



Genome sequence of the copper resistant and acid-tolerant *Desulfosporosinus* sp. BG isolated from the tailings of a molybdenum-tungsten mine in the Transbaikal area



Olga V. Karnachuk^{a,*}, Vitalii V. Kadnikov^b, Inna A. Panova^a, Andrey V. Mardanov^b, Alexey V. Beletsky^b, Erzhen V. Danilova^c, Marat R. Avakyan^a, Nikolai V. Ravin^b

^a Laboratory of Biochemistry and Molecular Biology, Tomsk State University, 634050 Tomsk, Russia

^b Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, 119071 Moscow, Russia

^c Institute of General and Experimental Biology, Siberian Branch Russian Academy of Sciences, 670047 Ulan-Ude, Buryatia, Russia

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ABSTRACT

Here, we report on the draft genome of a copper-resistant and acidophilic *Desulfosporosinus* sp. BG, isolated from the tailings of a molybdenum-tungsten mine in Transbaikal area. The draft genome has a size of 4.52 Mb and encodes transporters of heavy metals. The phylogenetic analysis based on concatenated ribosomal proteins revealed that strain BG clusters together with the other acidophilic copper-resistant strains *Desulfosporosinus* sp. OT and *Desulfosporosinus* sp. I2. The K⁺-ATPase, Na⁺/H⁺ antiporter and amino acid decarboxylases may participate in enabling growth at low pH. The draft genome sequence and annotation have been deposited at GenBank under the accession number NZ_MASS00000000.

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|---------------------------|--|
| Specifications | |
| Organism/cell line/tissue | <i>Desulfosporosinus</i> BG |
| Sex | N/A |
| Sequencer or array type | GS FLX (Roche) |
| Data format | Analyzed |
| Experimental factors | Bacterial strain |
| Experimental features | Assembled and annotated draft genome of <i>Desulfosporosinus</i> strain BG |
| Consent | N/A |
| Sample source location | Sediments of a tailings pond at a molybdenum-tungsten mine in Bom-Gorkhon, Transbaikal Area, Russia (51° 28'25"; 109° 49'15"). |

1. Direct link to deposited data

https://www.ncbi.nlm.nih.gov/nucleotide/NZ_MASS000000000.1

2. Introduction

Members of the genus *Desulfosporosinus* are often found in acidic mining environments [6,8,11,13,17,18,20–23]. These bacteria may participate in metal detoxification by precipitating them in the form of sulfides [5]. *Desulfosporosinus acidiphilus* [2] and *Desulfosporosinus acididurans* [19], both isolated from mining environments, represent the only two validly described, moderately acidophilic sulfate-reducing bacteria. Two other acidophilic *Desulfosporosinus* have been isolated and their genomes are available [1,15]. *Desulfosporosinus* spp. are also known for their tolerance to metals, in particular to copper [1,14,15]. Recently, a novel acidophilic, copper-resistant *Desulfosporosinus* sp. BG was isolated from the tailings pond sediment of the Bom-Gorkhon molybdenum-tungsten mine in the Transbaikal area [10]. The acidophilic isolate could tolerate up to 6 g Cu²⁺/L and grew at the pH range from 1.0 to 6.5. The purpose of this study was to sequence genome of

* Corresponding author.

E-mail address: olga.karnachuk@green.tsu.ru (O.V. Karnachuk).

Desulfosporosinus sp. BG to verify its phylogenetic relationship with the known acidophiles belonging to this genus and compare the putative mechanisms, which enable the bacteria to withstand acid and metal stress.

3. Experimental design, materials and methods

3.1. Sequencing and assembly of *Desulfosporosinus* sp. BG genome

Genomic DNA was extracted from *Desulfosporosinus* sp. BG biomass using the SDS-CTAB method [16]. Genomic DNA was sequenced with a Roche Genome Sequencer FLX (GS FLX), using the Titanium XL+ protocol for a shotgun library. About 189 Mb of sequences with an average read length of 495 nt were generated. The reads were *de novo* assembled into contigs using the Newbler Assembler version 2.9 (454 Life Sciences, Branford, CT). The resulting draft genome sequence of *Desulfosporosinus* sp. BG consists of 156 contigs longer than 500 bp, with a total length of 4,536,051 bp. The total length of all 345 obtained contigs is 4,579,156 bp. The N50 contig size of the genome is 70,527 bp. Gene search and annotation were performed for all contigs longer than 500 bp using the RAST server [3] following manual curation.

3.2. Features of the *Desulfosporosinus* sp. BG genome

The draft genome of *Desulfosporosinus* sp. BG was around 4.52 Mb, which is a relatively small size, compared to *Desulfosporosinus orientis* – 5.86 Mb (NC_016584), *Desulfosporosinus youngiae* – 5.66 Mb (NZ_CM001441), *Desulfosporosinus meridiei* – 4.87 Mb (NZ_CM001441.1), and *Desulfosporosinus acidiphilus* – 4.93 Mb (NC_018068). However, the acidophilic *Desulfosporosinus acididurans* has approximately the same size genome, 4.64 Mb (NZ_LDZY00000000.1), as strain BG. The GC content of the genome is 42.4%. The genome includes 4516 protein-coding genes, 67 tRNA genes, and 9 rRNA genes.

The phylogenetic analysis based on concatenation of 32 ribosomal proteins showed that strain BG clustered with the acidophilic, copper-resistant *Desulfosporosinus* sp. I2, and two uncultivated strains, *Desulfosporosinus* BRH_c37 and *Desulfosporosinus* BICA1-9. Their composite genomes were obtained from the metagenomic data from groundwater (Fig. 1). The closest relative of strain BG was

Desulfosporosinus sp. OT isolated previously from the Norilsk mining area [9].

Several major mechanisms notable for the acid- and metal-tolerance were detected in the *Desulfosporosinus* sp. BG genome. The acid-tolerance determinants included the K⁺-transporting ATPase KdpABC (DSBG_RS17700-KdpA, DSBG_RS17705-KdpB, DSBG_RS17710-KdpC), which participates in the generation of internal positive membrane potential preventing proton influx to the cytoplasm. Strain BG along with *Desulfosporosinus* sp. OT has an Na⁺/H⁺ antiporter (DSBG_RS05820), known as the key transporter in maintaining the pH of actively metabolizing cells. The Na⁺/H⁺ antiporter did not occur in any other available *Desulfosporosinus* genomes. Phylogenetic analysis shows that the antiporter was likely acquired from *Bacillus* via lateral gene transfer. Additionally, strain BG has proton-consuming decarboxylases – the arginine decarboxylase (DSBG_RS13090) and the lysine decarboxylase (DSBG_RS07860), which are involved in the mechanism of coping with low pH environment enterobacteria [4]. Orthologous decarboxylases are present in all available *Desulfosporosinus* genomes.

A copper ATPase occurs in the *Desulfosporosinus* sp. BG genome. It comprises an operon with transcriptional regulator CsoR (DSBG_RS14110) and copper chaperon CopZ (DSBG_RS14120). Orthologous genes for the Cu-ATPase are present in all available *Desulfosporosinus* genomes. Consistent with the phylogenetic position derived from ribosomal proteins, the closest relatives of the Cu-ATPase of strain BG were proteins from *Desulfosporosinus* sp. OT (sequence similarity 97%) and *Desulfosporosinus* sp. I2 (80%).

Several other heavy metal-transporting ATPases occur in the genome of strain BG. Two IB P-type ATPases primarily responsible for translocating Cd²⁺ and other closely-related divalent Co, Hg, Pb, and Zn ions, were found in strain BG genome. One of the ATPases (DSBG_RS14085) is present in all available *Desulfosporosinus* genomes and located close to the Cu-ATPase. Interestingly, another cadmium ATPase (DSBG_RS14615) has orthologous genes only in *Desulfosporosinus* sp. OT (95% similarity), *Desulfosporosinus* sp. I2 (88%), *Desulfosporosinus acidiphilus* (85%), and uncultivated *Desulfosporosinus* sp. BRHc37 (90%), but not in any other available *Desulfosporosinus* genomes. The phylogenetic analysis shows that the cadmium ATPase was likely transferred laterally from *Paenibacillus*. *Desulfosporosinus* sp. OT encodes two ATPases of this type (WP_009618081, WP_009624106). Physiological studies did not reveal outstanding tolerance to cobalt, nickel, and cadmium in strain BG [10].

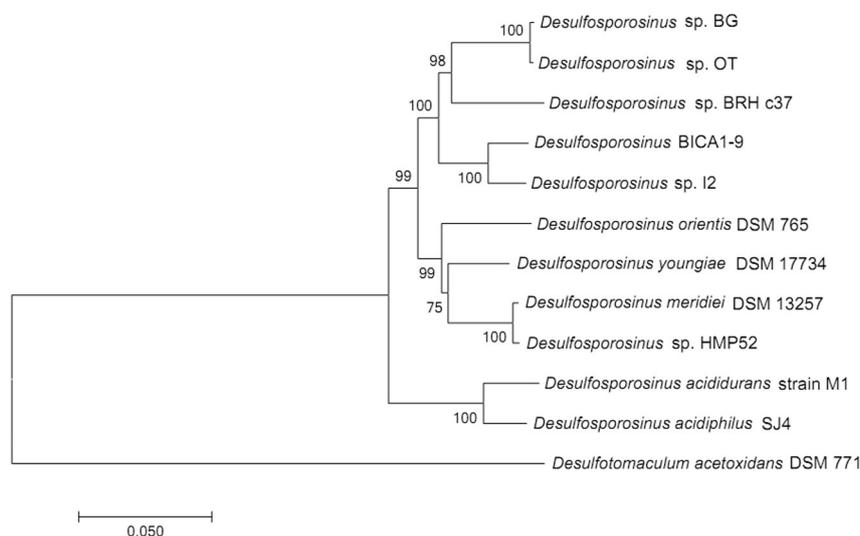


Fig. 1. Phylogenetic analysis of representatives of the genus *Desulfosporosinus* based on concatenated amino acid sequences of 32 ribosomal proteins. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model [7]. The tree with the highest log likelihood (–27,426.3925) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 12 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 4259 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [12].

In conclusion, we have revealed additional transporters that are not available genomes of other members of this genus. These may enable *Desulfosporosinus* sp. BG to withstand low pH or high metal concentrations. The strain has heavy metal transporters that can enable it to tolerate high concentrations of lead, mercury, or zinc. The latter is a subject to be studied in the future research.

Conflict of interest

The authors declare no conflicts of interest in this study.

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