Complete Genome Sequence of Strain WHRI 3811 Race 1 of *Xanthomonas campestris* pv. *campestris*, the Causal Agent of Black Rot of Cruciferous Vegetables

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Abstract

Xanthomonas campestris pv. *campestris* is an important bacterial pathogen that causes black rot and brings about enormous production loss for cruciferous vegetables worldwide. Currently, genome sequences for only a few *X. campestris* pv. *campestris* isolates are available, most of which are draft sequences. Based on the next-generation sequencing and single-molecule sequencing in real time technologies, we present here the complete genome sequence of strain WHRI 3811 race 1 of *X. campestris* pv. *campestris*, which is a type strain that has been extensively used. The genome data will contribute to our understanding of *X. campestris* pv. *campestris* genomic features and pave the way for research on *X. campestris* pv. *campestris*–host interactions.

Xanthomonas campestris, a member of the bacterial genus *Xanthomonas*, ranks as high as fifth in all the bacterial plant pathogens, based on their scientific and economic importance (Mansfield et al. 2012). *X. campestris* pv. *campestris* is the causal agent of black rot of many cruciferous crops, including cabbage, broccoli, cauliflower, and Chinese cabbage, and has caused considerable production losses worldwide every year (Hayward 1993; Vauterin et al. 1990; Vicente and Holub 2013).

Black rot caused by *X. campestris* pv. *campestris* was first described as a disease of cabbage in the United States and has now been found almost all over the world, wherever crucifers are grown (Garman 1894; Jensen et al. 2010; Vicente et al. 2001; Williams 1980). Until now, 11 races have been identified using differential hosts and molecular ways, with race 1 and 4 being most prevalent worldwide (Cruz et al. 2017; Fargier and Manceau 2007; Rouhrazi and Khodakaramian 2014; Singh et al. 2016; Vicente et al. 2001). Currently, genome sequences from only a few isolates with limited origins for races 1, 3, 4, and 9 are available, most of which are draft sequences (Bolot et al. 2015; da Silva et al. 2002; Desai et al. 2015; Horta de Passo 2016; Qian et al. 2005; Vorhölter et al. 2008).

Additional genome sequences for various *X. campestris* pv. *campestris* isolates will contribute to our knowledge of *X. campestris* pv. *campestris* genomic diversity. Here, the *X. campestris* pv. *campestris* strain WHRI 3811 (PHW 1205) race 1, isolated in the United States from *Brassica oleracea*, which has been widely used in previous studies as a type strain (Taylor et al. 2002; Vicente et al. 2001), was subjected to whole-genome sequencing. The pathogen was incubated on nutrient broth medium and genomic DNA was extracted with

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Keywords

bacterial pathogenesis, genomics

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the DNeasy blood and tissue kit (Qiagen Inc., Shanghai, China). To obtain the complete genome, two libraries were constructed: 20K of the single-molecule sequencing in real time (SMRT) library, specifying standard sequencing mode with insert size of 20 kb under the platform of PacBio (Pacific Biosciences Inc., Menlo Park, CA, U.S.A.), and PE400 of the next-generation sequencing (NGS) library, specifying a 2 × 251 bp paired-end with an insert size of 400 bp under the platform of Illumina Miseq (Illumina Inc., San Diego, CA, U.S.A.).

De novo assembling and annotation procedures included the following. i) For assembly of the NGS data, a total of 7,577,892 reads totaling 2.13-Gb bases were generated, using the Miseq platform. After data quality control and trimming, the high-quality data of 7,520,546 reads totaling 1.92-Gb bases were obtained and assembled to NGS scaffolds and contigs, using the software A5-miseq v20150522 (Coil et al. 2015) and SPAdes v3.9.0 (Bankevich et al. 2012). ii) For assembly of the SMRT data, a total of 94,617 sequences totaling 0.89-Gb bases were generated, using the PacBio platform, and were then assembled to SMRT scaffolds and contigs, using the tool Canu v1.4 (Koren et al. 2017). iii) For mix assembly, the NGS and SMRT data were assembled together to NGS-SMRT scaffolds and contigs, using SPAdes v3.9.0 (Bankevich et al. 2012). iv) For final assembly, all the scaffolds and contigs were aligned and assembled together, using MUMmer v3.1 (Kurtz et al. 2004), and the final assembly was confirmed using Pilon v1.18 (Walker et al. 2014). v) For annotation, GLIMMER 3.0 (Delcher et al. 1999), tRNAscan-SE v1.3.1 (Lowe and Eddy 1997), and RNAmmer v1.2 (Lagesen et al. 2007) were used for coding and noncoding gene prediction.

The final assembly of the chromosome genome was 5,072,566 bp in length, with 0 gaps and a GC content of 65.1%. There were, in total, 4,661 protein-coding genes, six ribosomal RNA genes, 53 transfer RNA genes, and 131 other noncoding RNA genes. In addition, one plasmid comprised 39,063 bp with GC content of 62.4% and 50 protein-coding genes. So far, complete genomes of three *X. campestris* pv. *campestris* strains have been sequenced, including ATCC 33913 (race 3) (da Silva et al. 2002), 8004 (race 9) (Qian et al. 2005), and B100 (race 1) (Vorhölter et al. 2008). The lengths of these genomes were 5,076,1875, 5,148,708, and 5,079,002 bp, respectively, harboring 4,181, 4,273, and 4,471 protein-coding genes, respectively. Compared with them, the number of predicted protein-coding genes was the largest in WHRI 3811 and only WHRI 3811 had one plasmid. These genomic data will contribute to our understanding of *X. campestris* pv. *campestris* genomic variation, *X. campestris* pv. *campestris*–host interactions, and, ultimately, will be useful for the development of strategies to prevent and control this disease.

This whole-genome shotgun project has been deposited in the GenBank database under accession number CP025750.

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Literature Cited

- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Pribelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., and Pevzner, P. A. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19:455-477.
- Bolot, S., Cerutti, A., Carrère, S., Arlat, M., Fischer-Le Saux, M., Portier, P., Poussier, S., Jacques, M. A., and Noël, L. D. 2015. Genome sequences of the race 1 and race 4 *Xanthomonas campestris* pv. *campestris* strains CFBP 1869 and CFBP 5817. *Genome Announc.* 3:e01023-15.
- Coil, D., Jospin, G., and Darling, A. E. 2015. A5-miseq: An updated pipeline to assemble microbial genomes from Illumina MiSeq data. *Bioinformatics* 31: 587-589.
- Cruz, J., Tenreiro, R., and Cruz, L. 2017. Assessment of diversity of *Xanthomonas campestris* affecting cruciferous plants in Portugal and disclosure of two novel *X. campestris* pv. *campestris* races. *J. Plant Pathol.* 99:403-414.
- da Silva, A. C., Ferro, J. A., Reinach, F. C., Farah, C. S., Furlan, L. R., Quaggio, R. B., Monteiro-Vitorello, C. B., Van Sluys, M. A., Almeida, N. F., Alves, L. M., do Amaral, A. M., Bertolini, M. C., Camargo, L. E., Camarotte, G., Cannavan, F., Cardozo, J., Chambergo, F., Ciapina, L. P., Cicarelli, R. M., Coutinho, L. L., Cursino-Santos, J. R., El-Dorry, H., Faria, J. B., Ferreira, A. J., Ferreira, R. C., Ferro, M. I., Formighieri, E. F., Franco, M. C., Greggio, C. C., Gruber, A., Katsuyama, A. M., Kishi, L. T., Leite, R. P., Lemos, E. G., Lemos, M. V., Locali, E. C., Machado, M. A., Madeira, A. M., Martinez-Rossi, N. M., Martins, E. C., Meidanis, J., Menck, C. F., Miyaki, C. Y., Moon, D. H., Moreira, L. M., Novo, M. T., Okura, V. K., Oliveira, M. C., Oliveira, V. R., Pereira, H. A., Rossi, A., Sena, J. A., Silva, C., de Souza, R. F., Spinola, L. A., Takita, M. A., Tamura, R. E., Teixeira, E. C., Tezza, R. I., Trindade dos Santos, M., Truffi, D., Tsai, S. M., White, F. F., Setubal, J. C., and Kitajima, J. P. 2002. Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature* 417:459-463.
- Delcher, A. L., Harmon, D., Kasif, S., White, O., and Salzberg, S. L. 1999. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.* 27:4636-4641.
- Desai, D., Li, J. H., van Zijl de Jong, E., Braun, R., Pitman, A., Visnovsky, S., Hampton, J., and Christey, M. 2015. Draft genome sequences of two New Zealand *Xanthomonas campestris* pv. *campestris* isolates, ICMP 4013 and ICMP 21080. *Genome Announc.* 3:e01247-15.

- Fargier, E., and Manceau, C. 2007. Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris* pv. *campestris*. Plant Pathol. 56:805-818.
- Garman, H. 1894. A bacterial disease of cabbage. Kentucky Agricultural Experiment Station Rep. 3:43-46.
- Hayward, A. C. 1993. The hosts of *Xanthomonas*. Pages 1-119 in: *Xanthomonas*. Swings, J. G., and Civerolo, E. L., eds. Chapman & Hall, London.
- Horta de Passo, V. 2016. Molecular genetics of interactions between *Xanthomonas campestris* pv. *raphani* and *Arabidopsis thaliana*. Ph.D. thesis. University of Warwick, Warwick, U.K.
- Jensen, B. D., Vicente, J. G., Manandhar, H. K., and Roberts, S. J. 2010. Occurrence and diversity of *Xanthomonas campestris* pv. *campestris* in vegetable brassica fields in Nepal. Plant Dis. 94:298-305.
- Koren, S., Walenz, B. P., Berlin, K., Miller, J. R., Bergman, N. H., and Phillippy, A. M. 2017. Canu: Scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. Genome Res. 27:722-736.
- Kurtz, S., Phillippy, A., Delcher, A. L., Smoot, M., Shumway, M., Antonescu, C., and Salzberg, S. L. 2004. Versatile and open software for comparing large genomes. Genome Biol. 5:R12.
- Lagesen, K., Hallin, P., Rødland, E. A., Staerfeldt, H. H., Rognes, T., and Ussery, D. W. 2007. RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100-3108.
- Lowe, T. M., and Eddy, S. R. 1997. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25:955-964.
- Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., and Foster, G. D. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. 13:614-629.
- Qian, W., Jia, Y., Ren, S. X., He, Y. Q., Feng, J. X., Lu, L. F., Sun, Q., Ying, G., Tang, D. J., Tang, H., Wu, W., Hao, P., Wang, L., Jiang, B. L., Zeng, S., Gu, W. Y., Lu, G., Rong, L., Tian, Y., Yao, Z., Fu, G., Chen, B., Fang, R., Qiang, B., Chen, Z., Zhao, G. P., Tang, J. L., and He, C. 2005. Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris*. Genome Res. 15:757-767.
- Rouhraz, K., and Khodakaramian, G. 2014. Genetic fingerprinting of Iranian *Xanthomonas campestris* pv. *campestris* strains inducing black rot disease of crucifers. Eur. J. Plant Pathol. 139:175-184.
- Singh, D., Rathaur, P. S., Vicente, J. G., Palleroni, N. J., Hildebrand, D. C., Stead, D. E., Civerolo, E. L., Hayward, A. C., Maraite, H., Stall, R. E., Vidaver, A. K., and Bradbury, J. F. 2016. Characterization, genetic diversity and distribution of *Xanthomonas campestris* pv. *campestris* races causing black rot disease in cruciferous crops of India. Plant Pathol. 65:1411-1418.
- Taylor, J. D., Conway, J., Roberts, S. J., Astley, D., and Vicente, J. G. 2002. Sources and Origin of Resistance to *Xanthomonas campestris* pv. *campestris* in Brassica Genomes. Phytopathology 92:105-111.
- Vauterin, L., Swings, J., Kersters, K., Gillis, M., Mew, T. W., and Schroth, M. N. 1990. Towards an improved taxonomy of *Xanthomonas*. Int. J. Syst. Bacteriol. 40:312-316.
- Vicente, J. G., Conway, J., Roberts, S. J., and Taylor, J. D. 2001. Identification and origin of *Xanthomonas campestris* pv. *campestris* races and related pathovars. Phytopathology 91:492-499.
- Vicente, J. G., and Holub, E. B. 2013. *Xanthomonas campestris* pv. *campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. Mol. Plant Pathol. 14:2-18.
- Vorhölter, F. J., Schneiker, S., Goesmann, A., Krause, L., Bekel, T., Kaiser, O., Linke, B., Patschkowski, T., Rückert, C., Schmid, J., Sidhu, V. K., Sieber, V., Tauch, A., Watt, S. A., Weisshaar, B., Becker, A., Niehaus, K., and Pühler, A. 2008. The genome of *Xanthomonas campestris* pv. *campestris* B100 and its use for the reconstruction of metabolic pathways involved in xanthan biosynthesis. J. Biotechnol. 134:33-45.
- Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., Young, S. K., and Earl, A. M. 2014. Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963.
- Williams, P. H. 1980. Black rot: A continuing threat to world crucifers. Plant Dis. 64: 736-742.