



Draft Genome Sequence of *Shewanella* sp. Strain T2.3D-1.1, Isolated from 121.8 Meters Deep in the Subsurface of the Iberian Pyrite Belt

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ABSTRACT *Shewanella* sp. strain T2.3D-1.1 was isolated from the deep subsurface of the Iberian Pyrite Belt. We report its draft genome sequence, consisting of 49 scaffolds, with a chromosome of \approx 4.6 Mb and a 23.8-kb plasmid. The chromosome annotation identified 4,068 coding DNA sequences, 1 rRNA operon, and 108 tRNA genes.

Shewanella is the only genus in the family *Shewanellaceae* within the order *Alteromonadales*. Species of this genus are Gram-negative, motile rods which are widely distributed in many environments. Their growth is facultatively anaerobic, and they can use a wide range of compounds as electron acceptors (1). *Shewanella* sp. strain T2.3D-1.1 was isolated from a strictly anaerobic denitrification enrichment culture using a 121.8-m-deep core sample obtained from a drilling project aimed at studying the microbial diversity existing in the deep subsurface of the Iberian Pyrite Belt (southwest Spain) (2). The borehole coordinates were 37°43'45.42"N and 6°33'23.57"W. Drilling was performed as described in reference 3. A powdered rock sample (\approx 6 g) was used as the inoculum. *Shewanella* sp. strain T2.3D-1.1 was isolated and grown under the conditions described in reference 4.

Genomic DNA of the strain T2.3D-1.1 was extracted by the cetyltrimethylammonium bromide (CTAB)-based method (5), and its concentration was determined using a Qubit v.2.0 fluorometer (Invitrogen, USA). The 16S rRNA gene was amplified under the conditions described in reference 4. Reads were edited and assembled as described in reference 6. The complete 16S rRNA gene sequence was compared with sequences in the NCBI GenBank database using BLAST (7). The closest sequence was found to belong to *Shewanella hafniensis* P010(T) (99.43% similarity) (8).

Library preparation and sequencing were carried out by the MicrobesNG company using the Illumina MiSeq platform, with a mean coverage of 73.37 \times . The sequencing run generated 763,338 paired-end reads of 2 \times 250 bp with a mean length of 537 bp. *De novo* assembly was carried out using SPAdes v.3.14.0 (“-careful” option) (9), and the extrachromosomal elements were assembled with Recycler, which uses the read coverage of contigs to distinguish between plasmids and chromosomes (10). Plasmid contigs were aligned against the chromosomal assembly with Mauve Aligner v.2.4.0 (11) to separate chromosomal and plasmid contigs. Contigs were extended and merged into scaffolds using SSPACE software (12). Gaps created with SSPACE were closed with GapFiller v.1-10 software (13). Default parameters were used for all software unless otherwise specified. This yielded a chromosome in 49 scaffolds, with an N_{50} value of 183,411 bp, a GC content of 44.42%, 1 chromosome of 4,660,397 bp, and 1 plasmid of 23,806 bp in 3 scaffolds.

The genome was annotated with Prokka v.1.12 software (14) and the RAST platform

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(15), using *Shewanella putrefaciens* strain 200 as the reference genome. A total of 4,068 coding DNA sequences, 108 tRNA genes, 1 rRNA operon, 1 transfer-messenger RNA (tmRNA), and 3 CRISPR sequences were identified. Genes involved in (i) denitrification, (ii) both assimilatory and dissimilatory sulfur reduction pathways, (iii) different reductase enzymes which are part of anaerobic respiration, (iv) a ferric ion reductase, and (v) heavy metal resistance were identified in the chromosome.

The analysis of the genome of *Shewanella* sp. strain T2.3D-1.1 should assist in identifying the mechanisms used by organisms to develop under the extreme oligotrophic conditions present in the dark biosphere.

Data availability. Reads were deposited at ENA/GenBank/DDBJ under the accession number [ERR3773752](https://www.ncbi.nlm.nih.gov/nuccore/ERR3773752), and the complete genome sequences were deposited under the accession numbers [CACVBT02000000](https://www.ncbi.nlm.nih.gov/nuccore/CACVBT02000000) (chromosome) and [CACVAL02000000](https://www.ncbi.nlm.nih.gov/nuccore/CACVAL02000000) (plasmid). All of them are included under study number [PRJEB35936](https://www.ncbi.nlm.nih.gov/nuccore/PRJEB35936).

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