes, *gyrB* (DNA gyrase subunit B), *dnaK* (chaperone protein), *rpoD* (RNA polymerase sigma factor), and *tonB* (outer membrane receptor), were selected and compared to

the corresponding gene sequences of *X. fastidiosa* subsp. *multiplex* strain M12 (5), *X. fastidiosa* subsp. *fastidiosa* strains M23, GB514, and Temecula (5, 6, 8), and *X. fastidiosa* subsp. *pauca* strain 9a5c (14). For all five loci, *X. fastidiosa* subsp. *multiplex* Griffin-1 is 100% identical to strain M12, indicating that the oak strain is a member of *X. fastidiosa* subsp. *multiplex*.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. **AVGA00000000**. The version described in this paper is version AVGA01000000.

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REFERENCES

- Wells JM, Raju BC, Huang H-Y, Weisburg WG, Mandelco-Paul L, Brenner DJ. 1987. *Xylella fastidiosa* gen. nov., sp. nov.: Gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. *Int. J. Syst. Bacteriol.* 37:136–143.
- Chang CJ, Walker JT. 1988. Bacterial leaf scorch of northern red oak: isolation, cultivation, and pathogenicity of xylem-limited bacterium. *Plant Dis.* 72:730–733.
- Hearon SS, Sherald JL, Kostka SJ. 1980. Association of xylem-limited bacteria with elm, sycamore, and oak leaf scorch. *Can. J. Bot.* 18: 1986–1993.
- Kostka SJ, Sherald JL, Tattar TA. 1984. Culture of fastidious, xylem-limited bacteria from declining oaks in the northeastern states. *Phytopathology* 74:803.
- 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. doi:[10.1128/JB.00651-10](https://doi.org/10.1128/JB.00651-10).
- Schreiber HL, IV, Koiralal M, Lara A, Ojeda M, Dowd SE, Bextine B, Morano L. 2010. Unraveling the first *Xylella fastidiosa* subsp. *fastidiosa* genome from Texas. *Southwest Entomol.* 35:479–483.

7. 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-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, et al. 2000. The *Xylella fastidiosa* consortium of the organization for nucleotide sequencing and analysis. *Nature* 406:151–159.
8. 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, Jr, Sasaki FT, Sena JA, de Souza AA, Truffi D, et al. 2003. Comparative analyses of the complete genomes sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. *J. Bacteriol.* 185:1018–1026.
9. 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, Jr, Walunas TL, Purcell A, Edwards RA, Hawkins T, Haselkorn R, Overbeek R, Kyrides NC, Predki PF. 2002. Draft sequencing and comparative genomics of *Xylella fastidiosa* strains reveal novel biological insights. *Genome Res.* 12:1556–1563.
10. 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.
11. Schaad NW, Postnikova E, Lacy G, Fatmi M, Chang CJ. 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. [correction] *fastidiosa* [correction] subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. *Syst. Appl. Microbiol.* 27:290–300.
12. Chen J, Lamikanra O, Chang CJ, Hopkins DL. 1995. Randomly amplified polymorphic DNA analysis of *Xylella fastidiosa* Pierce's disease and oak leaf scorch pathogens. *Appl. Environ. Microbiol.* 61:1688–1690.
13. Davis MJ, French WJ, Schaad NW. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. *Curr. Microbiol.* 6:309–314.
14. Maniatis T, Fritsch EF, Sambrook J. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
15. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makrilia VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437:376–380.
16. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formasma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. doi:[10.1186/1471-2164-9-75](https://doi.org/10.1186/1471-2164-9-75).
17. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402.