Draft genome sequence of Xanthomonas arboricola pv. pruni PVCT 262.1 isolated from Prunus dulcis in italy

Giulio Dimaria, Alexandros Mosca, Marcella Russo, Jaime Cubero, Joël F Pothier, Ralf Koebnik, Vittoria Catara

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

| Genomics and Proteomics | Announcement

Draft genome sequence of *Xanthomonas arboricola* pv. pruni PVCT 262.1 isolated from *Prunus dulcis* in italy

Giulio Dimaria,[1] Alexandros Mosca,[1] Marcella Russo,[2] Jaime Cubero,[3] Joël F. Pothier,[4] Ralf Koebnik,[5] Vittoria Catara[1]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT Here, we report the draft genome sequence of *Xanthomonas arboricola* pv. pruni strain PVCT 262.1, isolated from almond (*Prunus dulcis*) leaves affected by bacterial spots in Italy in 2020. Genome size is 5,076,418 bp and G+C content is 65.44%. A total of 4,096 protein-coding genes and 92 RNAs are predicted.

KEYWORDS *Xanthomonas*, plant pathology, genomics, *Prunus*

Xanthomonas arboricola pv. pruni (*Xap*) is the causal agent of bacterial spots of stone fruits and almonds (1). The disease is currently distributed across almost all stone fruit-producing countries and mainly affects peach, nectarine, apricot, and Japanese plum production (2). In Italy, it has been reported on cherry laurel (3), peach, plum (4), and more recently on almond (5).

We report the draft genome sequence of *Xap* strain PVCT 262.1, isolated from almond leaves exhibiting bacterial spot symptoms collected in a commercial orchard in the Agrigento province, Sicily, Italy [Global Positioning System (GPS) coordinates, 37.290807°N, 13.584432°E] in 2020.

For *Xap* isolation, leaf tissue (2 mm) from lesion margins was crushed with sterile water. The suspensions were spread onto Yeast-Peptone-Glucose Agar (YPGA). Plates were incubated at 28°C for 2–3 days.

Xap was identified using conventional duplex PCR with specific primers (*Xarb*Q-F/*Xarb*Q-R and *Xap*Y17-F/*Xap*Y17-R) (6), *rpoD* gene partial sequencing (99.63% identity with *Xap* 15-088, GenBank accession number CP044334.1), and pathogenicity tests on 1-year-old grafted almond plants and detached leaves (7). For total DNA extraction and whole-genome sequencing, a single bacterial colony was transferred from YPGA to lysogenic broth (LB) and incubated overnight (180 rpm, 28°C). DNA was extracted from 1 mL culture (1×10^9 cfu mL^{-1}) with Gentra Puregene bacterial DNA extraction kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

Sequencing libraries were constructed at Beijing Novogene Bioinformatics Technology Co., Ltd using Illumina NEBNext Ultra DNA Library Prep Kit (NEB, USA; 350 bp insert size), according to the manufacturer's recommendations. Sequencing was performed on Illumina NovaSeq 6000 platform (sequencing depth, 312×; paired-end read length, 150 bp). Raw reads (14,580,000 bp) were demultiplexed, recorded in FASTQ file using bcl2fastq v2.19 and quality filtered with Fastp v0.23.1 (8), obtaining 11,366,667 bp clean reads (Q_{30} = 94.62%).

Reads were assembled using SOAPdenovo v2.04 (9), SPAdes v3.11.1 (10), Abyss v1.5.2 (11), and CISA v1.3 (12) for integration. Gaps were solved with SOAP GapCloser v1.12 (9). The annotation was performed on the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.6 (13). Default parameters were used except where otherwise noted.

The draft genome assembly generated 60 scaffolds (>500 bp) with 5,076,418 bp total length (genome coverage, 99.99×; N_{50} length, 165,685 bp) and 65.44% GC content. Genome completeness was estimated at 99.8% using BUSCO v5.6.1 (14) and

Editor David A. Baltrus, The University of Arizona, Tucson, Arizona, USA

Address correspondence to Vittoria Catara, vcatara@unict.it.

The authors declare no conflict of interest.

Received 18 March 2024
Accepted 16 May 2024
Published 11 June 2024

xanthomonadales_odb10 (2024-01-08) lineage. PGAP predicted 4,096 protein-coding genes, 188 pseudogenes, and 92 RNAs.

PVCT 262.1 genome harbors copper resistance genes (e.g., *copB*, *copD*- and *copL*-family, *cutA*, *cutC*), suggesting copper resistance acquisition. The latter was observed *in vitro* for Italian *Xap* isolates, although *copLAB* gene cluster was not detected, suggesting different copper resistance mechanisms (4). The type III effector gene *xopE3*, specific to pathovar pruni within *X. arboricola* (15), was identified.

The genome sequence provided here will help further studies on bacterial spot ecology and epidemiology, contributing to elucidate almond-*Xap* interactions.

ACKNOWLEDGMENTS

This article is based upon work from COST Action CA16107 "EuroXanth," supported by COST (European Cooperation in Science and Technology). The work was financially supported by Incentive Plan for Research (PIA.CE.RI.) 2020–2022 line 2 (MEDIT-ECO 5A722192155 Research Project) of the University of Catania (Italy).

AUTHOR AFFILIATIONS

[1]Department of Agriculture, Food, and Environment, University of Catania, Catania, Italy
[2]Agrobiotech Soc. Coop., Catania, Italy
[3]Departamento de Protección Vegetal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria/Consejo Superior de Investigaciones Científicas (INIA/CSIC), Madrid, Spain
[4]Environmental Genomics and Systems Biology Research Group, Institute for Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland
[5]Plant Health Institute of Montpellier, University of Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France

AUTHOR ORCIDs

Giulio Dimaria http://orcid.org/0009-0004-5210-1070
Joël F. Pothier http://orcid.org/0000-0002-9604-7780
Vittoria Catara http://orcid.org/0000-0001-8076-258X

AUTHOR CONTRIBUTIONS

Giulio Dimaria, Conceptualization, Formal analysis, Investigation, Methodology, Resources, Software, Writing – original draft, Writing – review and editing | Alexandros Mosca, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Writing – original draft, Writing – review and editing | Marcella Russo, Investigation, Methodology | Jaime Cubero, Methodology, Supervision, Validation, Writing – review and editing | Joël F. Pothier, Methodology, Software, Supervision, Validation, Writing – review and editing | Ralf Koebnik, Methodology, Supervision, Validation, Writing – review and editing | Vittoria Catara, Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

This Whole-Genome Shotgun project was deposited in GenBank under the accession number JAZHEZ000000000, BioProject accession number PRJNA1061488, BioSample accession number SAMN39271251. The version described is JAZHEZ010000000. SRA accession number is SRR27587392.

REFERENCES

1. Garita-Cambronero J, Palacio-Bielsa A, Cubero J. 2018. *Xanthomonas arboricola* pv. pruni, causal agent of bacterial spot of stone fruits and almond: its genomic and phenotypic characteristics in the *X. arboricola* species context. Mol Plant Pathol 19:2053–2065. https://doi.org/10.1111/mpp.12679
2. Stefani E. 2010. Economic significance and control of bacterial spot/canker of stone fruits caused by *Xanthomonas arboricola* pv. pruni. J Plant Pathol:S99–S103. https://doi.org/10.4454/jpp.v92i1sup.2511
3. Marchi G, Cinelli T, Surico G. 2011. Bacterial leaf spot caused by the quarantine pathogen *Xanthomonas arboricola* pv. pruni on cherry laurel in central Italy. Plant Dis 95:74. https://doi.org/10.1094/PDIS-07-10-0529
4. Giovanardi D, Dallai D, Stefani E. 2017. Population features of *Xanthomonas arboricola* pv. pruni from *Prunus* spp. orchards in northern Italy. Eur J Plant Pathol 147:761–771. https://doi.org/10.1007/s10658-016-1040-5
5. Gerin D, Cariddi C, De Miccolis Angelini RM, Dongiovanni C, Faretra F, Pollastro S. 2019. First report of bacterial spot caused by *Xanthomonas arboricola* pv. pruni on almond in Italy. Plant Dis 103:1018. https://doi.org/10.1094/PDIS-11-18-2006-PDN
6. Pothier JF, Pagani MC, Pelludat C, Ritchie DF, Duffy B. 2011. A duplex-PCR method for species- and pathovar-level identification and detection of the quarantine plant pathogen *Xanthomonas arboricola* pv. pruni. J Microbiol Methods 86:16–24. https://doi.org/10.1016/j.mimet.2011.03.019
7. Randhawa PS, Civerolo EL. 1985. A detached-leaf bioassay for *Xanthomonas campestris* pv. pruni. Phytopathology 75:1060–1063. https://doi.org/10.1094/Phyto-75-1060
8. Chen S, Zhou Y, Chen Y, Gu J. 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560
9. Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, et al. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. Gigascience 1:1–6. https://doi.org/10.1186/2047-217X-1-18
10. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021
11. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123. https://doi.org/10.1101/gr.089532.108
12. Lin SH, Liao YC. 2013. CISA: contig integrator for sequence assembly of bacterial genomes. PLoS ONE 8:e60843. https://doi.org/10.1371/journal.pone.0060843
13. Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the prokaryotic genome annotation pipeline reach with protein family model curation. Nucleic Acids Res 49:D1020–D1028. https://doi.org/10.1093/nar/gkaa1105
14. Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM. 2021. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol Biol Evol 38:4647–4654. https://doi.org/10.1093/molbev/msab199
15. Hajri A, Pothier JF, Fischer-Le Saux M, Bonneau S, Poussier S, Boureau T, Duffy B, Manceau C. 2012. Type three effector gene distribution and sequence analysis provide new insights into the pathogenicity of plant-pathogenic *Xanthomonas arboricola*. Appl Environ Microbiol 78:371–384. https://doi.org/10.1128/AEM.06119-11