



# Complete Genome Sequence of *Xanthomonas campestris* pv. *campestris* SB80, a Race 4 Strain Isolated from White Head Cabbage in Turkey

 Songül Erken Meral,<sup>a</sup>  Shaheen Bibi,<sup>b</sup>  Carlos Andrés Díaz Rodríguez,<sup>c</sup>  Jelena Menković,<sup>d</sup>  Adriana J. Bernal,<sup>c</sup>  Ralf Koebnik<sup>e</sup>

<sup>a</sup>Department of Plant Health, Black Sea Agricultural Research Institute, Samsun, Turkey

<sup>b</sup>Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, Pennsylvania, USA

<sup>c</sup>Department of Biological Sciences, Universidad de Los Andes, Bogotá, Colombia

<sup>d</sup>University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

<sup>e</sup>Plant Health Institute of Montpellier, University of Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France

**ABSTRACT** Here, we report the complete genome sequence of the race 4 strain *Xanthomonas campestris* pv. *campestris* SB80, which was isolated from a symptomatic white head cabbage leaf in Samsun Province, Turkey, in 2019. The genome consists of a circular chromosome (5,129,762 bp) with a G+C content of 64.98%, for which 4,159 putative protein-coding genes, 2 rRNA operons, 54 tRNAs, and 86 noncoding RNAs (ncRNAs) were predicted.

**B**acteria of the species *Xanthomonas campestris* cause black rot, which is the bacterial disease that causes the most devastation to *Brassicaceae* family plants worldwide. The pathovar *X. campestris* pv. *campestris* has been divided into 11 races based on interactions with a differential set of *Brassica* cultivars, with races 1 and 4 being the most prevalent and destructive (1, 2). Only one draft genome sequence of a race 4 strain, isolated in Chile in 2001, is available at NCBI GenBank (3).

*X. campestris* pv. *campestris* strain SB80 was isolated from a symptomatic leaf of white head cabbage (*Brassica oleracea* var. *capitata*) growing in a field in Samsun Province, Turkey, in 2019, as described (4). The race of strain SB80 was determined by evaluating the reactions of various *Brassica* sp. genotypes (compatible, Miracle F1, SxD1, Wiroso F1; incompatible, FBLM2, PIC1, Seven Top Turnip, COB60, Just Right Hybrid Turnip) (1). For DNA isolation, a single colony was grown at 28°C on PSA medium (0.5% peptone, 2% sucrose, 1.5% agar) for 24 h. Bacteria were then resuspended in 10 mM MgCl<sub>2</sub> and diluted to an optical density at 600 nm of 1.0. Cells from 2 mL of this suspension were harvested by centrifugation, washed once with 10 mM MgCl<sub>2</sub>, and genomic DNA was isolated using the Genomic-tip 100/G protocol (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

For library construction and sequencing, performed by OhmX.bio (Ghent, Belgium), 1 μg DNA was mechanically fragmented using g-TUBE devices (Covaris, Woburn, MA) at approximately 13 kb. The sequencing library was prepared using the ligation sequencing kit (SQK-LSK110) and the native barcode expansion (PCR-free) pack (EXP-NBD114) based on the manufacturer's protocol (ONT, Oxford, UK). The samples were sequenced on a GridION R9.4 flow cell for a total of 3 days. Bases were called using MinKNOW v21.10.8. The demultiplexed sequence reads (34,944; N<sub>50</sub>, 14,461 bp) were provided by OhmX.bio as FASTQ files.

Adapter sequences were trimmed from the reads using Porechop v0.2.1 (5). The raw reads were checked for quality using NanoFilt (6). The sequences were assembled using Flye v2.9 (7). Default parameters were used for all software unless otherwise specified. Closer inspection revealed issues with homopolymeric nucleotide runs, some of which were manually changed to match the high-quality reference genome sequences for strains ATCC 33913 and 8004 (8, 9). In addition to a large contig of 5.1 Mbp, corresponding to the circular

**Editor** David A. Baltrus, University of Arizona

**Copyright** © 2022 Erken Meral 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 Ralf Koebnik, koebnik@gmx.de.

The authors declare no conflict of interest.

**Received** 13 January 2022

**Accepted** 7 February 2022

**Published** 22 February 2022

chromosome, the Flye assembly resulted in a second contig of 23 kb, which was almost identical to a region in the large contig and did not encode typical plasmid-associated genes, suggesting an assembly artifact. Notably, this 23-kb region contained a perfect tandem duplication of 1,792 bp in the chromosome but not in the smaller contig. Again, comparison with the two reference genomes prompted us to delete the smaller contig from the assembly and to remove one copy of the duplication in the chromosome.

Assembly and polishing yielded one circular chromosome of 5,129,762 bp with a typical G+C content of 64.98%, corresponding to 136× sequence coverage. The chromosome was annotated using GeneMarkS-2+ (10), as implemented in the NCBI Prokaryotic Genome Annotation Pipeline ([http://www.ncbi.nlm.nih.gov/genome/annotation\\_prok/](http://www.ncbi.nlm.nih.gov/genome/annotation_prok/)), which predicted a total of 4,520 genes, including 4,159 coding genes, 215 pseudogenes, 86 noncoding RNAs (ncRNAs), 54 tRNAs, and 2 rRNA operons (5S, 16S, 23S).

This genome sequence for *X. campestris* from Turkey will facilitate the identification of race-specific factors in *X. campestris* pv. *campestris* and thus contribute to the development and employment of resistant cabbage cultivars. Interestingly, this strain does not contain an endogenous plasmid, as the other sequenced race 4 strain does. Calculation of genome-wide average nucleotide identities demonstrates that both sequenced race 4 strains belong to two different clades of *X. campestris* pv. *campestris* (11, 12).

**Data availability.** The genome sequence and raw sequencing reads for strain SB80 were deposited under GenBank accession number [CP089952](#), BioProject accession number [PRJNA785926](#), BioSample accession number [SAMN23597367](#), and SRA accession number [SRR17407536](#).

## ACKNOWLEDGMENTS

We thank the Institut de Recherche pour le Développement, France, for supporting the North-South-South Network on Xanthomonads (NSSLN-X) within the International Scientific Coordination Network—South (GDRI-Sud). S.E.M., J.M., and R.K. thank the European Cooperation in Science and Technology (COST) program for support of the EuroXanth COST Action CA16107, which was key in initiating this collaborative genome project. J.M. was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, and Faculty of Agriculture contract number 451-03-9/2021-14/200116.

We thank Volkan Cevik (University of Bath, UK) for providing the seed material for race typing.

## REFERENCES

- Vicente JG, Holub EB. 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. <https://doi.org/10.1111/j.1364-3703.2012.00833.x>.
- Cruz J, Tenreiro R, Cruz L. 2017. Assessment of diversity of *Xanthomonas campestris* pathovars affecting cruciferous plants in Portugal and disclosure of two novel *X. campestris* pv. *campestris* races. *J Plant Pathol* 99:403–414. <https://doi.org/10.4454/jpp.v99i2.3890>.
- Bolot S, Cerutti A, Carrère S, Arlat M, Fischer-Le Saux M, Portier P, Poussier S, Jacques MA, Noël LD. 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. <https://doi.org/10.1128/genomeA.01023-15>.
- Schaad NW, Jones JB, Chun W. 2001. Laboratory guide for identification of plant pathogenic bacteria, 3rd ed. APS Press, St. Paul, MN.
- Wick RR. 2018. Porechop: an adapter trimmer for Oxford Nanopore reads. <https://github.com/rwick/Porechop>.
- De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics* 34:2666–2669. <https://doi.org/10.1093/bioinformatics/bty149>.
- Kolmogorov M, Bickhart DM, Behsaz B, Gurevich A, Rayko M, Shin SB, Kuhn K, Yuan J, Pevzner PA. 2020. metaFlye: scalable long-read metagenome assembly using repeat graphs. *Nat Methods* 17:1103–1110. <https://doi.org/10.1038/s41592-020-00971-x>.
- da Silva AC, Ferro JA, Reinach FC, Farah CS, Furlan LR, Quaggio RB, Monteiro-Vitorello CB, Van Sluys MA, Almeida NF, Alves LM, do Amaral AM, Bertolini MC, Camargo LE, Camarotte G, Cannavan F, Cardozo J, Chambergro F, Ciapina LP, Cicarelli RM, Coutinho LL, Cursino-Santos JR, El-Dorri H, Faria JB, Ferreira AJ, Ferreira RC, Ferro MI, Formighieri EF, Franco MC, Greggio CC, Gruber A, Katsuyama AM, Kishi LT, Leite RP, Lemos EG, Lemos MV, Locali EC, Machado MA, Madeira AM, Martinez-Rossi NM, Martins EC, Meidanis J, Menck CF, Miyaki CY, Moon DH, Moreira LM, Novo MT, Okura VK, Oliveira MC, Oliveira VR, Pereira HA, Rossi A, Sena JA, Silva C, de Souza RF, Spinola LA, Takita MA, Tamura RE, Teixeira EC, et al. 2002. Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature* 417:459–463. <https://doi.org/10.1038/417459a>.
- Qian W, Jia Y, Ren SX, He YQ, Feng JX, Lu LF, Sun Q, Ying G, Tang DJ, Tang H, Wu W, Hao P, Wang L, Jiang BL, Zeng S, Gu WY, Lu G, Rong L, Tian Y, Yao Z, Fu G, Chen B, Fang R, Qiang B, Chen Z, Zhao GP, Tang JL, He C. 2005. Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris*. *Genome Res* 15:757–767. <https://doi.org/10.1101/gr.3378705>.
- Lomsadze A, Gemayel K, Tang S, Borodovsky M. 2018. Modeling leaderless transcription and atypical genes results in more accurate gene prediction in prokaryotes. *Genome Res* 28:1079–1089. <https://doi.org/10.1101/gr.230615.117>.
- Guy E, Genissel A, Hajri A, Chabannes M, David P, Carrere S, Lautier M, Roux B, Boureau T, Arlat M, Poussier S, Noël LD. 2013. Natural genetic variation of *Xanthomonas campestris* pv. *campestris* pathogenicity on *Arabidopsis* revealed by association and reverse genetics. *mBio* 4:e00538–12. <https://doi.org/10.1128/mBio.00538-12>.
- Erken MS, Bibi S, Díaz RC, Menković J, Bernal AJ, Koebnik R. 2022. ANI-based phylogenetic tree of *Xanthomonas campestris* pv. *campestris*. *figshare* <https://doi.org/10.6084/m9.figshare.18144785.v2>.