



First Complete Genome Sequence of *Tenacibaculum dicentrarchi*, an Emerging Bacterial Pathogen of Salmonids

Horst Grothusen, Alejandro Castillo, Patricio Henríquez, Esteban Navas, Harry Bohle, Carolina Araya, Fernando Bustamante, Patricio Bustos, Marcos Mancilla

Laboratorio de Diagnóstico y Biotecnología, ADL Diagnostic Chile Ltda., Puerto Montt, Chile

Tenacibaculum-like bacilli have recently been isolated from diseased sea-reared Atlantic salmon in outbreaks that took place in the XI region (Región de Aysén) of Chile. Molecular typing identified the bacterium as *Tenacibaculum dicentrarchi*. Here, we report the complete genome sequence of the AY7486TD isolate recovered during those outbreaks.

Received 24 December 2015 Accepted 5 January 2016 Published 18 February 2016

Citation Grothusen H, Castillo A, Henríquez P, Navas E, Bohle H, Araya C, Bustamante F, Bustos P, Mancilla M. 2016. First complete genome sequence of *Tenacibaculum dicentrarchi*, an emerging bacterial pathogen of salmonids. Genome Announc 4(1):e01756-15. doi:10.1128/genomeA.01756-15.

Copyright © 2016 Grothusen et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Marcos Mancilla, mmancilla@adldiagnostic.cl.

embers of the genus Tenacibaculum are Gram-negative, marine fish pathogens, responsible for tenacibaculosis. Ulcerative lesions on different parts of the body, especially on the skin surface as pathognomonic signs, manifest the disease (1). Long, slender, filamentous rods are the typical morphology, which are tough to isolate using standard media for marine bacteria. Standard phylogeny determined by the sequencing of the 16S rRNA gene placed several species out of the genus Flexibacter; therefore, the genus Tenacibaculum was proposed (2), which currently comprises 21 environmental and pathogenic species (3). T. maritimum is recognized as a major cause of tenacibaculosis in marine fish, and has been isolated from several host species worldwide (4). Tenacibaculum are slow growing bacteria, a trait related to the underdiagnosis of the disease based on culture techniques. Moreover, the closed phylogenetic relationship between species makes their differentiation by conventional approaches difficult. In Chile, even though there is no official report on the isolation of T. maritimum, the regulatory agency has informed PCR-based detections since 2012.

Outbreaks in Atlantic salmon (*Salmo salar*) reared in the XI region (Región de Aysén) occurred in the second half of 2015. Clinical findings were ascribed to tenacibaculosis, but molecular screening for *T. maritimum* was negative. *Tenacibaculum*-like bacteria were isolated as pure culture from a skin lesion. Typing by 16S rRNA sequencing revealed the presence of *T. dicentrarchi* (5). Here, we present the complete genome sequence of the *T. dicentrarchi* AY7486TD field isolate.

Sequencing was performed at Macrogen, Inc. (Seoul, South Korea) using the Pacific Bioscience single-molecule real-time (SMRT) cell 8Pac v3 and DNA polymerase binding kit P6 v2 for library preparation. 62,255 mapped reads (~13,000-bp average length) from a total of 63,904 reads were *de novo* assembled using SMRT Analysis v2.3.0.1 (http://www.pacb.com) into a circular chromosome of 2,918,253 bp ($N_{50} = 18,803$). The genome depicts a G+C content of 31.5%. The assembled data were annotated with the NCBI Prokaryotic Genome Annotation Pipeline using the best-placed reference protein set as the annotation method imple-

mented in GeneMarkS+ revision 3 0 software. Annotated features were 2,542 genes, 2,420 coding sequences (CDSs), 22 pseudogenes, 10 rRNAs, 1 small noncoding RNA (ncRNA), and 69 tRNAs. An *oriC* region was predicted by the Ori-Finder tool (6).

A relevant feature from the annotation was the presence of genes encoding several metallopeptidases and collagen-binding proteins. These genes, along with those encoding hemolysins are likely involved in the extensive surface tissue damage observed in affected fish. Interestingly, genes encoding structural components of a type IX secretion system (T9SS) were also identified. Recent studies have shown that the T9SS not only plays a role in gliding motility, but also in the delivery of effector proteases of related bacteria (7). Further research will shed more light on these and others virulence aspects of this emerging pathogen.

This first complete genome sequence will serve to improve diagnostics and contribute to a better understanding of the biology of the *Tenacibaculum* genus.

Nucleotide sequence accession numbers. The sequence of the AY7486TD isolate is part of a sequencing project, which has been deposited at DDBJ/EMBL/GenBank under the accession no. CP013671. The version described in this paper is the first version, CP013671.1.

ACKNOWLEDGMENT

This work was partially funded by the 14IDL2-30005 project from The Chilean Economic Development Agency, CORFO.

FUNDING INFORMATION

Corporación de Fomento de la Producción (CORFO) provided funding to Horst Grothusen, Alejandro Castillo, Patricio Henríquez, Esteban Navas, Harry Bohle, and Marcos Mancilla under grant number 14IDL2-30005.

REFERENCES

 Olsen AB, Nilsen H, Sandlund N, Mikkelsen H, Sørum H, Colquhoun DJ. 2011. *Tenacibaculum* sp. associated with winter ulcers in sea-reared Atlantic salmon *Salmo salar*. Dis Aquat Org 94:189–199. http://dx.doi.org/ 10.3354/dao02324.

- Suzuki M, Nakagawa Y, Harayama S, Yamamoto S. 2001. Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. Int J Syst Evol Microbiol 51:1639–1652. http://dx.doi.org/10.1099/00207713-51 -5-1639.
- Habib C, Houel A, Lunazzi A, Bernardet JF, Olsen AB, Nilsen H, Toranzo AE, Castro N, Nicolas P, Duchaud E. 2014. Multilocus sequence analysis of the marine bacterial genus *Tenacibaculum* suggests parallel evolution of fish pathogenicity and endemic colonization of aquaculture systems. Appl Environ Microbiol 80:5503–5514. http://dx.doi.org/10.1128/ AEM.01177-14.
- Avendaño-Herrera R, Toranzo AE, Magariños B. 2006. Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: a review. Dis Aquat Org 71:255–266. http://dx.doi.org/10.3354/dao071255.
- Piñeiro-Vidal M, Gijón D, Zarza C, Santos Y. 2012. Tenacibaculum dicentrarchi sp. nov., a marine bacterium of the family Flavobacteriaceae isolated from European sea bass. Int J Syst Evol Microbiol 62:425-429. http://dx.doi.org/10.1099/ijs.0.025122-0.
- Gao F, Zhang CT. 2008. Ori-finder: a web-based system for finding oriCs in unannotated bacterial genomes. BMC Bioinformatics 9:79. http:// dx.doi.org/10.1186/1471-2105-9-79.
- McBride MJ, Nakane D. 2015. Flavobacterium gliding motility and the type IX secretion system. Curr Opin Microbiol 28:72–77. http://dx.doi.org/ 10.1016/j.mib.2015.07.016.