

Draft Genome Sequence of Virgibacillus sp. Strain AGTR, Isolated from Hypersaline Lake Acıgöl in Turkey

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ABSTRACT Virgibacillus sp. strain AGTR, which is a haloalkaliphilic microorganism, was isolated from a sediment sample collected in hypersaline Lake Acıgöl in Turkey. It has the potential to produce biotechnologically essential proteases. Here, the whole-genome sequence and its annotations are reported.

The use of enzymes from halophiles in industrial applications is not limited to their stability at high salt concentrations, as these extremozymes are also tolerant to high temperatures or low temperatures and alkali pH and are stable in the presence of organic solvents [\(1](#page-1-0)–[4\)](#page-1-1). Virgibacillus sp. strain AGTR is a Gram-positive, endospore-forming, motile, and halophilic microorganism that was isolated from hypersaline Lake Acıgöl (the salinity rate varies between 5.8% and 13% [wt/vol]). Sediment samples were collected from hypersaline Lake Acıgöl in Turkey (37.837100N, 29.861E). Sediment samples were diluted, and 100 μ L diluted sample was plated on protease activity screening plates, which are nutrient agar (NA) plates containing 1% (wt/vol) skim milk and 10% NaCl (wt/vol). The plates were incubated at 30°C for 7 days. Colonies were isolated by repeated streaking (three times) on fresh screening plates. The isolate showing the maximum hydrolysis zone was selected for whole-genome sequencing. The isolate was cultivated in 100 mL nutrient broth (NB) (with 10% NaCl) at 30°C for 24 h, and DNA isolation was carried out with a MoBio kit (catalog number 12888-50). One hundred nanograms of genomic DNA was used to create sequencing libraries with the TruSeq Nano DNA low-throughput library preparation kit (catalog number 20015964; Illumina). Quality control, in terms of size distribution and quantity of the libraries, was performed by using a 2100 Bioanalyzer (Agilent Technologies, USA). Sequencing by synthesis (SBS) was performed using the HiSeq Rapid SBS kit v2 (catalog number FC-402-4023; Illumina). The Illumina HiSeq 2500 platform was used for sequencing. The raw sequence data were checked with FastQC ([5](#page-1-2)), in terms of sequence quality and the presence of any adapter sequences. A total of 4,625,053 raw reads were obtained.

Raw paired-end sequencing data were used for de novo assembly with the Unicycler genome assembler v0.4.8 ([6](#page-1-3)). The assembled genome size was 4,708,499 bp, with a GC content of 36.66%, which is in good accordance with values for previously published Virgibacillus genomes. The assembled genome was annotated using Prokka v1.13 ([7](#page-1-4)). The annotation pipeline with Prokka uses several external tools, such as Prodigal ([8](#page-1-5)) for coding sequences, RNAmmer v1.2 [\(9\)](#page-1-6) for rRNA genes, ARAGORN [\(10](#page-1-7)) for tRNA genes, and Infernal v1.1 ([11](#page-1-8)) for noncoding RNAs. The draft genome was predicted to have 4,536 coding sequences, 64 tRNA genes, and 5 rRNA sequences. Assembled genome features are indicated in [Table 1](#page-1-9). The average nucleotide identity (ANI) was calculated by using EzBioCloud TruBac ID software v1 [\(12\)](#page-1-10), which revealed 99.44% similarity to Virgibacillus marismortui. Default parameters were used for all software unless otherwise noted.

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TABLE 1 Genome features of Virgibacillus sp. strain AGTR

Data availability. The whole-genome sequence of Virgibacillus sp. strain AGTR was submitted to GenBank under BioProject accession number [PRJNA701885,](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA701885) BioSample accession number [SAMN17916045](https://www.ncbi.nlm.nih.gov/biosample/SAMN17916045), and nucleotide accession number [JAJERH000000000](https://www.ncbi.nlm.nih.gov/nuccore/JAJERH000000000). The raw data were deposited in the Sequence Read Archive (SRA) under SRA accession number [SRP376786.](https://www.ncbi.nlm.nih.gov/sra/SRP376786)

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REFERENCES

- 1. Huo YY, Rong Z, Jian SL, Xu CD, Li J, Xu XW. 2017. A novel halotolerant thermoalkaliphilic esterase from marine bacterium Erythrobacter seohaensis SW-135. Front Microbiol 8:2315. [https://doi.org/10.3389/fmicb.2017.02315.](https://doi.org/10.3389/fmicb.2017.02315)
- 2. Noby N, Hussein A, Saeed H, Embaby AM. 2020. Recombinant cold-adapted halotolerant, organic solvent-stable esterase (estHIJ) from Bacillus halodurans. Anal Biochem 591:113554. [https://doi.org/10.1016/j.ab.2019.113554.](https://doi.org/10.1016/j.ab.2019.113554)
- 3. Borba TM, Machado TB, Brandelli A, Kalil SJ. 2018. Thermal stability and catalytic properties of protease from Bacillus sp. P45 active in organic solvents and ionic liquid. Biotechnol Prog 34:1102–1108. [https://doi.org/10](https://doi.org/10.1002/btpr.2672) [.1002/btpr.2672.](https://doi.org/10.1002/btpr.2672)
- 4. Wang M, Ai L, Zhang M, Wang F, Wang C. 2020. Characterization of a novel halotolerant esterase from Chromohalobacter canadensis isolated from salt well mine. 3 Biotech 10:430. <https://doi.org/10.1007/s13205-020-02420-0>.
- 5. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- 6. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. [https://doi.org/10.1371/journal.pcbi.1005595.](https://doi.org/10.1371/journal.pcbi.1005595)
- 7. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. [https://doi.org/10.1093/bioinformatics/btu153.](https://doi.org/10.1093/bioinformatics/btu153)
- 8. Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. [https://doi.org/10.1186/1471-2105-11-119.](https://doi.org/10.1186/1471-2105-11-119)
- 9. Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. <https://doi.org/10.1093/nar/gkm160>.
- 10. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. [https://doi.org/10.1093/nar/gkh152.](https://doi.org/10.1093/nar/gkh152)
- 11. Kolbe DL, Eddy SR. 2011. Fast filtering for RNA homology search. Bioinformatics 27:3102–3109. <https://doi.org/10.1093/bioinformatics/btr545>.
- 12. Ha S-M, Kim CK, Roh J, Byun J-H, Yang S-J, Choi S-B, Chun J, Yong D. 2019. Application of the whole genome-based bacterial identification system, TrueBac ID, using clinical isolates that were not identified with three matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems. Ann Lab Med 39:530–536. [https://doi.org/10.3343/alm.2019.39.6.530.](https://doi.org/10.3343/alm.2019.39.6.530)