



Genome Sequence of *Oenococcus oeni* UNQOe19, the First Fully Assembled Genome Sequence of a Patagonian Psychrotrophic Oenological Strain

Néstor G. Iglesias,^{a,b} Danay Valdés La Hens,^{c,d} Nair T. Olguin,^{b,c} Bárbara M. Bravo-Ferrada,^{b,c} Natalia S. Brizuela,^{b,c} E. Elizabeth Tymczyszyn,^{b,c} Horacio Bibiloni,^e Adriana C. Caballero,^f Lucrecia Delfederico,^c Liliana Semorile^{c,d}

^aLaboratorio de Ingeniería Genética y Biología Celular y Molecular, Instituto de Microbiología Básica y Aplicada, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Argentina

^bConsejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

^cLaboratorio de Microbiología Molecular, Instituto de Microbiología Básica y Aplicada, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Argentina

^dComisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC-BA), La Plata, Argentina

^eBodega Humberto Canale, General Roca, Río Negro, Argentina

^fFacultad de Ciencia y Tecnología de los Alimentos, Universidad Nacional del Comahue and PROBIEN-CONICET, Neuquén, Argentina

ABSTRACT *Oenococcus oeni* UNQOe19 is a native strain isolated from a Patagonian pinot noir wine undergoing spontaneous malolactic fermentation. Here, we present the 1.83-Mb genome sequence of *O. oeni* UNQOe19, the first fully assembled genome sequence of a psychrotrophic strain from an Argentinean wine.

Oenococcus oeni UNQOe19 is a native psychrotrophic strain isolated from a pinot noir wine undergoing spontaneous malolactic fermentation (MLF) at the oldest commercial winery in General Roca, North Patagonia, Argentina. Pinot noir grapes grow very well in the North Patagonian region due to the agroecological conditions there. In a previous work, we showed the prevalence of *O. oeni* and *Lactobacillus plantarum* strains in Patagonian wines undergoing spontaneous MLF, which suggests that both species are involved in leading the MLF process (1). In this work, we report the first fully assembled genome sequence of a psychrotrophic *O. oeni* strain, UNQOe19, which showed a good capacity for implantation in microvinification assays, driving the MLF at low incubation temperatures. This finding suggests its potential to be used as a malolactic starter culture at environmental temperatures below 15°C (2).

Strain UNQOe19 was grown in *Leuconostoc oenos* medium (MLO) (3) at 28°C for 7 days. To obtain DNA, 1 mg/ml of lysozyme with 1% sodium dodecyl sulfate was used. Proteins were removed with 0.1 µg/ml of proteinase K, followed by phenol-chloroform-isoamyl alcohol (25:24:1) extraction. A total of 16 µg of high-quality genomic DNA was required for library preparation and sequencing. A whole-genome shotgun library was constructed using a 20-kb SMRTbell version 1.0 template prep kit, followed by single-molecule real-time (SMRT) sequencing conducted on an RS II (Pacific Biosciences) sequencer. A total of 103,710 reads (588-fold coverage and a polymerase read N_{50} size of 14,765 bp), with an average length of 10,359 bp and an estimated accuracy of 85%, were used as input for *de novo* assembly with the Canu package (4). The Canu output consisted of a single circular contig without gaps; the chromosomal contig was 1,826,824 bp long with a 37.9% G+C content. Prediction and annotation of the coding sequences were conducted with GeneMarkS (5). Genome annotation was done using the NCBI Prokaryotic Genome Annotation Pipeline (6), and gene ontology relationships were estimated using Blast2GO version 5.1.1 (7). The Bacterial Pan-Genome Analysis (BPGA) pipeline (8) was used

Received 25 June 2018 Accepted 13 July 2018 Published 9 August 2018

Citation Iglesias NG, Valdés La Hens D, Olguin NT, Bravo-Ferrada BM, Brizuela NS, Tymczyszyn EE, Bibiloni H, Caballero AC, Delfederico L, Semorile L. 2018. Genome sequence of *Oenococcus oeni* UNQOe19, the first fully assembled genome sequence of a Patagonian psychrotrophic oenological strain. Microbiol Resour Announc 7:e00889-18. <https://doi.org/10.1128/MRA.00889-18>.

Editor J. Cameron Thrash, Louisiana State University

Copyright © 2018 Iglesias 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 Néstor G. Iglesias, gabriel.iglesias@unq.edu.ar, or Danay Valdés La Hens, dvhens@unq.edu.ar.

N.G.I. and D.V.L.H. contributed equally to this work.

to compare the presence/absence of genes in strain UNQOe19 with other *O. oeni* strains. Out of 1,891 predicted genes, 1,721 were identified as protein-coding DNA sequences, 118 were potential pseudogenes, and 52 were RNA coding genes (43 tRNAs, 6 rRNAs, and 3 noncoding RNAs); these findings are comparable to those for other *O. oeni* strains (9–13).

Compared with the genome annotation of *O. oeni* strain PSU-1 (14), 160 unique genes in *O. oeni* UNQOe19 were detected. Among them, 19 genes were related to cellular components, 29 to metabolic processes, 23 to DNA molecular functions, 11 to plasmid and related conjugal transfer proteins, 13 to transmembrane transporter activities, 21 to putative phage-related proteins, and 38 to hypothetical proteins. Interestingly, the remaining six genes were related to homeostasis and oxidoreductase activity stress response, which is similar to earlier results described for other lactic acid bacterial species (15–17).

Further detailed evaluation will help elucidate the molecular basis of *O. oeni* strain UNQOe19's potential as a malolactic starter culture and its ability to adapt to the stressful wine conditions, including low environmental temperatures, in the North Patagonian region.

Data availability. The whole-genome sequence of *O. oeni* strain UNQOe19 has been deposited at GenBank under the accession number [CP027431](https://www.ncbi.nlm.nih.gov/nuccore/CP027431).

ACKNOWLEDGMENTS

This work was funded by grants from the Universidad Nacional de Quilmes (Programa Microbiología Molecular Básica y Aplicada–Resolución [R] number 954/17), the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC-PBA; project PIT-AP-BA number 173/16), CIN–CONICET (PDTs CIN CONICET 2014 number 173), and ANPCyT (PICT 2014 number 1395, PICT 2016 number 3435).

D.V.L.H. and L.S. are members of the Research Career of CIC-BA, and N.G.I., N.T.O., B.M.B.-F., N.S.B., and E.E.T. are members of the Research Career of CONICET.

REFERENCES

- Valdés La Hens D, Bravo-Ferrada BM, Delfederico L, Caballero AC, Semorile LC. 2015. Prevalence of *Lactobacillus plantarum* and *Oenococcus oeni* during spontaneous malolactic fermentation in Patagonian red wines revealed by polymerase chain reaction-denaturing gradient gel electrophoresis with two targeted genes. *Aust J Wine Grape Res* 21:49–56. <https://doi.org/10.1111/ajgw.12110>.
- Manera C, Bravo-Ferrada BM, Tymczyszyn E, Delfederico L, Olguín N, Semorile LC, Valdés La Hens D. 2017. Aislamiento y selección de cepas psicrotolerantes de bacterias lácticas enológicas de la región patagónica. *In* IV Congreso Internacional Científico y Tecnológico–CONCyT 2017. <http://digital.cic.gba.gov.ar/handle/11746/6684>.
- Maicas S, González-Cabo P, Ferrer S, Pardo I. 1999. Production of *Oenococcus oeni* biomass to induce malolactic fermentation in wine by control of pH and substrate addition. *Biotechnol Lett* 21:349–353. <https://doi.org/10.1023/A:1005498925733>.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Res* 27:722–736. <https://doi.org/10.1101/gr.215087.116>.
- Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. *Nucleic Acids Res* 29:2607–2618. <https://doi.org/10.1093/nar/29.12.2607>.
- 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>.
- Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21:3674–3676. <https://doi.org/10.1093/bioinformatics/bti610>.
- Chaudhari NM, Gupta VK, Dutta C. 2016. BPGA—an ultra-fast pan-genome analysis pipeline. *Sci Rep* 6:24373. <https://doi.org/10.1038/srep24373>.
- Lamontanara A, Orrù L, Cattivelli L, Russo P, Spano G, Capozzi V. 2014. Genome sequence of *Oenococcus oeni* OM27, the first fully assembled genome of a strain isolated from an Italian wine. *Genome Announc* 2(4):e00658-14. <https://doi.org/10.1128/genomeA.00658-14>.
- Capozzi V, Russo P, Lamontanara A, Orrù L, Cattivelli L, Spano G. 2014. Genome sequences of five *Oenococcus oeni* strains isolated from Nero di Troia wine from the same terroir in Apulia, southern Italy. *Genome Announc* 2(5):e01077-14. <https://doi.org/10.1128/genomeA.01077-14>.
- Mendoza LM, Saavedra L, Raya RR. 2015. Draft genome sequence of *Oenococcus oeni* strain X₂L (CRL1947), isolated from red wine of north-west Argentina. *Genome Announc* 3(1):e01376-14. <https://doi.org/10.1128/genomeA.01376-14>.
- Jara C, Romero J. 2015. Genome sequences of three *Oenococcus oeni* strains isolated from Maipo Valley, Chile. *Genome Announc* 3(4):e00866-15. <https://doi.org/10.1128/genomeA.00866-15>.
- Mills DA, Rawsthorne H, Parker C, Tamir D, Makarova K. 2005. Genomic analysis of *Oenococcus oeni* PSU-1 and its relevance to winemaking. *FEMS Microbiol Rev* 29:465–475.
- Zhai Q, Xiao Y, Zhao J, Tian F, Zhang H, Narbad A, Chen W. 2017. Identification of key proteins and pathways in cadmium tolerance of *Lactobacillus plantarum* strains by proteomic analysis. *Sci Rep* 7:1182. <https://doi.org/10.1038/s41598-017-01180-x>.
- Tomita S, Lee I-C, van Swam II, Boeren S, Vervoort J, Bron PA, Kleerebezem M. 2016. Characterization of the transcriptional regulation of the *tarJKL* locus involved in ribitol-containing wall teichoic acid biosynthesis in *Lactobacillus plantarum*. *Microbiology* 162:420–432. <https://doi.org/10.1099/mic.0.000229>.
- Ramos JL, Martínez-Bueno M, Molina-Henares AJ, Terán W, Watanabe K, Zhang X, Gallegos MT, Brennan R, Tobes R. 2005. The TetR family of transcriptional repressors. *Microbiol Mol Biol Rev* 69:326–356. <https://doi.org/10.1128/MMBR.69.2.326-356.2005>.
- Margalef-Català M, Araque I, Bordons A, Reguán C, Bautista-Gallego J. 2016. Transcriptomic and proteomic analysis of *Oenococcus oeni* adaptation to wine stress conditions. *Front Microbiol* 7:1554. <https://doi.org/10.3389/fmicb.2016.01554>.