Draft Genome Sequence of a Sulfate-Reducing Bacterium, “Desulfofundulus salinum” 435T, Isolated from a High-Temperature Gas Field in Russia

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ABSTRACT The draft genome sequence of the thermophilic sulfate-reducing bacterium “Desulfofundulus salinum” strain 435T, isolated from condensate water of the Igrim high-temperature gas field (Western Siberia, Russia), is presented here. The genome is annotated to elucidate the taxonomic position of strain 435T.

Gram-positive spore-forming sulfate-reducing bacteria of the genus Desulfotomaculum are commonly revealed in deep subsurface environments, including petroleum and gas reservoirs, by culture-based and molecular approaches (1, 2). Due to their ability to produce endospores and to grow on a range of organic substrates or H2-CO2 by reducing metals or sulfate and other oxidized sulfur compounds, or by fermentation, bacteria of the genus Desulfotomaculum (“D. salinum”, D. kuznetsovii, and D. thermocisternum) remain viable and geochemically active in deep anoxic environments (1, 3–6).

The thermophilic spore-forming sulfate-reducing Desulfotomaculum nigrificans strain 435T was isolated in 1973 from gas condensate water of the Igrim gas field in Russia (4). Later, the halotolerant strain 435T was reclassified as a new species, Desulfotomaculum salinum (7). Since the strain 435T was deposited in a single international collection, this species is not validly described. On the phylogenetic tree of 16S rRNA gene sequences, strain 435T forms an independent branch within the Desulfotomaculum genus cluster, sharing 94.9% similarity with the 16S rRNA gene of the most closely related type strain, Desulfotomaculum sp. strain DSM 6115T (7–9). As a result of the recent revision of the genus Desulfotomaculum, species of the D. kuznetsovii cluster were assigned to the new genus Desulfofundulus (10). Strain 435T is currently deposited in two microbial collections (VKM B 1492T and DSM 23196). The aim of the present study was to sequence the genome of the strain 435T in order to elucidate its taxonomic position.

Strain 435T was grown anaerobically at 60°C in bicarbonate-buffered medium with lactate and sulfate as the substrates (7). Genomic DNA was extracted using Wilson’s method (11) with minor modifications. Cells were harvested from 2 liters of culture medium by centrifugation after 7 days of incubation, and the cell pellet was resuspended in 400 μl of Tris-EDTA (TE) buffer. Thereafter, 25 μl of 10% SDS and 20 μl of proteinase K solution were added, and the mixture was incubated at 37°C for 60 min. After incubation, 125 μl of 4 M NaCl, 160 μl of 5% cetyltrimethylammonium bromide (CTAB) and 20 μl of RNase (10 mg/ml) were added. The mixture was then incubated for 10 min at 65°C and cooled to room temperature; thereafter, the
mixture was treated with chloroform, followed by centrifugation for 10 min at 9,000 × g. DNA from the supernatant was recovered by adding 0.6 volume of isopropanol. The dried DNA was dissolved in 50 μl of Milli-Q water (MQ). The libraries were constructed with the NEBNext DNA library prep reagent set for Illumina, according to the protocol for the kit. Sequencing was undertaken using the Illumina HiSeq 1500 platform with 250-bp single-end reads. Raw reads were quality checked with FastQC version 0.11.7 (12), and low-quality reads were trimmed using Trimmomatic version 0.36, with default settings (13). Subsequently, the quality-filtered reads were de novo assembled with SPAdes version 3.11.0 using the default settings (14). A total of 1,788,819 reads were assembled into 71 contigs larger than 500 bp. The MeDuSa scaffolder, with default parameters (15), was used to generate scaffolds from the contigs and to perform the mapping against Desulfofundulus kuznetsovii DSM 6115T (GenBank assembly accession no. GCF_000214705.2) as a reference genome. The final assembled 2,886,683-bp-long genome comprised 10 scaffolds, with an N50 value of 2,856,498 bp, G+C content of 55.1 mol%, and coverage of 137×. Annotations of the contigs were carried out using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (16), which identified 2,909 genes, 2,755 coding sequences, 101 pseudogenes, and 43 tRNA genes. The average nucleotide identity (ANI) (17) and digital DNA-DNA hybridization (dDDH) (18) values of 92.4% and 51.1%, respectively, to the genome of the closest strain D. kuznetsovii DSM 6115T were below the criteria for assignment to separate species (95 to 96% for ANI and 70% for dDDH) (19), which indicate that strain 435T belongs to a new Desulfofundulus species.

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. RBWE00000000. The version described in this paper is the first version, RBWE00100000. The raw FASTQ reads have been deposited in the NCBI SRA database under the accession no. SRR8069234.

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**REFERENCES**

1. Aüllo T, Ranchou-Peyruse A, Ollivier B, Magot M. 2013. **Desulfofundulus** spp. and related gram-positive sulfate-reducing bacteria in deep subsurface environments. Front Microbiol 4:362. https://doi.org/10.3389/fmicb.2013.00362.
2. Gray ND, Sherry A, Hubert C, Dolfing J, Head IM. 2010. Methanogenic degradation of petroleum hydrocarbons in subsurface environments remediation, heavy oil formation, and energy recovery. Adv Appl Microbiol 72:137–161. https://doi.org/10.1016/S0065-2164(10)72005-0.
3. Plugge CM, Zhang W, Scholten JC, Stams AJ. 2011. Metabolic flexibility of sulfate-reducing bacteria. Front Microbiol 2:81. https://doi.org/10.3389/fmicb.2011.00081.
4. Nazina TN, Rozanova EP. 1978. Thermophilic sulfate-reducing bacteria from oil strata. Microbiology 47:113–118.
5. Nazina TN, Ivanova AE, Kanchaveli LP, Rozanova EP. 1988. A new spore-forming thermophilic methylotrophic sulfate-reducing bacterium, **Desulfotomaculum** kuznetsovii sp. nov. Microbiology 75:659–663.
6. Nilsen RK, Torsvik T, Lien T. 1996. **Desulfotomaculum thermocisternum** sp. nov., a sulfate reducer isolated from a hot North Sea oil reservoir. Int J Syst Bacteriol 46:397–402. https://doi.org/10.1099/00207713-46-2-397.
7. Nazina TN, Rozanova EP, Belyakova EV, Lysenko AM, Poltaraus AB, Tourova TP, Osipov GA, Belyaev SS. 2005. Description of **Desulfotomaculum kuznetzovii** subsp. salinus” as a new species, **Desulfotomaculum salinus** sp. nov. Microbiology 7:659–663.
8. Tourova TP, Kuznetzov BB, Novikova EV, Poltaraus AB, Nazina TN. 2001. Heterogeneity of the nucleotide sequences of the 16S rRNA genes of the type strain of **Desulfotomaculum kuznetzovii**. Microbiology 70:678–684. https://doi.org/10.1023/A:1013135831669.
9. Visser M, Worm P, Muyzer G, Pereira IA, Schaua P, Plugge CM, Kuever J, Parshina SN, Ivanova AE, Bernier-Latmani R, Goodwin LA, Grouzdev et al. 2001. A new spore-forming methylotrophic sulfate-reducing bacterium, **Desulfotomaculum thermocisternum** sp. nov. Int J Syst Bacteriol 51:678–684. https://doi.org/10.1099/00207713-46-2-397.
10. Watanabe M, Kojima H, Fukui M. 2018. Review of **Desulfotomaculum** species and proposal of the genera **Desulfotomaculum** gen. nov., **Desulfolocicmen** gen. nov. and **Desulfohalotomaculum** gen. nov. Int J Syst Evol Microbiol 68:2891–2899. https://doi.org/10.1099/ijsem.0.002915.
11. Wilson K. 2001. Preparation of genomic DNA from bacteria. Curr Protoc Mol Biol Chapter 2 Unit 2.4.
12. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
13. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
14. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
15. Bosi E, Donati B, Galardini M, Brunetti S, Sagot M-F, Lió P, Crescenzi P, Fani R, Fondi M. 2015. MeDuSa: a multi-draft based scaffold. Bioinformatics 31:2443–2451. https://doi.org/10.1093/bioinformatics/btv171.
16. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Ciufo S, Li W. 2013. Prokaryotic genome annotation pipeline. The NCBI handbook, 2nd ed. National Center for Biotechnology Information, Bethesda, MD.
17. Rodriguez-R LM, Konstantinidis KT. 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. PeerJ Preprints 4:e1900v1. https://doi.org/10.7287/peerj.preprints.1900v1.
18. Auch AF, von Jan M, Klenk HP, Göker M. 2010. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2:117–134. https://doi.org/10.4056/sigs.531120.
19. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijsem.0.002516.