

Genome Sequence of the Psychrophilic Bacterium *Tenacibaculum ovolyticum* Strain da5A-8 Isolated from Deep Seawater

Maki Teramoto,^a Zhenyu Zhai,^a Ayumi Komatsu,^a Keigo Shibayama,^b Masato Suzuki^b

Oceanography Section, Kochi University, Kochi, Japan^a; Department of Bacteriology II, National Institute of Infectious Diseases, Tokyo, Japan^b

Some bacterial species of the genus *Tenacibaculum*, including *Tenacibaculum ovolyticum*, have been known as fish pathogens in the sea. So far, the only published genome sequence for this genus is for *Tenacibaculum dicentrarchi*, which could also be a fish pathogen. Strain da5A-8, showing 100% identity to the 16S rRNA gene sequence of *T. ovolyticum* DSM 18103^T, was isolated from seawater at a depth of 344 m in Kochi, Japan, and grew optimally at 10 to 20°C. The genome sequence of strain da5A-8 revealed the possible virulence genes commonly observed in the genus *Tenacibaculum*.

Received 15 May 2016 Accepted 17 May 2016 Published 30 June 2016

Citation Teramoto M, Zhai Z, Komatsu A, Shibayama K, Suzuki M. 2016. Genome sequence of the psychrophilic bacterium *Tenacibaculum ovolyticum* strain da5A-8 isolated from deep seawater. *Genome Announc* 4(3):e00644-16 doi:10.1128/genomeA.00644-16.

Copyright © 2016 Teramoto 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 Maki Teramoto, maki.teramoto@kochi-u.ac.jp, or Masato Suzuki, suzuki-m@nih.go.jp.

The genus *Tenacibaculum* currently comprises 20 species of marine origin (<http://www.bacterio.net/tenacibaculum.html>). In this genus, *T. maritimum* (1), *T. ovolyticum* (2, 3), *T. discolor*, and *T. gallaicum* (4, 5) are fish pathogens. *T. soleae* (6) and *T. dicentrarchi* (7, 8) from diseased fish could also be pathogens. So far, the genome sequence has been published only for *T. dicentrarchi* AY7486TD, isolated from a diseased salmon (8).

A bacterial strain, designated da5A-8, was isolated from seawater from a depth of 344 m in Kochi, Japan (33°18'N, 134°13'E), in January 2013. The 16S rRNA gene sequence (accession number LC144619) showed 100% identity to that of *T. ovolyticum* DSM 18103^T (NR_040912), indicating that strain da5A-8 belongs to *T. ovolyticum*.

T. ovolyticum has been shown to be abundant, 4.5% of the total bacteria, at a depth of 320 m (at 1 to 2°C; Argo JAMSTEC) in the Japan Sea Proper Water, while *Tenacibaculum* spp. other than *T. ovolyticum* constituted <0.1% of the total bacteria in the water (unpublished data). Also, *T. ovolyticum* has not been detected from surface seawater in Japan (unpublished data). Consistently, strain da5A-8 was psychrophilic: it grew at 4, 10, 15, 20, and 25°C on dR2A-SW plates (9), growing optimally at 10 to 20°C. Other *Tenacibaculum* spp. grow optimally at a temperature of 25°C or higher (<http://www.bacterio.net/tenacibaculum.html>). Another characteristic of the Japan Sea Proper Water with a characteristic of low temperature is that it can harbor a large amount of eggs and larvae of Atlantic halibut, for which *T. ovolyticum* is a pathogen (2, 3). Proteolytic activity of *T. ovolyticum* dissolves the egg shell, which could lead to the death of the embryo (2, 3).

To examine the virulence-associated genes in strain da5A-8, whole-genome shotgun sequencing was performed using the Illumina HiSeq 2500 pyrosequencing platform (500- to 750-bp insert size). Paired-end reads (2 × 150-bp) were assembled *de novo* using CLC Genomics Workbench version 8.5 (Qiagen). The draft genome sequence consisted of 282 contigs, yielding a total sequence of 4,148,120 bp with an N_{50} contig size of 44,580 bp. The mean G+C content was 29.5%. A total of 3,969 coding DNA

sequences were annotated by RASTtk (<http://rast.nmpdr.org>). One copy of the 16S rRNA gene was present, showing 95.7 to 96.6% sequence identity to the genes (9 copies) in strain AY7486TD (8).

Genes encoding metalloproteases and hemolysins were detected in strain da5A-8 by RASTtk. Gene clusters encoding structural components of the type IX secretion system (T9SS) were detected by RASTtk and also by TXSScan (<http://mobyle.pasteur.fr/cgi-bin/portal.py#forms::txsscan>). Bacterial pathogens frequently use such a protein secretion system to interact with their hosts. T9SS is found in 61.9% of the species of the phylum *Bacteroidetes* (10) and is required for gliding motility and secretion of the surface adhesins. The detected genes are also found in strain AY7486TD (8) and are thus possible virulence genes commonly observed in *Tenacibaculum*. More details of strain da5A-8 will be reported in a future publication.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number BDCW00000000. The version described in this paper is the first version, BDCW00000000.1.

ACKNOWLEDGMENT

This study was performed through the Program to Disseminate Tenure Tracking System of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

FUNDING INFORMATION

This work, including the efforts of Maki Teramoto, was funded by MEXT.

REFERENCES

- 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. <http://dx.doi.org/10.3354/dao071255>.
- Bergh Ø, Hansen GH, Tøxt RE. 1992. Experimental infection of eggs and yolk sac larvae of halibut, *Hippoglossus hippoglossus* L. *J Fish Dis* 15: 379–391. <http://dx.doi.org/10.1111/j.1365-2761.1992.tb01237.x>.
- Hansen GH, Bergh O, Michaelsen J, Knappskog D. 1992. *Flexibacter*

- ovolyticus* sp. nov., a pathogen of eggs and larvae of Atlantic halibut, *Hippoglossus hippoglossus* L. Int J Syst Bacteriol 42:451–458. <http://dx.doi.org/10.1099/00207713-42-3-451>.
4. Piñeiro-Vidal M, Centeno-Sestelo G, Riaza A, Santos Y. 2007. Isolation of pathogenic *Tenacibaculum maritimum*-related organisms from diseased turbot and sole cultured in the Northwest of Spain. Bull Eur Assoc Fish Pathol 27:29–35.
 5. Piñeiro-Vidal M, Riaza A, Santos Y. 2008. *Tenacibaculum discolor* sp. nov. and *Tenacibaculum gallaicum* sp. nov. isolated from sole (*Solea senegalensis*) and turbot (*Psetta maxima*) culture systems. Int J Syst Evol Microbiol 58:21–25. <http://dx.doi.org/10.1099/ijs.0.65397-0>.
 6. Piñeiro-Vidal M, Carballas CG, Gómez-Barreiro O, Riaza A, Santos Y. 2008. *Tenacibaculum soleae* sp. nov., isolated from diseased sole (*Solea senegalensis* Kaup). Int J Syst Evol Microbiol 58:881–885. <http://dx.doi.org/10.1099/ijs.0.65539-0>.
 7. 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>.
 8. 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. <http://dx.doi.org/10.1128/genomeA.01756-15>.
 9. Teramoto M, Nishijima M. 2014. *Amylibacter marinus* gen. nov., sp. nov., isolated from surface seawater. Int J Syst Evol Microbiol 64:4016–4020. <http://dx.doi.org/10.1099/ijs.0.065847-0>.
 10. Abby SS, Cury J, Guglielmini J, Néron B, Touchon M, Rocha EP. 2016. Identification of protein secretion systems in bacterial genomes. Sci Rep 6:23080. <http://dx.doi.org/10.1038/srep23080>.