ISSN 0026-2617, Microbiology, 2015, Vol. 84, No. 5, pp. 677–686. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © O.V. Karnachuk, I.A. Kurganskaya, M.R. Avakyan, Y.A. Frank, O.P. Ikkert, R.A. Filenko, E.V. Danilova, N.V. Pimenov, 2015, published in Mikrobiologiya, 2015, Vol. 84, No. 5, pp. 595–605.

EXPERIMENTAL ARTICLES

An Acidophilic *Desulfosporosinus* Isolated from the Oxidized Mining Wastes in the Transbaikal Area

O. V. Karnachuk[a, 1], I. A. Kurganskaya[a], M. R. Avakyan[a], Y. A. Frank[a], O. P. Ikkert[a], R. A. Filenko[b], E. V. Danilova[c], and N. V. Pimenov [d]

[a] *Department of Plant Physiology and Biotechnology, Tomsk State University, Tomsk, Russia*
[b] *Institute of Natural Resources, Ecology, and Cryology, Siberian Branch, Russian Academy of Sciences, Chita, Russia*
[c] *Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences, Ulan-Ude, Russia*
[d] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia*

Received November 28, 2014

Abstract—Dissimilatory sulfate reduction plays an important role in removal of dissolved metals from acidic mine waters. Although this process was convincingly shown to occur in acidic waste of metal recovery, few isolates of acidophilic/acid-tolerant sulfate reducers are known. We isolated a new acidophilic sulfidogen, strain BG, from the oxidized acidic waste of the Bom-Gorkhon tungsten deposit, Transbaikalia, Russia. Phylogenetic analysis of its 16S rRNA gene sequence made it possible to identify it as a member of the genus *Desulfosporosinus*. Unlike other known acidophilic sulfate reducers of this genus, strain BG was tolerant to high copper concentrations (up to 5 g/L), could grow on organic acids at low ambient pH, and formed crystalline copper sulfides (covellite and chalcopyrite). Molecular analysis of the phylotypes predominating in oxidized waste and in enrichment cultures confirmed the presence of various *Desulfosporosinus* strains.

Keywords: dissimilatory sulfate reduction, acidophilic sulfidogen, *Desulfosporosinus*, heavy metals, acidic mine waters

DOI: 10.1134/S0026261715050112

Recovery and processing of sulfide ores results in large amounts of solid and liquid waste (Blowes et al., 2003). Mine water with high concentrations of protons and metals is formed in the course of biological and chemical oxidation of residual metal sulfides. Microbial sulfidogenesis plays an important part in natural self-purification of mine wastes. Sulfate-reducing prokaryotes (SRP) produce considerable amounts of sulfide, precipitate metals as sulfides, and increase pH due to proton consumption. This process is used in biotechnologies for treatment of metal-contaminated water in bioreactors (Jong and Parry, 2003; Kaksonen et al., 2004; Van Houten et al., 2009; Bijmans et al., 2009; Sahinkaya et al., 2009; Ňancucheo and Johnson, 2014).

Active sulfate reduction at low pH has been confirmed by measurements of reduction of radioactively labeled sulfate under conditions close to in situ (see review Koschorreck, 2008). Pure cultures of acidophilic or even acid-tolerant SRP have not been isolated for a long time. Some authors attributed this phenomenon to toxicity of lactate, the major organic substrate in the media for SRP, at low pH values (Koschorreck, 2008). Replacement of lactate by alcohols and especially by glycerol was subsequently proposed (Alazard et al., 2010; Sánchez-Andrea et al., 2014). The first acidophilic SRP species, *Desulfosporosinus acidiphilus*, was described in 2010 (Alazard et al., 2010). Several acidophilic/acid-tolerant *Desulfosporosinus* strains were isolated (Karnachuk et al., 2009). *Desulfosporosinus* sp. OT, resistant to high copper concentrations, was the first organism of this genus for which the genome sequence was determined (Abicht et al., 2011). The genome of *D. acidiphilus* was subsequently also sequenced (Pester et al., 2011). Another acidophilic species, *Desulfosporosinus acididurans* was described recently (Sánchez-Andrea et al., 2014). However, none of the validly described sulfate-reducing acidophiles grows in the presence of heavy metals (copper, cobalt, nickel, or cadmium) or arsenic. High concentration of these cations, which are solubilized at low pH, is the major factor preventing the application of microorganisms for treatment of acidic mine waters.

Apart from high concentrations of sulfate and metal ions, acidic waste of sulfide ore processing is characterized by the presence of strong oxidizers, especially Fe(III). We have previously shown that oxidized conditions did not prevent SRP activity and formation of biogenic sulfides in the oxidized metal mining waste (Karnachuk et al., 2005). Active microbial sulfate reduction in oxidized systems by the organisms

[1] Corresponding author; e-mail: olga.karnachuk@green.tsu.ru

Fig. 1. Location of the Bom-Gorkhon deposit on the maps of Russia and the Transbaikal Area.

previously considered strict anaerobes is presently considered possible (see review Dolla et al., 2006; Sass and Cypionka, 2007).

The goal of the present work was to search for new acidophilic SRP in acidic environments of the areas of intense oxidation of the wastes of metal recovery and processing. Since resistance to high concentrations of heavy metals is potentially advantageous for development of the relevant biotechnologies, acidophiles were isolated in the presence of elevated concentrations of copper, cadmium, and molybdenum. Waste storage areas of the Bom-Gorkhon sulfide-tungsten deposit (Transbaikalia, Russia) were the subjects of research.

MATERIALS AND METHODS

The Bom-Gorkhon tungsten deposit is located in the western Transbaikalia, 14 km from the Novopavlovka railway station and 35 km east of the Petrovsk-Zabaikal'skii city (Fig. 1). Orohydrographycally it is located at the southern slope of the Tsagan-Khurt ridge in the upper basin of the Bom-Gorkhon River, a right tributary to Khilok. The deposit belongs to the hubnerite–sulfide–quartz mineral type of the greisen formation. The tungsten-bearing quartz ledges are localized in late Jurassic granites of the Gudzhir magmatic complex. Quartz, microcline, muscovite, pyrite, and hubnerite are the main minerals of the ledges. Among the main minerals of the oxidation zone, goethite, hydrogoethite, tungstite, and jarosite were revealed. Fluorite, thin-scale muscovite, biotite, albite, epidote, adular, chlorite, molybdenite, sphalerite, bismuthine and cosalite, scheelite, etc. are the minor minerals. Martite, hydrohematite, bismutite and ferrimilybdenite, chalcocite, and covellite are the oxidized minor minerals. Accessory minerals include beryl, apatite, triplite, tinstone, magnetite, haematite, galena, stannite, pyrrhotite, tetratimite, native bismuth, kannizzarite, gladite, and hammarite (Barabanov, 1975; Ontoev, 1974). The deposit was discovered in 1962. Active recovery and ore-dressing have been carried out since the 1980s. Wolframite and sulfide concentrates are obtained by ore processing using the flotation–gravity technological scheme. Loose waste is stored in the upper valleys of the Bom-Gorkhon and Barun-Tignya springs. Mill tailings undergo repeated secondary processing for additional recovery of valuable components. Interacting with atmospheric precipitation, ore sulfides, concentrates, and tailings are oxidized, forming various sulfates at the geochemical barriers. Among newly formed minerals, X-ray analysis and microprobe investigation revealed such sulfates as hanningite, wilcoxite ($MgAl(SO_4)_2F \cdot 18H_2O$), rostite, starkeite, boyleite, rozenite, siderotile, chalcanthite, halochlorite, alum ($(K,Na)Al(SO_4)_2 \cdot 12\,H_2O$), etc. Cadmium, molybde-

num, lead, arsenic, selenium, tellurium, and other toxic elements were present in sulfates as admixtures (Eremin et al., 2014).

Sediment samples from the storage pond accumulated after wolframite concentrate production were collected on August 22, 2012. Temperature, pH, and Eh of the sample were determined on site using a HANNA HI 8314F pH meter. For elemental and mineralogical analyses, the samples were collected in sterile 50-mL test tubes and stored at 4°C prior to analysis. For analysis of metal content in near-bottom water, it was filtered through a 0.45-μm membrane. Elemental analysis of the water was carried out at the Plasma Chemical Analytic Center (Tomsk, Russia) by inductively coupled plasma mass spectrometry (ICP-MS). Prior to analysis, the samples were stored at 4°C. Prior to electron microscopic, elemental, and diffraction analyses, the sediments were air-dried and homogenated. Elemental composition of obtained powder was determined by energy dispersion analysis on an EDAX microanalyzer coupled to a Philips SEM 515 scanning electron microscope (Philips, The Netherlands). Microscopy was carried out at 30 kV, 12 mm focal length, and 50–100 nm probe size. Mineralogical analysis of the samples and construction of diffraction patterns were carried out using a Shimadzu XRD 6000 diffractometer (Shimadzu, Japan) enabling X-ray phase analysis. Analysis of numerous possible combinations of different phases was carried out using the Crystallographica-SearchMatch software package with the PDF-2 database. Prior to analysis, calibration with the standard sample was carried out in order to exclude the possible shift of the diffraction pattern. The standard sample was made of pure silicon with the known peak position required for the calibration curve (28.46°). The measurements were taken with 0.02° step, rate of 1° per minute, and exposure at a point of 1.2 s. Ultrathin sectioning and transmission electron microscopy were carried out as described previously (Ikkert et al., 2013).

For enrichment cultures and DNA isolation, sediment samples were collected in sterile 50-mL test tubes and stored at 4 and –20°C, respectively. To obtain SRP enrichment cultures, the material was inoculated into liquid Widdel medium for freshwater organisms (Widdel and Bak, 1992) with lactate or glycerol as electron donors. Penicillin vials filled to capacity contained iron wire as an additional iron source and in order to maintain low redox values due to formation of cathode hydrogen. For the isolation of acidophilic/acid-tolerant SRP, the medium was modified by removal of the $NaHCO_3$ buffer solution. Required pH values were adjusted with H_2SO_4 solution. Preparation of the medium with elevated metal concentrations was described previously (Karnachuk et al., 2009). Metal chlorides ($CuCl_2 \cdot 2H_20$, $CdCl_2 \cdot 2.5H_2O$, $CoCl_2 \cdot 6H_2O$, and $NiCl_2$) were used for this purpose.

Cultivation was carried out at 28°C. Purity of the culture was determined microscopically, by plating on rich media (Plate Count Agar and Anaerobic Agar), as well as by determination of the almost complete 16S rRNA gene sequence.

DNA from the sediments and SRP cultures was isolated using the MO BIO Power Soil DNA Kit (MO BIO Laboratories, Carlsbad, United States) according to the manufacturer's recommendations. Phylogenetic position of bacteria from the sediments and cultures was determined by separation of the PCR-amplified 16S rRNA gene fragments by denaturing gradient gel electrophoresis (PCR–DGGE). Prior to separation, nested PCR was carried out. The producers of PCR with the first primer pair 27F (5'-AGAGTTTGATCCTGGCTCAG-3') -1492R (5'-GGTTACCTTGTTACGACTT-3') (DeLong, 1992; Weisburg et al., 1991) were used as a template in the subsequent reaction with BacV3f (Weisburg et al., 1991) and 907r primers (Lane, 1991). The forward primer contained a GC sequence (5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGC-CGCCCCCGCCCC-3') (Lane, 1991). The PCR mixture (50 μL) contained 1× *Taq* buffer (Fermentas, Lithuania), 2.5 mM $MgCl_2$ (Fermentas, Lithuania), 100 μM of dNTP mixture (Fermentas, Lithuania), 1.25 U recombinant *Taq* DAN polymerase (Fermentas, Lithuania), and 0.2 μM primers (Syntol, Moscow, Russia). The amplification mode is shown in Table 1.

The amplified DNA fragments were separated using DCodeSystem (BioRad, United States) in the linear gradient of urea and formamide from 40 to 60% (the 100% denaturing solution contains 7 M urea and 40% formamide) in 8% polyacrylamide gel. Electrophoresis was carried out at 60°C and 110 V for 19 h in 1× TAE buffer. The gel fragments were washed with nuclease-free sterile water (Fermentas, Lithuania) for 2 h at room temperature, and DNA was extracted for 12 h at 4°C. The 16S rRNA gene fragments were used as templates for PCR with the primers BacV3f and 907r. In this case the forward primer contained no GC sequence. The presence of PCR products before and after DGGE was visualized in 1% agarose gel stained with ethidium bromide .

For sequencing of the 16S rRNA gene of the pure SRP culture, amplification was carried out with the primer pair 27F (5'-AGAGTTTGATCCTGGCTC AG-3') and 1492R (5'-GGTTACCTTGTTAC-GACTT-3') (Weisburg et al., 1991; Muyzer et al., 1996) according to the previously described protocols (Karnachuk et al., 2009). Sequencing of the DNA fragments was carried out by Syntol (Moscow). The sequences were analyzed using the BioEdit software package and the BLASTGenBank tool (http://www.ncbi.nlm.nih.gov/) (Altschul, 1997). Phylogenetic analysis was carried out using MEGA6 (Tamura et al., 2013). To determine the branching order, bootstrap analysis was carried out using 1000 alternative trees. The 16S rRNA gene sequence of the

Table 1. Modes of PCR amplification used

	Initial denaturing	Number of cycles	Denaturing	Primer annealing	Elongation	Final elongation
27F-1492R	95°C, 20 s	6	95°C, 10 s	45°C, 20 s	72°C, 90 s	72°C, 3 min
		30	95°C, 10 s	55°C, 20 s	72°C, 1.5 min	
BacV3f-907R	95°C, 5 min	30	95°C, 20 s	50°C, 1 min	72°C, 1.5 min	72°C, 10 min

Table 2. Some physicochemical parameters of the sample from the storage pond

Parameter	Units	Values
pH water		3.1
pH sediments		3.04
T water	°C	12.3
T sediments	°C	12.4
Eh water	mV	+470
Eh sediments	mV	+487
Fe	mg/L	172
Mn	mg/L	60.1
Al	mg/L	211
Zn	mg/L	172
Cu	mg/L	2.28
Cd	mg/L	3.21
Co	mg/L	0.443
Ni	mg/L	0.32
Pb	mg/L	0.268
As	mg/L	<0.002
Sr	mg/L	2.04
Th	mg/L	0.798
U	mg/L	0.468
Ag	mg/L	0.0012

pure culture was deposited to GenBank NCBI under accession no. KP184717.

RESULTS

Characterization of the oxidized sediment of the waste of tungsten processing. The sediments from the storage pond for liquid waste were of intense orange color, with a whitish film on the surface; pH of the near-bottom water and of the sediment (3–4 mm from the surface) was 3.04 and 3.1, respectively (Table 2). The temperature of the upper sediment layer and of the overlaying water was almost the same (12.4 and 12.3°C, respectively). Both the water and the sediment (5 mm from the surface) were oxidized, with Eh +470 and +487 mV, respectively. Compared to other similar environments, the water of the pond contained relatively low concentrations of dissolved metals (Table 2). Zn (172 mg/L), Al (211 mg/L), and Fe (172 mg/L) predominated in the water. The concentrations of Cu and Cd were 2.28 and 3.21 mg/L, respectively. Considerable concentrations of rare earth elements and of radioactive uranium and thorium were detected. Apart from turnerite, fluorite may also be a source of rare earth elements at the Bom-Gorkhon deposit (Eremin et al., 2014). Thorium and uranium probably originated from a number of uranium- and thorium-containing minerals, especially from thorite, chalcolamprite, and cyrtolite.

Diffraction analysis revealed the presence of muscovite ($KAl_2(AlSi_3O_{10})(OH)_2$, orthoclase ($K(AlSi_3O_8)$), and other rock-forming aluminum silicates in the sediments. The sediments also contained secondary urainium minerals typical of the oxidized zones of polymetallic deposits: uranium phosphate, saleite ($Mg(UO_2)_2((P,As)O_4)_2$, uranium vanadate tyuyamunite, ($Ca(UO_2)_2V_2O_8 \cdot 8H_2O$), molybtotumochoite ($UO_2MoO_4 \cdot 4H_2O$), and uranium-containing niobateliandratite $U_{+6}(Nb,Ta)_2O_8$. The presence of

the secondary sulfate, jarosite $KFe_3(SO_4)_2(OH)_6$, confirms active oxidation of residual sulfides in the waste. Unexpectedly, bernardite, a rare thallium arsenic sulfide ($TlAs_5S_8$), was the only sulfide present. Other common sulfides were probably completely oxidized or were separated in the course of migration.

Enrichment and Pure SRP Cultures

Inoculation of lactate- or glycerol-containing media with sediment samples resulted in enrichment cultures exhibiting indications of sulfate reduction (darkening of the medium and sulfide formation). For the isolation of pure cultures, the enrichment with lactate and initial Mo^{2+} and Cu^{2+} concentrations of 200 mg/L at pH 3.1 was chosen. Sequential transfer of terminal dilutions into the medium with lactate and glycerol at low pH (1.3–2.0) was carried out. Lower pH than in the enrichment cultures was used in order provide for selection of the culture with the highest tolerance to high proton concentrations. A morphologically uniform glycerol-growing culture with nonmotile rod-shaped cells was obtained. It was heated for 20 min at 90°C with subsequent transfer of serial dilutions. This procedure resulted in a morphologically uniform culture designated strain BG. Its purity was confirmed by the absence of growth on rich media—Plate Count Agar (aerobic) and Anaerobic Agar (anaerobic).

The cells of strain BG are nonmotile rods, straight or slightly curved, 1.5–3.5 μm long and 0.75–1.0 μm wide (Figs. 2a, 2b). During the stationary growth phase, the culture formed oval subterminal spores (Fig. 2c). The culture grew at initial pH 2 with 10-day lag phase duration. Increasing pH to 4.5–5.5 or decreasing it to 1 resulted in considerably decreased cell numbers and lag phase increase to 25 days (Fig. 3). At initial pH 6.5, no growth occurred, and only spores were present in the culture. Importantly, the medium was alkalinized in the course of cultivation. Long lag phase is possibly associated with certain intracellular processes, resulting in rapid growth during the subsequent 24–48 h, followed by rapid lysis and release of spores. The data presented on Fig. 3 indicate that active growth of strain BG occurs within pH range from 2 to 4.5 with the optimum at pH 2.0.

Growth of the strain on other substrates was therefore studied in the medium with initial pH 2.

Apart from lactate and glycerol, strain BG grew on pyuvate, acetate + CO_2, and on alcohols (ethanol, butanol, and propanol). In all cases pH of the medium increased to 5.0–5.5, and then active spore formation and cell lysis occurred.

Strain BG was highly resistant to heavy metal ions (Table 3). At up to 3000 mg/L copper in the medium, active growth occurred with 8–10-day lag phase. When Cu^{2+} concentration was increased to 6000 mg/L, lag phase elongated to 20–27 and the cell yield almost halved. Apart from Cu^{2+}, strain BG grew in the presence of other heavy metal ions: Cd^{2+} (10–150 mg/L), Ni^{2+} and Co^{2+} (20–100 mg/L) (Table 3). X-ray phase analysis of the precipitate formed by the culture grown in the presence of copper (initial Cu^{2+} concentration 250 mg/L) revealed the presence of crystalline copper sulfides, covellite and chalcopyrite (Fig. 4).

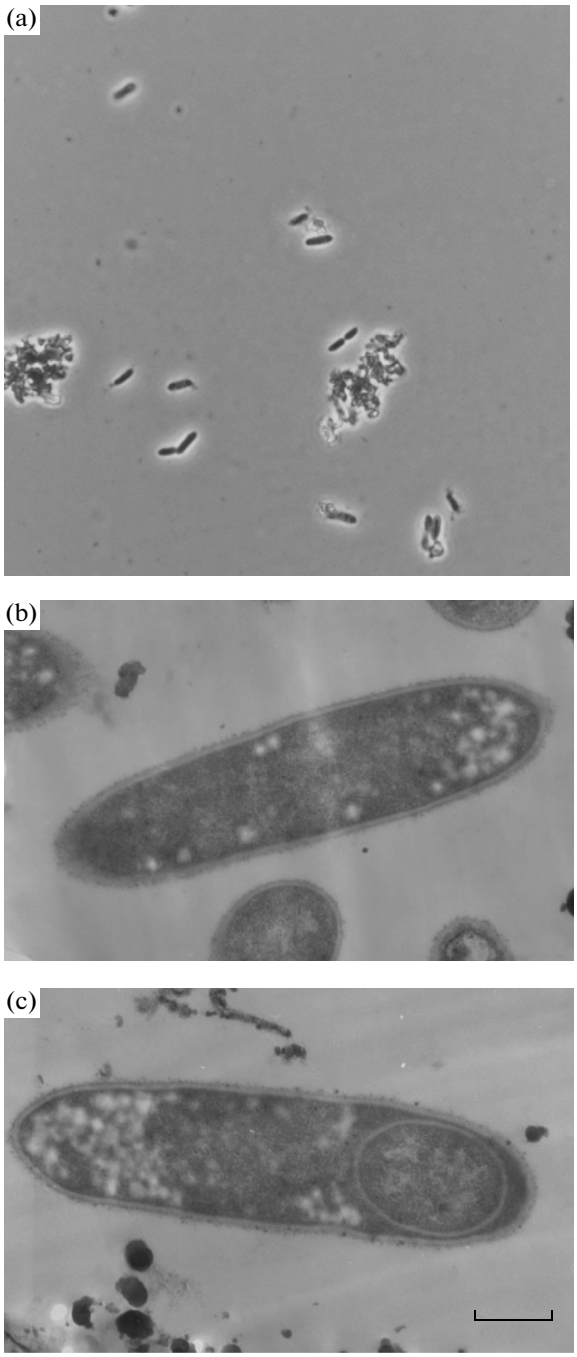

Fig. 2 Cells of strain BG, phase contrast (a) and ultrathin sections (b, c), transmission electron microscopy. Scale bar, 0.5 μm.

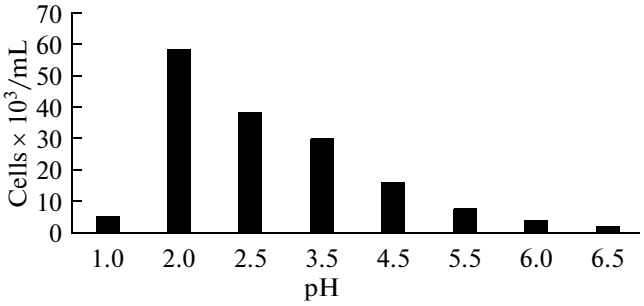

Fig. 3. Cell numbers of strain BG at different pH values.

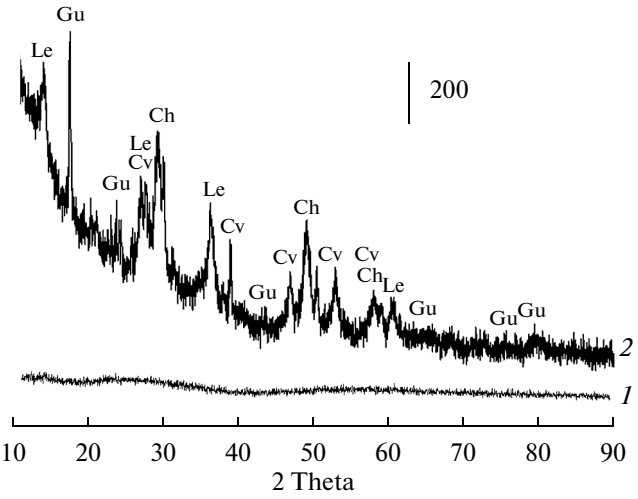

Fig. 4. Diffractograms of the sediments obtained by incubation of the cell-free chemical control (Widdel medium with 250 mg/L Cu^{2+}) for 17 days (*1*) and incubation of strain BG for 21 day (Widdel medium with 150 mg/L Cu^{2+}) (*2*). Designations: Ch, chalcopyrite, $CuFeS_2$; Cv, covellite, CuS; Le, lepidocrocite, FeO(OH); Gu, guildite, $CuFe(SO_4)_2(OH)(H_2O)_4$.

Bacterial Phylotypes Revealed in the Sediments and Enrichment Cultures

Apart from isolation of pure cultures, in enrichment cultures the dominant phylotypes of the 16S r RNA gene fragments were analyzed by PCR–DGGE. The phylotypes in the sediment samples were also analyzed. Sequencing of the amplified fragments revealed the presence of microorganisms of the genus *Desulfosporosinus* in enrichment cultures of sulfidogens under both acidic and neutral conditions (Table 4). The closest relatives of all the phylotypes obtained were different, indicating diversity of the microorganisms of this genus in the sediment community. Sulfidogenic cultures growing in neutral medium contained a phylotype most closely related to *Desulfotomaculum sapomandens* belonging to another SRP genus. The sequence similarity, however, did not exceed 91%. Since only a ~600-bp gene fragment was sequenced, the detected organism probably belonged to some presently unknown genus, rather than to *Desulfotomaculum*. Comparison of the phylotypes from the sediments and enrichment cultures indicated that spore-forming SRP of the phylum *Firmicutes* did not predominate in the sediment, where iron-oxidizing acidophilic *Acidithiobacillus ferrooxidans* were the most abundant microorganisms (Table 4).

Phylogenetic Position of Strain BG

Genetic homogeneity of strain BG was confirmed by the 16S rRNA gene sequencing. Phylogenetic analysis of the almost complete 16S rRNA gene fragment (1440 bp) placed it within the phylum *Firmicutes*, class *Clostridia*, order *Clostridiales*, family *Peptococcacaea*, genus *Desulfosporosinus* (Fig. 5). *Desulfosporosinus lacus* was the most closely related validly described species (97% similarity). Some uncultured organisms related to strain BG were detected in the waste of metal processing. Thus, the clone P3IB-51 (GenBank accession no. AF414568) was detected in the sediments of uranium mines (Suzuki and Banfield, unpublished data), while the clone RBC-13B (AB583916.1) was found in groundwater (Murakami et al., unpublished data).

DISCUSSION

An opinion was expressed that lactate, a traditionally used organic electron donor for SRP, was unsuitable for the isolation of acidophilic cultures (Koschorreck, 2008; Alazard et al., 2010; Sánchez-Andrea et al., 2014). Lactate does not dissociate at low pH and is presumably toxic to bacteria. Easier penetration through the cell membrane resulting in decreased intracellular pH is the putative mechanism of the action of undissociated lactate molecules. The original enrichment culture used for the isolation of strain BG contained lactate as the sole electron donor. *Desulfosporosinus* sp. BG grew in the media with lactic acid. Weak growth on lactate was shown for *D. acidiphilus* (Alazard et al., 2010). *D. acididurans* grew well on pyruvate, which, according to the authors' hypothesis, should have also inhibited growth (Sánchez-Andrea et al., 2014). Thus, the presence of lactate and other carboxylic acids does not prevent growth of some SRP at low pH. *Desulfosporosinus* probably possess a mechanism limiting the penetration of undissociated acids into the cell.

Recent research indicates that spore-forming SRP of the genus *Desulfosporosinus* are important components of the microbial community carrying out dissimilatory sulfate reduction in the low-pH ecosystems associated with recovery. *Desulfosporosinus* sp. Lau III, isolated from an acidic lake formed as a result of sur-

face coal mining in Lusatia, Germany, was one of the first SRP cultures able to grow at low pH (Küsel et al., 2001). It grew within a narrow pH range from 4.9 to 6.1 with the optimum at pH 5.5. This strain was also isolated on lactate. Two validly described acidophiles and several SRP strains with pH optima below 5 belong to the genus *Desulfosporosinus* and were isolated from the ecosystems associated with metal recovery.

In the ecosystems associated with sulfide recovery, low pH usually results in the dissolution of metals in the liquid phase. Microorganisms from such habitats should be tolerant to high concentrations of both protons and metal ions. Tolerance to metals is also important for the possible application of acidophilic strains for bioremediation. While the recently described *D. acididurans* grew at up to 2800 mg/L Fe^{2+} and 279 mg/L Al^{3+} (Sánchez-Andrea et al., 2014), its growth was completely inhibited by 63.5 mg/L Cu^{2+}. Tolerance of *D. acidiphilus* to metals was not determined (Alazard et al., 2010). The strain *Desulfosporosinus* sp. DB isolated from gold recovery waste (Kuznetsk Basin, Russia) grew at initial Cu^{2+} concentration of 650 mg/L (Karnachuk et al., 2009). *Desulfosporosinus* sp. OT grew at the highest copper concentration, up to 15 g/L (Abicht et al., 2011). Thus, strain BG, which is tolerant to copper (up to 5 g/L) is among the most metal-resistant acidophilic SRP. Formation of crystalline copper sulfides, covellite (CuS) and chalcopyrite ($CuFeS_2$), is also of biotechnological importance. These sulfides are traditional raw materials for the pyrometallurgic industry and may be used in the known technological cycles. Moreover, crystalline phases are less prone to oxidation than amorphous sulfides and are preferable for waste burial.

The new acidophilic strain BG was isolated from oxidized sediment layers. Oxygen tolerance has not been reported for *Desulfosporosinus* isolates. Although numerous and diverse SRP have long been known to occur in the oxidized zone of the storage facility for Cu–Zn recovery waste (Fortin and Beveridge, 1997), the presence and activity of SRP in highly oxidized wastes of metal recovery receives insufficient attention. Indirect evidence indicates the presence of *Desulfosporosinus* in oxidized ecosystems with low pH. This SRP group was detected by molecular genetic techniques in microbial filaments (streamers) developing on the surface of Rio Tinto (Garcia-Moyano et al., 2007). Rio Tinto, which flows through the Iberian pyrite belt, is known for its low pH and active processes of ion oxidation. Suzuki et al. (2003) investigated development of *Desulfosporosinus* in uranium-contaminated oxidized soils and suggested bacterial survival under oxidized conditions in the form of spores. Further research is required for the understanding of the role of *Desulfosporosinus* in the low-pH oxidized zones of metal recovery waste.

Table 3. Effect of different concentrations of metal ions on lag phase duration and growth of strain BG

Initial Me^{2+} ion concentrations, mg/L	Cell number, $\times 10^3$/mL	Lag phase duration, days
Cu 500	55.4	10
Cu 1000	54.4	10
Cu 2000	55.7	10
Cu 3000	56.5	8
Cu 4000	50.0	20
Cu 5000	38.9	25
Cu 6000	25.0	27
Cd 10	45.4	15
Cd 20	46.3	18
Cd 60	44.9	21
Cd 100	44.4	25
Cd 150	18.5	30
Cd 200	2.00	NG
Ni 20	64.9	12
Ni 50	55.6	10
Ni 100	41.7	8
Co 20	47.3	15
Co 50	33.4	23
Co 100	46.5	19

* Bacterial cell number at inoculation was 2.0×10^3 cells/mL. NG indicates no growth.

Table 4. Phylotypes of the 16S rRNA genes detected in the sediments and in enrichment cultures

Sample	Phylotype	Closest relative (NCBI accession no.)/16S rRNA gene similarity, %	Closest validly described relative (NCBI accession no.)/16S rRNA gene similarity, %
Sediments of the storage pond	Uncultured *Firmicutes*	Strain MCF99 from mine water of the Iberian pyrite belt (JX412370)/100%	*Alicyclobacillus contaminans* (AB681941)/90%
	Uncultured *Acidithiobacillus* sp.	Clone OY04C1-057 from acidic volcanic soil, Japan (AB552190)/100%	*Acidithiobacillus ferrooxidans* strain PS1 (KC954526)/99%
Enrichment cultures; pH 7			
Peptone + 200 mg/L Cu^{2+} + 200 mg/L Mo^{2+}	*Desulfosporosinus* sp.	Clone MS9 from an enrichment culture anaerobically growing on hydrocarbons (FJ842586)/99%	*Desulfosporosinus orientis* DSM 765 (ÑÇ003108)/99%
Lactate + 200 mg/L Mo^{2+}	*Desulfosporosinus* sp.	Clone SB15 from soil contaminated by heavy metals and uranium (HM992486)/98%	*Desulfosporosinus lacus* DSM15449 (NR_042202)/96%
Lactate + 200 mg/L Mo^{2+}	*Desulfotomaculum* sp.	Clone EMIRGE_OTU_s8b4e_3915 from an underground aquifier (JX225716)/99%	*Desulfotomaculum sapomandens* DSM 3223 (NR_025026)/91
Lactate + 200 mg/L Mo^{2+}	*Sporotalea* sp.	Clone EMIRGE_OTU_s2b2b_7442 from an underground aquifier (JX222484)/99%	*Sporotalea propionica* TM1 (FN689723)/99%
Lactate + 200 mg/L Mo^{2+}	*Gracilibacter* sp.	Clone SmB5 from an organic waste-treating bioreactor (AB291265)/100%	*Gracilibacter thermotolerans* strain JW/YJL-S1/ (DQ117469)/95%
Peptone + 200 mg/L Mo^{2+}	*Acidithiobacillus* sp.	Clone SX2-15 from acidic mine water (DQ469222)/100%	*Acidithiobacillus thiooxidans* ABRM2011 (JQ034367)/99%
Enrichment cultures; pH 3			
Formate + 100 mg/L Cd^{2+}	*Desulfosporosinus* sp.	Clone RBC-13B from groundwater (AB583916)/100%	*Desulfosporosinus hippei* DSM 8344 (NR_044919)/97%
Formate + 100 mg/L Cd^{2+}	*Clostridium* sp.	Clone D12_34 from groundwater contaminated with heavy hydrocarbons (EU266838)/100%	*Clostridium sufflavum* CDT-1 (AB267266)/93%
Formate + 100 mg/L Cd^{2+}	*Firmicutes*	Clone 5_68_B3_b from groundwater contaminated with hydrocarbons (JQ087045)/100%	*Cryptanaerobacter phenolicus* ATCC BAA-820 (NR_025757)/91%
Lactate + 100 mg/L Cd^{2+}	*Acidithiobacillus* sp.	Clone KM33 from metal recovery waste (DQ240272)/100%	*Acidithiobacillus ferrivorans* SS3 (NR_074660)/99%
Formate + 200 mg/L Cu^{2+} + 200 mg/L Mo^{2+}	*Leptospirillum*	Clone placa1_d5 from surface streamers, acidic river Rio Tinto (EF446244)/100%	*Leptospirillum ferrooxidans* Chil-Lf2 (AF356835)/100%
Formate + 200 mg/L Cu^{2+} + 200 mg/L Mo^{2+}	*Gammaproteobacteria*	Clone ADK-MOe02-89 from a lake subject to acidic waste (EF520576)/99%	*Legionella pneumophila* GC-C5 (JN983403)/94%

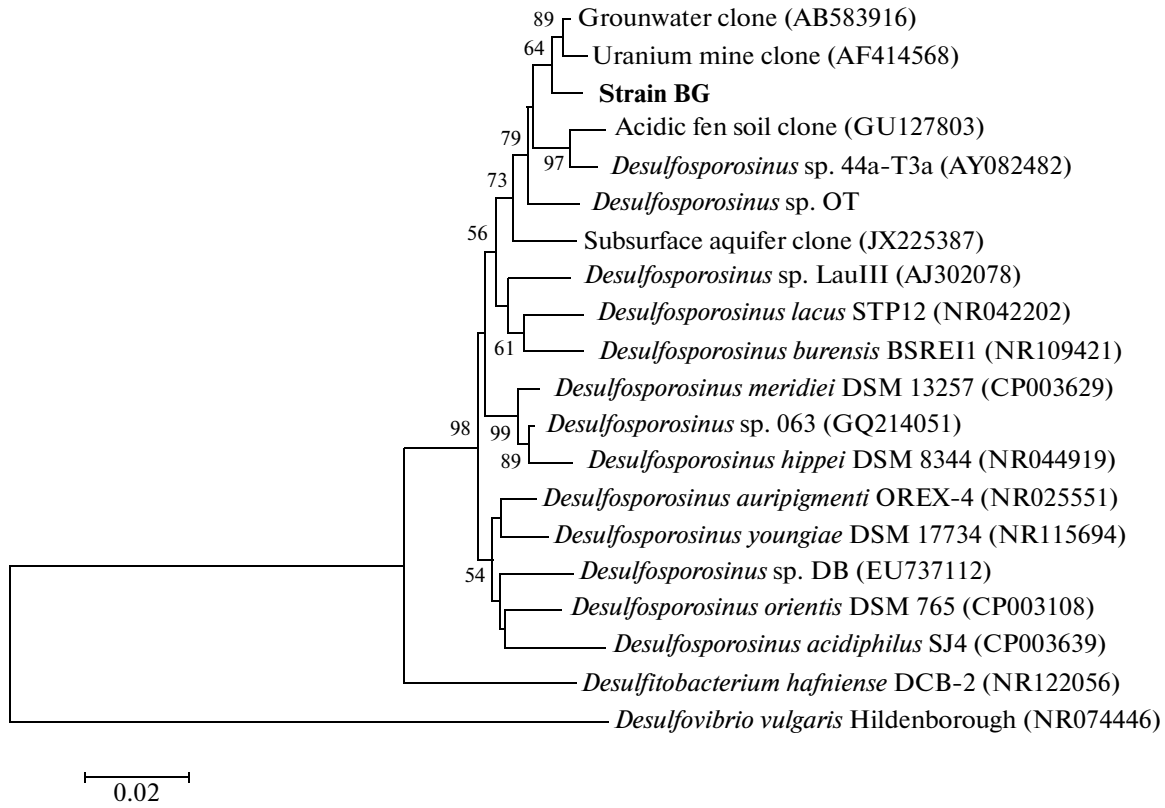

Fig. 5. Phylogenetic position of strain BG determined by the neighbor-joining method. *Desulfovibrio vulgaris* NR074446 was used as an outgroup.

ACKNOWLEDGMENTS

The work was supported by the Federal Target Program grant, agreement no. 14.575.21.0067 of 07.08.2014, project RFMEFI57514X0067.

REFERENCES

Abicht, H.K., Mancini, S., Karnachuk, O.V., and Solioz, M., Genome sequence of *Desulfosporosinus* sp. OT, an acidophilic sulfate reducing bacterium from copper mining waste in Norilsk, Northern Siberia, *J. Bacteriol.* 2011, vol. 193, no. 21, pp. 6104–6105.

Alazard, D., Joseph, M., Battaglia-Brunet, F., Cayol, J.-L., and Ollivier, B., *Desulfosporosinus acidiphilus* sp. nov.: a moderately acidophilic sulfate-reducing bacterium isolated from acid mining drainage sediments, *Extremophiles*, 2010, vol. 14, no. 3, pp. 305–312.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acid Res.*, 1997, vol. 25, pp. 3389–3402.

Barabanov, V.F., *Mineralogiya vol'framitovykh mestorozhdenii Zabaikal'ya* (Mineralogy of Transbaikalia Wolframite Deposits), vol. 2, Leningrad: Len. Gos. Univ., 1975.

Bijmans, M.F.M., van Helvoort, P.-J., Dar, S.A., Dopson, M., Lens, P.N.L., and Buisman, C.J.N., Selective recovery of nickel over iron from a nickel–iron solution using microbial sulfate reduction in a gas-lift bioreactor, *Water Res.* 2009, vol. 43, no. 3, pp. 853–861.

Blowes, D.W., Ptacek, C.J., Jambor, J.L., and Weisener, C.G., The geochemistry of acid mine drainage, in *Treatise on Geochemistry*, Heinrich, D.H. and Karl, K.T, Eds., Oxford: Pergamon, 2003, pp. 149–204.

DeLong, E.F. Archaea in costal marine environments, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, vol. 89, pp. 5685–5689.

Dolla, A., Fournier, M., and Dermoun, Z., Oxygen defense in sulfate-reducing bacteria, *J. Biotechnol.*, 2006, vol. 126, no. 1, pp. 87–100.

Eremin, O.V., Epova, E.S., Yurgenson, G.A., and Smirnova, O.K., Predicted geoeclogical consequences of development of the Bom_Gorkhon tungsten deposit (Transbaikalia), *Khim. Interesah Ustoich. Razvit.*, 2014, vol. 22, pp. 125–131.

Fortin, D. and Beveridge, T.J., Microbial sulfate reduction within sulfidic mine tailings: Formation of diagenetic Fe sulfides, *Geomicrobiol. J.*, 1997, vol. 14, pp. 1–21.

García-Moyano, A., González-Toril, E., Aguilera, A., and Amils, R., Prokaryotic community composition and ecology of floating macroscopic filaments from an extreme acidic environment, Río Tinto (SW, Spain), *Syst. Appl. Microbiol.*, 2007, vol. 30, no. 8, pp. 601–614.

Van Houten, B.H.G.W., van Doesburg, W., Dijkman, H., Copini, C., Smidt, H., and Stams, A.J.M., Long-term performance and microbial community analysis of a full-scale synthesis gas fed reactor treating sulfate- and zinc-rich

wastewater, *Appl. Microbiol. Biotechnol.*, 2009, vol. 84, no. 3, pp. 555–563.

Ikkert, O.P., Gerasimchuk, A.L., Bukhtiyarova, P.A., Tuovinen, O.H., and Karnachuk, O.V., Characterization of precipitates formed by H_2S-producing, Cu-resistant *Firmicute* isolates of *Tissierella* from human gut and *Desulfosporosinus* from mine waste, *Anton. Leeuw. Int. J. G.*, 2013, vol. 103, pp. 1221–1234.

Jong, T. and Parry, D.L., Removal of sulfate and heavy metals by sulfate reducing bacteria in short term bench scale upflow anaerobic packed bed reactor runs, *Water Res.*, 2003, vol. 37, no. 14, pp. 3379–3389.

Kaksonen, A.H., Plumb, J.J., Franzmann, P.D., and Puhakka, J.A., Simple organic electron donors support diverse sulfate-reducing communities in fluidized-bed reactors treating acidic metal- and sulfate-containing wastewater, *FEMS Microbiol. Ecol.*, 2004, vol. 47, no. 3, pp. 279–289.

Karnachuk, O.V., Frank, Y.A., Pimenov, N.V., Yusupov, S.K., Ivanov, M.V., Kaksonen, A.H., Puhakka, J.A., Lindström, E.B., and Tuovinen, O.H., Sulfate reduction potential in sediments in the Norilsk mining area, Northern Siberia, *Geomicrobiol. J.,* 2005, vol. 22, nos. 1–2, pp. 11–25.

Karnachuk, O.V., Gerasimchuk, A.L., Banks, D., Frengstad, B., Stykon, G.A., Tikhonova, Z.L., Kaksonen, A., Puhakka, J., Yanenko, A.S., and Pimenov, N.V., Bacteria of the sulfur cycle in the sediments of gold mine tailings, Kuznetsk Basin, Russia, *Microbiology* (Moscow). 2009, vol. 78. no. 4. pp. 492–497.

Koschorreck, M., Microbial sulphate reduction at a low pH, *FEMS Microbiol. Ecol.*, 2008, vol. 64, no. 3, pp. 329–342.

Küsel, K., Roth, U., Trinkwalter, T., and Peiffer, S., Effect of pH on the anaerobic microbial cycling of sulfur in mining-impacted freshwater lake sediments, *Environ. Exp. Bot.,* 2001, vol. 46, pp. 213–223.

Lane, D.J., 16S/23S rRNA Sequencing, *Nucleic Acid Techniques in Bacterial Systematics*, Stackebrandt, E. and Goodfellow, M., Eds., New York: Wiley, 1991, pp. 115–175.

Muyzer, G., Hottenträger, S., Teske, A., and Wawer, C., Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA—a new molecular approach to analyse the genetic diversity of mixed microbial communities, in *Molecular Microbial Ecology Manual*, Akkermans, A.D.L. et al., Eds., Dordrecht: Kluwer Academic Publishers, 1996, pp. 1–23.

Ňancucheo, I. and Johnson, D.B., Removal of sulfate from extremely acidic mine waters using low pH sulfidogenic bioreactors, *Hydrometallurgy*, 2014. vol.150, pp. 222–226.

Ontoev, D.O., *Stadiinost' mineralizatsii i zonal'nost' mestorozhdenii Zabaikal'ya* (Staging of Mineralization and Zonality of Transbaikalia Deposits), Moscow: Nauka, 1974.

Pester, M., Brambilla, E., Alazard, D., Rattei, T., Weinmaier, T., Han, J., Lucas, S., Lapidus, A., Cheng, J.F., Goodwin, L., Pitluck, S., Peters, L., Ovchinnikova, G., Teshima, H., Detter, J.C. et al., Complete genome sequences of *Desulfosporosinus orientis* DSM765^T, *Desulfosporosinus youngiae* DSM17734^T, *Desulfosporosinus meridiei* DSM13257^T, and *Desulfosporosinus acidiphilus* DSM22704^T, *J. Bacteriol.*, 2012, vol. 194, no. 22, pp. 6300–6301.

Sahinkaya, E., Gungora, M., Bayrakdara, A., Yucesoya, Z., and Uyanik, S., Separate recovery of copper and zinc from acid mine drainage using biogenic sulfide, *J. Hazar. Mater.*, 2009, vol. 171, nos. 1–3, pp. 901–906.

Sánchez-Andrea, I., Stams, A.J.M, Hedrich, S., Ňancucheo, I., and Johnson, D.B., *Desulfosporosinus acididurans* sp. nov.: an acidophilic sulfate-reducing bacterium isolated from acidic sediments, *Extremophiles*, 2015, vol. 19, no. 1, pp. 39–47.

Sass, H. and Cypionka, H., Response of sulphate-reducing bacteria to oxygen, in *Sulphate Reducing Bacteria: Environmental and Engineered Systems*, Barton, L.L. and Hamilton, W.A., Eds., Cambridge: Cambridge Univ. Press, 2007, pp. 167–183.

Suzuki, Y., Kelly, S.D., Kemner, K.M., and Banfield, J.F., Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment, *Appl. Environ. Microbiol.*, 2003, vol. 69, pp. 1337–1346.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S., MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0., *Mol. Biol. Evol.,* 2013, vol. 30, pp. 2725–2729.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J., 16S ribosomal DNA amplification for phylogenetic study, *J. Bacteriol.*, 1991, vol. 173, pp. 697–703.

Widdel F.F. and Bak, R., Gram-negative mesophilic sulfate-reducing bacteria, in *The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd ed.*, Balows, A. et al., Eds., Berlin: Springer, 1992, vol. 4, pp. 3352–3378.

Translated by P. Sigalevich