





Draft Genome Sequence of *Tenacibaculum ovolyticum* To-7Br, Recovered from a Farmed Atlantic Salmon (*Salmo salar*)

Ruben Avendaño-Herrera,^{a,b,c} Mónica Saldarriaga-Córdoba,^{b,d} Rute Irgang^{a,b}

^aUniversidad Andrés Bello, Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias de la Vida, Viña del Mar, Chile ^bCentro FONDAP, Interdisciplinary Center for Aquaculture Research, Universidad Andrés Bello, Viña del Mar, Chile ^cCentro de Investigación Marina Quintay, Universidad Andrés Bello, Valparaíso, Chile ^dCentro de Investigación en Recursos Naturales y Sustentabilidad, Universidad Bernardo O'Higgins, Santiago, Chile

ABSTRACT We present the draft genome sequence of *Tenacibaculum ovolyticum* isolate To-7Br, recovered from a gill of a farmed specimen of Atlantic salmon (*Salmo salar*) showing signs of tenacibaculosis. This study provides the first detailed insights into the genomic characteristics of *T. ovolyticum* isolated for the first time from fish farmed in Chile.

S pecies of the genus *Tenacibaculum* are Gram-negative, filamentous bacteria with gliding motility and are commonly found in marine environments (1). To date, 31 *Tenacibaculum* species are validly described (https://www.bacterio.net/), including several microorganisms recognized as putative pathogens associated with tenacibaculosis in fish (2, 3). Studies on the bacterial microbiota associated with fish diseases have described the presence of *Tenacibaculum dicentrarchi* (4), *Tenacibaculum finnmarkense* (5), *Tenacibaculum maritimum* (6, 7), and *Tenacibaculum piscium* (8) in Chilean salmon aquaculture. The present study describes the presence and genome sequence of a *T. ovolyticum* isolate retrieved from a gill of Atlantic salmon (*Salmo salar*) farmed in southern Chile (Seno Gala, 44°11′21″S, 73°8′1″W) in April 2018.

Strain To-7Br was obtained during a tenacibaculosis outbreak, with isolation on a marine agar 2216 (BD Difco) plate incubated at 18°C for 48 h, which resulted in mixed cultures. The dominant colony morphotype was streaked onto new marine agar 2216 plates to obtain pure cultures, which were stored at -80° C in marine broth supplemented with 10% (vol/ vol) glycerol. The taxonomic position of the organism was first determined by 16S rRNA gene sequencing by Macrogen. Two pure colonies were subjected to DNA extraction by employing the InstaGene matrix (Bio-Rad) according to the manufacturer's instructions. The 16S rRNA gene was PCR amplified using the universal primer pair pA and pH (9). The full-length 16S rRNA gene sequence (1,341 nucleotides) with the accession number OM420283 revealed 99.87% identity to *T. ovolyticum* strain da5A-8 (LC144619) and 99.85% identity to the type strain, IFO 15947 (NR_040912).

For genomic sequencing, DNA was purified with the Mag-Bind universal pathogen 96 kit (Omega Bio-Tek Inc.), and concentration was measured using the QuantiFluor dsDNA system on a Quantus fluorometer (Promega). Prior to *de novo* genome assembly, raw Illumina sequence data were quality checked with FastQC v.0.11.9 (https://www.bioinformatics .babraham.ac.uk/projects/fastqc/). Next-generation sequencing reads were preprocessed with the Geneious Prime v.2020.2.2 software (10), using the following workflow: (i) reads were paired using the "set paired reads" function (insert size = 300 bp); (ii) poor-quality bases were trimmed from read ends using the BBDuk Trimmer plugin (reads of <20 bp and those with a minimum quality score of <30 were removed), with paired-read overlaps trimmed to ensure complete adapter removal; (iii) coverage was normalized by down-sampling reads in high-depth genome areas of the genome with the "error correct" and "normalize reads" functions using BBNorm; and (d) duplicate reads were removed using the Dedupe plugin.

Editor Irene L. G. Newton, Indiana University, Bloomington

Copyright © 2022 Avendaño-Herrera et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Ruben Avendaño-Herrera, reavendano@yahoo.com, ravendano@unab.cl.

The authors declare no conflict of interest.

Received 14 March 2022 **Accepted** 18 May 2022 **Published** 2 June 2022 Assembly was performed with SPAdes v.3.15.2 run in Geneious Prime, and quality was checked by QUAST version 5.1 (11). This generated 97 contigs and 4,054,048 bp with a G+C content of 29.58%. To quantitatively evaluate the assembly quality, the following measures were considered: minimum (i.e., 1,026 bp), maximum (i.e., 323,162 bp), and average (i.e., 41,794 bp) contig length. The total assembly size coincided with the expected size of the genome (12). The draft genome sequence contained 3,706 coding sequences, 244 subsystems, and 46 RNAs (42 tRNAs, 3 pseudo-tRNAs, and 1 16S rRNA), as determined by using the RAST server v.2.0 (13, 14).

The *in silico* genome analysis revealed genes related to secretion systems, including the type IV secretion system (T4SS), T1SS, T6SS, and T9SS, as detected by TXSScan (http://mobyle .pasteur.fr/cgi-bin/portal.py#forms::txsscan). In addition, iron-related protein families involved in iron transport, siderophore synthesis, siderophore transport, transcriptional regulation, and iron storage were identified. Furthermore, hemolysis genes and genes associated with antibiotic resistance (i.e., resistance to tetracycline and fluoroquinolones), the stress response, and other metabolic pathways were found. The present study provides the first detailed insights into the genomic characteristics of *T. ovolyticum* isolated for the first time from fish farmed in Chile. Considering the culture viability of isolate To-7Br, more details about virulence potential will be reported in a future publication.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ ENA/GenBank under the accession number JAKLCZ00000000.1 (https://www.ncbi.nlm.nih .gov/nuccore/JAKLCZ000000000). The version described in this paper is version JAKLCZ010000000. BioProject ID PRJNA801316 is publicly available at https://www.ncbi.nlm .nih.gov/bioproject/PRJNA801316, containing BioSample SAMN25339059 (https://www.ncbi .nlm.nih.gov/biosample/SAMN25339059). The GenBank accession number for the 16SrRNA gene sequence is OM420283.

ACKNOWLEDGMENTS

This study was supported by the Agencia Nacional de Investigación y Desarrollo (ANID, Chile) through FONDECYT 1190283 and FONDAP grant 15110027.

REFERENCES

- Suzuki M. 2015. *Tenacibaculum*, p 1–7. *In* Whitman WB, Rainey F, Kämpfer P, Trujillo M, Chun J, DeVos P, Hedlund B, Dedysh S (ed), Bergey's manual of systematics of archaea and bacteria. John Wiley & Sons, Ltd., Chichester, United Kingdom.
- Avendaño-Herrera R, Toranzo AE, Magariños B. 2006. Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: a review. Dis Aquat Organ 71:255–266. https://doi.org/10.3354/dao071255.
- Nowlan JP, Lumsden JS, Russell S. 2020. Advancements in characterizing Tenacibaculum infections in Canada. Pathogens 9:1029. https://doi.org/10 .3390/pathogens9121029.
- Avendaño-Herrera R, Irgang R, Sandoval C, Moreno-Lira P, Houel A, Duchaud E, Poblete-Morales M, Nicolas P, Ilardi P. 2016. Isolation, characterization and virulence potential of *Tenacibaculum dicentrarchi* in salmonid cultures in Chile. Transbound Emerg Dis 63:121–126. https://doi.org/ 10.1111/tbed.12464.
- Bridel S, Olsen AB, Nilsen H, Bernardet J-F, Achaz G, Avendaño-Herrera R, Duchaud E. 2018. Comparative genomics of *Tenacibaculum dicentrarchi* and "*Tenacibaculum finnmarkense*" highlights intricate evolution of fish-pathogenic species. Genome Biol Evol 10:452–457. https://doi.org/10.1093/gbe/evy020.
- Apablaza P, Frisch K, Brevik ØJ, Småge SB, Vallestad C, Duesund H, Mendoza J, Nylund A. 2017. Primary isolation and characterization of *Tenacibaculum maritimum* from Chilean Atlantic salmon mortalities associated with *Pseudochattonella* spp. algal bloom. J Aquat Anim Health 29:143–149. https://doi.org/10 .1080/08997659.2017.1339643.
- Valdés S, Irgang R, Barros MC, Ilardi P, Saldarriaga-Córdoba M, Rivera-Bohle J, Madrid E, Gajardo-Córdova J, Avendaño-Herrera R. 2021. First report and characterization of *Tenacibaculum maritimum* isolates recovered from rainbow trout (*Oncorhynchus mykiss*) farmed in Chile. J Fish Dis 44:1481–1490. https:// doi.org/10.1111/jfd.13466.

- Avendaño-Herrera R, Olsen AB, Saldarriaga-Córdoba M, Colquhoun DJ, Duchaud E, Irgang R. 2021. First report of Tenacibaculum piscium isolates recovered from outbreaks in Chilean salmonids, p 160. *In* 20th International Conference on Diseases of Fish and Shellfish. EAFP 2021, 20–23 September.
- Edwards U, Rogall T, Blöcker H, Emde M, Böttger EC. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17: 7843–7853. https://doi.org/10.1093/nar/17.19.7843.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton 0.039w?>S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10 .1093/bioinformatics/bts199.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
- Aguilar-Bultet L, Falquet L. 2015. Secuenciación y ensamblaje de novo de genomas bacterianos: una alternativa para el estudio de nuevos patógenos. Rev Salud Anim 37:125–132.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. https://doi.org/10 .1186/1471-2164-9-75.
- Lee I, Kim YO, Park SC, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103. https://doi.org/10.1099/ijsem.0.000760.