



High-Quality Genome Sequence of *Xanthomonas axonopodis* pv. *glycines* Strain 12609 Isolated in Taiwan

Shu-Fen Weng,^a An-Chi Luo,^a Che-Jui Lin,^a Tsai-Tien Tseng^b

Institute of Molecular Biology, National Chung Hsing University, Taichung, Taiwan^a; Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, Georgia, USA^b

ABSTRACT The genomic sequence was determined for *Xanthomonas axonopodis* pv. *glycines* strain 12609, isolated in Taiwan. Based on the genome sequence, we predicted the encoded genes, rRNA, tRNA, a plasmid sequence, secretion systems, cyclic GMP- and cyclic di-GMP-mediated pathways, and the gene cluster *rpfABCHGDE* (regulation of pathogenicity factor).

The genus *Xanthomonas* contains 27 closely related plant-pathogenic species (1). *Xanthomonas axonopodis* pv. *glycines* is the bacterium causing pustules in soybeans, causing tremendous losses (2). It exhibits rapid cell death (RCD) response in rich media, in which the intracellular cyclic AMP (cAMP) levels are high until the onset of stationary phase but decline rapidly afterward (3). These features render *Xanthomonas axonopodis* pv. *glycines* suitable for comparative study with other xanthomonads, e.g., *Xanthomonas campestris* pv. *campestris*, which infects crucifers, does not exhibit RCD, and has no detectable levels of cAMP (4), although the adenylate cyclase (Cya) predicted for *X. axonopodis* pv. *glycines* 12609 (Cya_{Xag12609}) shares 92% identity with the Cya of *X. campestris* pv. *campestris* strain 17 (Cya_{Xcc17}) responsible for cAMP synthesis (5). For future study, the genome sequence of *X. axonopodis* pv. *glycines* 12609 (also named BCRC 12609) was determined by next-generation sequencing. Twenty-eight contigs were obtained from 30-fold coverage, giving 5,183,780 nucleotides (nt) (G+C content, 65%). Annotation with best-placed reference protein set and GeneMarkS+ version 3.3 (6) predicted 4,475 genes, 4,220 proteins, four rRNA genes, 52 tRNA genes, and a DNA sequence 99% identical to the plasmid unnamed2 (5,753 bp) in *X. axonopodis* pv. *glycines* CFBP2526 (7) and 100% identical to the 6-kb chromosomal copy of *X. axonopodis* pv. *glycines* strain 8ra (8).

Cyclic GMP (cGMP)- and cyclic di-GMP (c-di-GMP)-mediated pathways regulate multiple functions, including pathogenesis in xanthomonads (9). These pathways at least involve (i) diguanylate cyclase (DGC) and guanylate cyclase (GCase) required for the synthesis of c-di-GMP and cGMP, respectively; and (ii) cAMP receptor protein-like protein (Clp), the transcription factor required for the expression of hundreds of genes in *X. campestris* pv. *campestris* (10). In the presence of c-di-GMP, the Clp-promoter binding activity *in vitro* is lowered (11). Homologs of these proteins are found and are highly conserved in *X. axonopodis* pv. *glycines* 12609, suggesting that cGMP- and c-di-GMP-mediated pathways are operative in *X. axonopodis* pv. *glycines* 12609.

Protein secretion plays a central role in modulating the interactions of bacteria with their environments, including pathogenesis; six classes are known in Gram-negative bacteria (12). All these systems are present in *X. axonopodis* pv. *glycines*; in contrast, *X. campestris* pv. *campestris* has no type VI secretion system (T6SS). Whether T6SS is involved in functions other than pathogenesis, e.g., RCD and host specificity determination, deserves further study.

Received 15 December 2016 Accepted 23 December 2016 Published 23 February 2017

Citation Weng S-F, Luo A-C, Lin C-J, Tseng T-T. 2017. High-quality genome sequence of *Xanthomonas axonopodis* pv. *glycines* strain 12609 isolated in Taiwan. *Genome Announc* 5:e01695-16. <https://doi.org/10.1128/genomeA.01695-16>.

Copyright © 2017 Weng 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 Shu-Fen Weng, sfweng@dragon.nchu.edu.tw, or Tsai-Tien Tseng, tseng@kennesaw.edu.

The gene cluster *rpfABCHGDE* (regulation of pathogenicity factor) is involved in the synthesis of diffusible signal factor, which is required for the production of virulence factors (13). *X. axonopodis* pv. *glycines* 12609 also contained the *rpf* cluster but not *rpfI*, whose homolog in *X. campestris* pv. *campestris* encodes a regulatory protein for the production of protease and endoglucanase (14). However, our plate assay indicated that the levels of these enzymes in *X. axonopodis* pv. *glycines* 12609 were as high as those in the virulent *X. campestris* pv. *campestris* strain 17 (15), suggesting that their production in *X. axonopodis* pv. *glycines* is regulated differently.

Filamentous phage phiLf of *X. campestris* pv. *campestris* can integrate into the host chromosome by site-specific integration using the *dif* (deletion induced filamentation of chromosome) sequence as the *attB* site (16). Prophages similar to phiLf are present in the genomes of *X. axonopodis* pv. *glycines* strain 8ra (8) and *X. campestris* pv. *vesicatoria* strain 85-10 (17) but not in that of *X. axonopodis* pv. *glycines* 12609.

Accession number(s). The draft genome sequence of *X. axonopodis* pv. *glycines* 12609 (Bioresource Collection and Research Center, Taiwan) is now available in the GenBank database under accession number [MKCQ00000000](https://doi.org/10.1128/genomeA.01466-15).

ACKNOWLEDGMENTS

This work was supported by grants NSC-99-2321-B-005-010-MY3 and NSC-102-2313-B-005-027 from the Ministry of Science and Technology, Taiwan.

REFERENCES

- Royer M, Koebnik R, Marguerettaz M, Barbe V, Robin GP, Brin C, Carrere S, Gomez C, Hügelland M, Völler GH, Noëll J, Pieretti I, Rausch S, Verdier V, Poussier S, Rott P, Süßmuth RD, Cociancich S. 2013. Genome mining reveals the genus *Xanthomonas* to be a promising reservoir for new bioactive non-ribosomally synthesized peptides. *BMC Genomics* 14:658. <https://doi.org/10.1186/1471-2164-14-658>.
- Vauterin L, Hoste B, Kersters K, Swings J. 1995. Reclassification of *Xanthomonas*. *Int J Syst Bacteriol* 45:472–489. <https://doi.org/10.1099/00207713-45-3-472>.
- Raju KK, Gautam S, Sharma A. 2006. Molecules involved in the modulation of rapid cell death in *Xanthomonas*. *J Bacteriol* 188:5408–5416. <https://doi.org/10.1128/JB.00056-06>.
- de Crecy-Lagard V, Glaser P, Lejeune P, Sismeiro O, Barber CE, Daniels MJ, Danchin A. 1990. A *Xanthomonas campestris* pv. *campestris* protein similar to catabolite activation factor is involved in regulation of phytopathogenicity. *J Bacteriol* 172:5877–5883. <https://doi.org/10.1128/jb.172.10.5877-5883.1990>.
- Liu YC, Wang SC, Yu YJ, Fung KM, Yang MT, Tseng YH, Tsai SF, Sun HS, Lyu PC, Chou SH. 2015. Complete genome sequence of *Xanthomonas campestris* pv. *campestris* strain 17 from Taiwan. *Genome Announc* 3(6):e01466-15. <https://doi.org/10.1128/genomeA.01466-15>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 44: 6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Darrasse A, Bolot S, Serres-Giardi L, Charbit E, Boureau T, Fisher-Le Saux M, Briand M, Arlat M, Gagnevin L, Koebnik R, Noël LD, Carrère S, Jacques MA. 2013. High-quality draft genome sequences of *Xanthomonas axonopodis* pv. *glycines* strains CFBP 2526 and CFBP 7119. *Genome Announc* 1(6):e01036-13. <https://doi.org/10.1128/genomeA.01036-13>.
- Lee JH, Shin H, Park HJ, Ryu S, Han SW. 2014. Draft genome sequence of *Xanthomonas axonopodis* pv. *glycines* 8ra possessing transcription activator-like effectors used for genetic engineering. *J Biotechnol* 179: 15–16. <https://doi.org/10.1016/j.jbiotec.2014.03.009>.
- An SQ, Chin KH, Febrer M, McCarthy Y, Yang JG, Liu CL, Swarbrick D, Rogers J, Maxwell Dow J, Chou SH, Ryan RP. 2013. A cyclic GMP-dependent signalling pathway regulates bacterial phytopathogenesis. *EMBO J* 32:2430–2438. <https://doi.org/10.1038/emboj.2013.165>.
- He YW, Ng AY, Xu M, Lin K, Wang LH, Dong YH, Zhang LH. 2007. *Xanthomonas campestris* cell-cell communication involves a putative nucleotide receptor protein Clp and a hierarchical signalling network. *Mol Microbiol* 64:281–292. <https://doi.org/10.1111/j.1365-2958.2007.05670.x>.
- Chin KH, Lee YC, Tu ZL, Chen CH, Tseng YH, Yang JM, Ryan RP, McCarthy Y, Dow JM, Wang AH, Chou SH. 2010. The cAMP receptor-like protein CLP is a novel c-di-GMP receptor linking cell-cell signaling to virulence gene expression in *Xanthomonas campestris*. *J Mol Biol* 396:646–662. <https://doi.org/10.1016/j.jmb.2009.11.076>.
- Tseng TT, Tyler BM, Setubal JC. 2009. Protein secretion systems in bacterial-host associations, and their description in the gene ontology. *BMC Microbiol* 9:S2. <https://doi.org/10.1186/1471-2180-9-S1-S2>.
- Dow JM, Crossman L, Findlay K, He YQ, Feng JX, Tang JL. 2003. Biofilm dispersal in *Xanthomonas campestris* is controlled by cell-cell signaling and is required for full virulence to plants. *Proc Natl Acad Sci U S A* 100:10995–11000. <https://doi.org/10.1073/pnas.1833360100>.
- Dow JM, Feng JX, Barber CE, Tang JL, Daniels MJ. 2000. Novel genes involved in the regulation of pathogenicity factor production within the *rpf* gene cluster of *Xanthomonas campestris*. *Microbiology* 146:885–891. <https://doi.org/10.1099/00221287-146-4-885>.
- Yang BY, Tseng YH. 1988. Production of exopolysaccharide and levels of protease and pectinase activity in pathogenic and non-pathogenic strains of *Xanthomonas campestris* pv. *campestris*. *Bot Bull Acad Sin* 29:93–99.
- Lin NT, Chang RY, Lee SJ, Tseng YH. 2001. Plasmids carrying cloned fragments of RF DNA from the filamentous phage phiLf can be integrated into the host chromosome via site-specific integration and homologous recombination. *Mol Genet Genomics* 266:425–435. <https://doi.org/10.1007/s004380100532>.
- Thieme F, Koebnik R, Bekel T, Berger C, Boch J, Büttner D, Caldana C, Gaigalat L, Goesmann A, Kay S, Kirchner O, Lanz C, Linke B, McHardy AC, Meyer F, Mittenhuber G, Nies DH, Niesbach-Klösgen U, Patschkowski T, Rückert C, Rupp O, Schneiker S, Schuster SC, Vorhölter FJ, Weber E, Pühler A, Bonas U, Bartels D, Kaiser O. 2005. Insights into genome plasticity and pathogenicity of the plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* revealed by the complete genome sequence. *J Bacteriol* 187:7254–7266. <https://doi.org/10.1128/JB.187.21.7254-7266.2005>.