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EXPERIMENTAL ARTICLES

Genotypic and Phenotypic Polymorphism of Environmental Strains of the Moderately Thermophilic Bacterium *Sulfobacillus sibiricus*

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Abstract—Five cultures of moderately thermophilic spore-forming acidophilic chemolithotrophic bacteria were isolated from the zones of spontaneous heating of pyrrhotite-containing pyrite-arsenopyrite gold-arsenic sulfide ores in an operating open pit (strains B1, B2, B3, OFO, and SSO). Analysis of the chromosomal DNA structure revealed the differences between these cultures at the strain level (apart from B3 and SSO, which had identical restriction profiles). All the strains had a similar G+C DNA molar content (47.4–48.3%). The level of DNA reassociation was 85 to 95%. The similarity between the DNA of the type strain *Sulfobacillus sibiricus* N1 isolated from arsenopyrite ore concentrate and that of these strains (83–93%) indicates that they belong to the same species. The strains had similar values of pH and temperature optimal for growth on ferrous iron (1.6–2.0 and 45–55°C, respectively). They were mixotrophs; Fe(II), S^0, and sulfide minerals along with organic compounds were used as energy sources and electron donors. However, the kinetic parameters of growth and substrate oxidation varied from strain to strain. Genetic variety of the strains from diverse ecosystems and environments is possibly the result of the different rates of microevolution processes.

Key words: isolates, genotypic and phenotypic characteristics, strains, *Sulfobacillus sibiricus.*

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Sulfide ore deposits are natural environments with well-established microbial communities. In mined quarries, in the presence of water (snow), carbon dioxide, and oxygen or other oxidizers, e.g., Fe(III), zones of spontaneous heating develop; depending on the ore type, the rate of exothermic reactions, and ambient temperature, the temperature in these zones may reach 60°C or more (up to inflammation). Microbial acidophilic communities include chemolithotrophic and organotrophic eubacteria and archaea [1]. Chemolithotrophs include bacteria of the genera *Acidithiobacillus*, *Sulfobacillus*, *Leptospirillum*, *Acidimicrobium*, and *Hydrogenobacter* and archaea of the genera *Acidianus*, *Sulfolobus*, *Ferroplasma*, and *Metallosphaera*. Chemoorganotrophic bacteria belong to the genera *Alicyclobacillus*, *Acidisphaera*, *Acidocella*, "*Ferromicrobium*," and *Acidiphilium* [2–6].

Analysis of the structure of chromosomal DNA by pulse electrophoresis indicates the importance of adaptation to new energy substrates as a selective factor which can result in both reversible [7–9] and irreversible [10] changes in the genome structure. Irreversible changes in the DNA result in strain polymorphism. For instance, a new strain *Acidithiobacillus ferrooxidans* TFO-2 emerged when the characteristics of the energy source changed [10]. The rates of energy source oxidation are the key taxonomic feature for strain identification of acidophilic chemolithotrophs [7–9].

The goal of the present work was to investigate the phenotypic and genotypic characteristics of the strains of thermoacidophilic chemolithotrophic bacteria isolated from the zones of spontaneous heating in the quarry of a developed deposit of pyrrhotite-containing pyrite–arsenopyrite gold–arsenic sulfide ores.

MATERIALS AND METHODS

Isolation of pure cultures and cultivation conditions. Ore samples were collected in the quarry of a pyrrhotite-containing pyrite–arsenopyrite gold–arsenic deposit. The ore samples (10 g) were placed in 250-ml Erlenmeyer flasks with 100 ml of nutrient media. The media used were Silverman and Lundgren 9K medium [11] and Manning MM medium [12] supplemented with $FeSO_4 \cdot 7H_2O$ (44.2 and 33.4 g l^{-1}, respectively) or elemental sulfur (5 g l^{-1}). Yeast extract (0.2 g l^{-1}) was added to create mixotrophic conditions. The flasks were incubated with shaking (180 rpm) at 48°C.

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Pure cultures were obtained by the method of serial tenfold dilutions and inoculation on the special medium initiating sporulation in sulfobacilli (liquid and with 0.5% of agarose) [13]. The spores were boiled for 35 min and inoculated on 9K and MM media with yeast extract; three repeats of serial tenfold dilutions were used. To ascertain the axenic nature of the cultures, transfers were performed to agarose medium [14], to media with high concentrations (10 g l^{-1}) of organic substrates (glucose, yeast extract, or tryptone), which suppress growth of oligotrophic sulfobacilli, and to media with $FeSO_4 \cdot 7H_2O$ or elemental sulfur without yeast extract. Pure cultures of sulfobacilli survived three transfers on such mineral media. Growth of heterotrophic bacterial satellites after six to eight transfers or of autotrophic ones after the fourth transfer was an indication of insufficient purification of the isolates.

The pure cultures were maintained on 9K medium with ferrous iron or sulfur and yeast extract (0.2 g l^{-1}). In some experiments, sulfide minerals and sulfide ore concentrates (10 g l^{-1}) were added as energy sources. The reference strains included type strains of *Sulfobacillus sibiricus* $N1^T$ (isolated from pyrite–arsenopyrite concentrate) [15], *S. thermosulfidooxidans* 1269, *S. acidophilus* NAL, and *S. thermotolerans* Kr1; they were also cultivated on 9K medium with ferrous iron and yeast extract.

Mineral and organic growth substrates. The mineral substrates used in the present work included ferrous iron, elemental sulfur, sulfide minerals (FeS, FeS_2, FeAsS, $CuFeS_2$, PbS, AsS, As_2S_3, MoS_2, ZnS, Sb_2S_3), and sulfide ore concentrates. Pure sulfide minerals were obtained from the collection of the Fersman Mineralogical Museum, Russian Academy of Sciences. The pyrrhotite pyrite–arsenopyrite concentrate contained the following: pyrrhotite (FeS), 36.5%; pyrite (FeS_2), 11.0%; arsenopyrite (FeAsS), 10.0%; antimonite (Sb_2S_3), 6.5%; sphalerite (ZnS), 0.3%; halenite (PbS), 0.3%; and chalcopyrite ($CuFeS_2$), 0.3%. The pyrite–arsenopyrite concentrate contained the following: FeS_2, 24.3%; FeAsS, 10.4%; PbS, 0.8%; ZnS, 0.3%; $CuFeS_2$, 0.4%; and Sb_2S_3, 0.1%. The particles of the minerals and sulfide concentrates had the size of 0.044 mm (80%). The amount introduced into the media was 10 g l^{-1}. In experiments on growth kinetics and substrate oxidation under mixotrophic conditions, the initial bacterial concentration was $0.8–1.0 \times 10^7$ cells ml^{-1}.

Organotrophic growth of the strains on carbohydrates, organic acids, and amino acids was determined; their carbon content was equivalent to 0.025% glucose. The concentrations of yeast extract and tryptone were 0.25 g l^{-1} when they were used together and 0.5 g l^{-1} when used individually. For mixotrophic cultivation with organic compounds, 5 g l^{-1} of Fe(II) as $FeSO_4 \cdot 7H_2O$ was added.

Cell counting. The number of cells in liquid cultures were determined by direct count under a Lumam I1 phase contrast microscope (LOMO, Russia). Specific growth rates, generation times, and the rates of substrate oxidation were calculated according to the known equations [16].

Determination of DNA nucleotide composition. DNA preparations were extracted from the cells and purified according to Marmur [17]. Nucleotide composition was determined in three repeats by the thermal denaturation method (determination of the melting point) [18]; the accuracy of the method is ±0.3–0.5%. DNA hybridization was determined by the De Ley standard method for DNA–DNA reassociation [19]; relations and phylogenetic homogeneity of the strains within a species, as well as interspecific differences, are determined with an accuracy of ±5–7% [20]. Analyses were performed at least three times. Genome size was determined according to De Ley [19]. The spectroscopic parameters were determined on a Pye-Unicam-1500 spectrophotometer (United States).

Analysis of the chromosomal DNA structure. After removal of accompanying microflora, the cultures were maintained on 9K medium with yeast extract and ferrous iron for at least five transfers. The structure of chromosomal DNA was then analyzed by pulsed-field gel electrophoresis. Analysis of DNA structure was carried out according to the modified method of Schwartz and Cantor [21]. Since the content of G+C nucleotide pairs in the DNA of bacteria under study did not exceed 50 mol %, *Not*I endonuclease with the $GC^{\downarrow}GGCCGC$ sequence in the restriction site was used. Stability of the genomes of the bacterial isolates obtained for three years has been confirmed by all the subsequent work with these strains, including the experiments not described in the present paper.

Growth temperature and pH. Bacterial isolates were grown in flasks with shaking on 9K medium with ferrous iron and yeast extract (pH 1.8–2.0, temperature range 14–65°C) or with elemental sulfur and yeast extract (48°C, pH range 1–6). Samples were taken daily within the higher temperature range (30–65°C) and every two to three days within the lower (14–25°C) temperature range. The highest specific growth rates corresponded to the optimal pH and temperature values. To determine the optimum, samples were taken every three to six hours.

Chemical analyses. The concentrations of Fe(II), Fe(III), and sulfates were determined as described in [15, 22–24].

All the values of growth parameters, substrate oxidation, and concentrations are the average of at least 3–5 independent experiments repeated two or three times. The differences between the average values were considered reliable when their confidence intervals didn't overlap. The reliability of the results was determined using the Student t criterion at $P \leq 0.05$ [25].

Table 1. DNA nucleotide base composition and DNA–DNA hybridization of *S. sibiricus* strains

S. sibiricus, strain	DNA G+C content, mol %	DNA–DNA hybridization, %				
		$N1^T$	B1	B2	OFO	SSO
$N1^T$	48.2 ± 0.3	100				
B1	47.5 ± 0.3	92 ± 7	100			
B2	47.7 ± 0.4	90 ± 5	89 ± 7	100		
OFO	48.3 ± 0.4	93 ± 5	95 ± 5	91 ± 6	100	
SSO	47.4 ± 0.5	83 ± 5	85 ± 6	90 ± 5	89 ± 5	100

RESULTS

Isolation of bacterial cultures. At the time of sampling, the quarry surface was covered with snow; the ambient air temperature was –15°C. Zones of heating were detected on the ore surface; melting of snow and emission of vapor were detected. At a depth of 10–20 cm, the temperature was as high as 12–14°C; pH of an aqueous soil–ore extract was 2.0–3.0. The samples for microbiological analysis were collected at a depth of 20 cm; prior to their arrival at the laboratory, they were stored at 4°C.

Enrichment cultures were obtained by inoculating 10 g of wet ore into 100 ml of 9K or MM medium; cell numbers determined by the serial dilution method in two rows were 10^4–10^5 cells ml^{-1}. The subsequent procedures (tenfold serial dilutions in three repeats, transfer to the medium for sporulation initiation, heating of the spores at 100°C, and repeated serial dilutions) resulted in isolation of five axenic bacterial strains (see Materials and Methods). Isolated colonies were formed on agarose medium; the absence of growth in the media with high concentrations of organic compounds indicated the absence of heterotrophic satellites. The presence of obligately autotrophic satellites was also not detected; no bacterial cells were found after 4–5 transfers on mineral media.

The strains were designated B1, B2, B3, OFO, and SSO. Attempts were made to cultivate these strains under autotrophic, heterotrophic, and mixotrophic conditions. Only in the latter case (in the presence of a mineral substrate and 0.2 g l^{-1} of yeast extract) did these cultures grow and develop normally. Autotrophic growth was maintained for no more than three transfers, and organoheterotrophic for two to six transfers.

Genotypic characteristics of the strains. The DNA G+C base content of the strains (47.5–48.3 mol % on the average) did not vary significantly (Table 1). Strains SSO and OFO, isolated from the same zone of heated ore, had a DNA G+C base content of 47.4 ± 0.5 and 48.3 ± 0.4 mol %, respectively. Strains B1 and B2, isolated from another site of the ore quarry, had a DNA G+C base content of 47.5 ± 0.3 and 47.7 ± 0.4 mol %, respectively.

Results of DNA–DNA reassociation are presented in Table 1. The degree of DNA reassociation between the new strains and the type strain *S. sibiricus* $N1^T$ [15] (VKM B-2280=DSM 17363) was close to intraspecific, 83 to 95% (Table 1, average values); the species boundaries are between 70 and 100% [20]. Strains B1 and OFO were the closest to the type strain in DNA homology (92 ± 7 and 93 ± 5%, respectively); strain SSO was the most remote (83 ± 5%). The DNA from other species of sulfobacilli was used for comparative analysis of the degree of DNA–DNA hybridization (the data are not presented in the table). No similarity at the species level was found between genomic DNA of the new isolates and type cultures of these species (10–38% similarity by DNA–DNA hybridization). Reassociation between the DNA of the new strains was 85 to 95%. The genome of the strains contained $5.6–5.92 \times 10^3$ kb. Thus, on the basis of genomic characterization all the newly obtained strains can be assigned to the species *S. sibiricus*.

Analysis of the chromosomal DNA structure. The results of pulse-field gel electrophoresis analysis of restriction fragments of the chromosomal DNA of five new isolates are presented on Fig. 1. Strains B1, B2, OFO, and SSO differed in the size and number of *Not*I-cleaved chromosomal DNA fragments. The restriction pattern of strain B3 was identical to that of strain SSO. The major fragments of bacterial chromosomal DNA were determined. Their size in all the cultures was from 90 to 330 kb.

Morphology. In the exponential growth phase, the cells were rods with rounded ends, single or in short chains. Cell sizes determined by light microscopy are presented in Table 2. The strains are polymorphic in the stationary growth phase, as well as at low and high cultivation temperatures. The cells are nonmotile, and the Gram reaction is positive. Endospores are usually oval, sometimes spherical, terminally or subterminally located; the sporangium is extended.

Growth temperature. The optimal growth temperatures for strains B1 and SSO were 45–50 and 50–55°C, respectively; for strains B2 and OFO, the optimal temperature was 48–50°C (Table 2; Fig. 2).

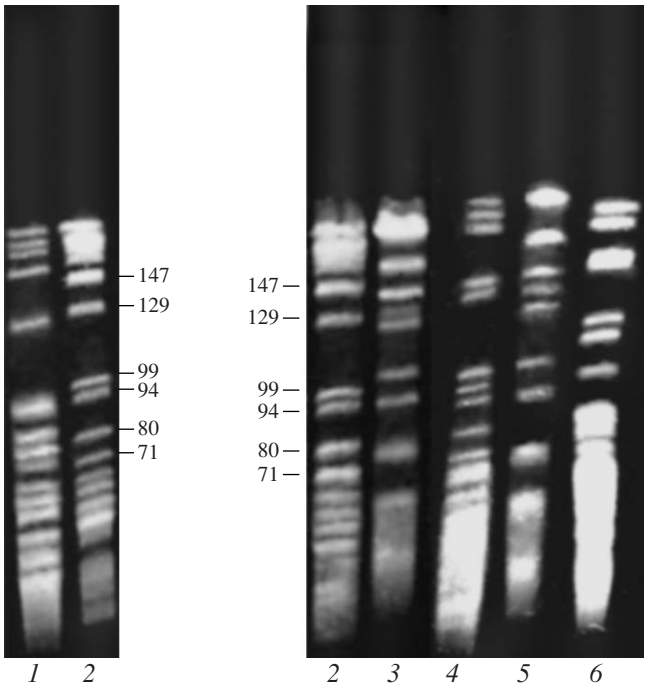

Fig. 1. Pulse-field gel electrophoresis of the chromosomal DNA fragments obtained from *S. sibiricus* strains by cleavage with *NotI* restriction endonuclease: B1 (*1*); size marker—*A. ferrooxidans* chromosomal DNA fragments cleaved by *Xba*I restriction endonuclease (*2*); B2 (*3*); OFO (*4*); SSO (*5*); and $N1^T$ (*6*). Electrophoresis conditions: voltage, 10.4 V/cm; pulse duration, 10 s; temperature, 18°C; duration, 68 h. Side markers indicate the fragment size, kb.

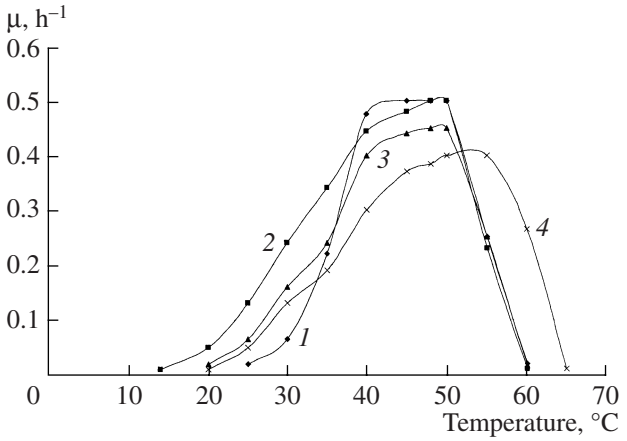

Fig. 2. Specific growth rate of *S. sibiricus* strains at various temperatures: B1 (*1*), B2 (*2*), OFO (*3*), and SSO (*4*). Cultivation on the medium with Fe(II) and yeast extract.

Thus all the strains are moderately thermophilic. The temperature growth range of the strains varied somewhat. Strain B2 could grow within the range from 14 to 58°C; strain B1 had a narrower range, from 25 to 58°C. Growth of strains OFO and SSO was possible at temperatures of 20–58 and 20–65°C, respectively.

Acidity. Strains B2 and B1 could grow on media with Fe(II) at pH from 1.2 to 2.6 and 1.5 to 2.7, respectively; strains SSO and OFO grew at lower pH values (pH 1.0–1.1) (Table 2, Fig. 3). Under such acidic conditions (pH 1.0–1.1), numerous chains of coccoid-like cells were observed. On media with iron, the optimal pH values for all the isolates except strain OFO were within the range from 1.8 to 2.0. Strain OFO exhibited the highest specific growth rate (0.45 h^{-1}) at the initial medium pH 1.6–1.8. Specific growth rates (μ_{max}) of the new strains on media with Fe(II) under optimal conditions of temperature and pH were within the range of 0.4–0.5 h^{-1}. Under similar growth conditions, the μ_{max} value for the type strain *S. sibiricus* $N1^T$ was 0.5 h^{-1} (Table 3).

On media with S^0, the optimal pH for strains B1 and B2 was 2.4–2.5 (μ_{max} = 0.33–0.35 h^{-1}); for strain OFO, 2.2–2.5 (μ_{max} = 0.30 h^{-1}); for strain SSO, 2.2–2.8 (μ_{max} = 0.4 h^{-1}); and for the type strain *S. sibiricus* N1—2.2–2.5 (μ_{max} = 0.2 h^{-1}). For two of the new strains, OFO and SSO, pH range for growth with S^0 (from 1.0 to 5.0–5.5) was wider than for strains B1 and B2 (from 1.3–1.5 to 4.0). However, at pH 1.8–3.5 the latter two strains grew relatively well (μ_{max} = 0.15–0.2 h^{-1}).

Table 2. Some differentiating characteristics of *S. sibiricus* strains

Strain	Cell size, μm	*T*°C optimum (range)	pH, optimum		pH, range	
			Fe(II)	S^0	Fe(II)	S^0
$N1^T$	2.0 ± 1.0 × 0.9 ± 0.2	55 (17–60)	2.0	2.2–2.5	1.1–2.6	2.0–3.5
B1	2.2 ± 0.8 × 0.9 ± 0.2	45–50 (25–60)	1.8–2.0	2.4–2.5	1.5–2.7	1.5–4.0
B2	2.05 ± 0.75 × 0.85 ± 0.15	48–50 (14–60)	1.8–2.0	2.5	1.2–2.6	1.3–4.0
OFO	2.75 ± 0.75 × 1.0 ± 0.1	48–50 (20–60)	1.6–1.8	2.2–2.5	1.1–2.7	1.0–5.5
SSO	1.75 ± 0.25 × 0.8 ± 0.1	50–55 (20–65)	1.8–2.0	2.2–2.8	1.0–2.6	1.0–5.0

Growth and Substrate Oxidation

Mixotrophic growth. Stable growth of the isolates with high growth rates occurred when both inorganic energy source and electron donor (Fe(II), S^0, or metal sulfides) and organic substrates were present. Growth of strains OFO and SSO under mixotrophic conditions was supported by yeast extract, tryptone, glucose, sucrose, fructose, glutathione, alanine, glutamate, and aspartate; strains B1 and B2, apart from these substrates, also utilized citrate and acetate. The spectrum of organic substrates utilized by the type strain *S. sibiricus* $N1^T$ under mixotrophic conditions includes yeast extract, glucose, sucrose, fructose, glutathione, glutamate, and sorbitol [15]. Specific growth rates of the strains depended on the oxidized substrates and varied from 0.11 to 0.5 h^{-1}.

Organotrophic growth. Organotrophic growth was maintained for three to six transfers on media with the above organic substrates, albeit at a low rate (0.02–0.1 h^{-1} in the first transfer). On citrate or acetate, strains B1 and B2 grew slowly (0.015 h^{-1}) for two to three transfers. All four strains survived four to five transfers on media with yeast extract; strains B2 and SSO had the highest growth yield (up to $4–5 \times 10^7$ ml^{-1}). The growth rate decreased in subsequent transfers; finally, individual living cells were detected, unable to grow when transferred to fresh media.

Lithotrophic growth and oxidative activity. Lithotrophic growth of the isolates on 9K medium with ferrous iron or elemental sulfur at optimal values of pH and temperature was maintained for two to three transfers. It intensity was variable. Growth of strains B1 and OFO on media with Fe(II) was more active; in the first transfer into autotrophic conditions, the cell yield was $1–2 \times 10^7$ ml^{-1} and the rate of iron oxidation was 0.15–0.18 g l^{-1} h^{-1}. The oxidation of S^0 to SO_4^{2-} was more active in strain SSO; in the first transfer into autotrophic conditions, the cell yield was 3×10^7 ml^{-1} and the rate of sulfate production was 0.02 g l^{-1} h^{-1}. Strain B2 oxidized actively both sulfur and iron; cell numbers reached $1.5–2 \times 10^7$ ml^{-1}, the rate of sulfate accumulation was 0.025 g l^{-1} h^{-1}, and the rate of iron oxidation was 0.16 g l^{-1} h^{-1}.

Oxidation of sulfur and ferrous iron under mixotrophic conditions. The rates of oxidation of mineral substrates by all *S. sibiricus* strains on 9K medium with yeast extract, Fe(II)/S^0, or sulfide minerals are presented in Table 3. The rate of iron oxidation decreases in the order $N1^T$ > B2 > B1 > OFO > SSO. The specific rate of iron oxidation by strain $N1^T$ was 0.8 g l^{-1} h^{-1}, i.e., 1.2–2.2 times higher than by other strains. The growth rates of the strains had the same order, although the quantitative dependence was not maintained.

The Table 3 also demonstrates that strain B2 exhibited the highest rate of S^0 oxidation (0.95 g SO_4^{2-} l^{-1} day^{-1}). The rate of elemental sulfur oxidation decreased in the order B2 > SSO > B1 > OFO > $N1^T$. On media with sulfur, strain B2 had a somewhat lower growth rate than strain SSO.

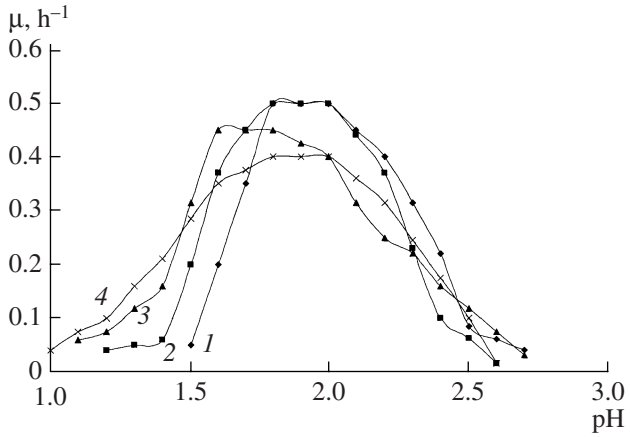

Fig. 3. Specific growth rate of *S. sibiricus* strains at various pH values: B1 (*1*), B2 (*2*), OFO (*3*), and SSO (*4*). Cultivation on the medium with Fe(II) and yeast extract.

Oxidation of metal sulfides and sulfide ore concentrates. In subsequent experiments, preferable growth substrates were also revealed among other mineral compounds (Table 3).

On pyrrhotite (FeS), strain B1 had the highest growth rate (μ = 0.28 h^{-1}); it had the highest oxidation rates of both sulfur (0.9 g l^{-1} day^{-1}) and iron (3.8 g l^{-1} day^{-1}) of this mineral. This strain exhibited the highest rate of iron oxidation on pyrite (FeS_2) and arsenopyrite (FeAsS), 3.7 and 3.35 g l^{-1} day^{-1}, respectively. When cultured on aurypigment As_2S_3, it also exhibited the highest rate of sulfur oxidation (0.7 g l^{-1} day^{-1}). Strain OFO had the highest growth rate of 0.2 and 0.26 h^{-1} on media with chalcopyrite $CuFeS_2$ and pyrrhotite concentrate; it had the highest oxidation rate of the iron of this mineral (0.52 g l^{-1} day^{-1}). The rates of iron oxidation in pyrrhotite concentrate (0.8 g l^{-1} day^{-1}) and of sulfur oxidation in pyrite–arsenopyrite concentrate (0.68 g l^{-1} day^{-1}), as well as in molybdenite MoS_2 and in realgar AsS (0.5 g l^{-1} day^{-1}), were high and close to those of strain B1. The third strain, SSO, oxidized sulfur in pyrite, arsenopyrite, chalcopyrite, sphalerite (ZnS), and pyrrhotite concentrate at high rates of 1.0, 1.5, 0.7, 0.22, and 1.4 g l^{-1} day^{-1}, respectively. Strain B2 oxidized reduced sulfur of pyrrhotite concentrate at the same rate; its rate of elemental sulfur oxidation (0.95 g l^{-1} day^{-1}) was the highest.

The growth rate of strain $N1^T$ on the medium with pyrite–arsenopyrite concentrate was high (0.25 h^{-1}) and exceeded those of the newly isolated strains (except for strain B2). Its cell yield was the highest; the rate of iron oxidation in the concentrate was also high (0.43 g l^{-1} day^{-1}). When grown on halenite PbS and sphalerite ZnS, reduced sulfur was oxidized at high rates of 0.5

Table 3. Growth rates of *S. sibiricus* strains and the rates of oxidation of reduced iron and sulfur compounds under mixotrophic conditions

Substrate	B1			B2			OFO			SSO			$N1^T$		
	μ_{max}, h^{-1}	*	**	μ_{max}, h^{-1}	*	**	μ_{max}, h^{-1}	*	**	μ_{max}, h^{-1}	*	**	μ_{max}, h^{-1}	*	**
Fe(II)	0.50	0.58	–	0.50	0.62	–	0.45	0.53	–	0.40	0.36	–	0.50	0.80	–
S^0	0.33	–	0.75	0.35	–	0.95	0.30	–	0.65	0.40	–	0.85	0.20	–	0.40
FeS	0.28	3.80	0.90	0.14	3.00	0.80	0.20	3.60	0.70	0.18	2.60	0.60	0.13	0.40	0.30
FeS_2	0.20	3.70	0.80	0.30	2.00	0.80	0.30	3.50	0.60	0.25	0.25	1.00	0.30	0.50	0.40
FeAsS	0.20	3.35	0.90	0.25	3.25	0.70	0.25	3.00	0.80	0.30	1.20	1.50	0.28	2.60	1.40
$CuFeS_2$	0.18	0.45	0.40	0.13	0.30	0.50	0.20	0.52	0.30	0.17	0.32	0.70	0.15	0.50	0.30
Cu_5FeS_4	0.17	ND	0.42	0.15	ND	0.29	0.18	ND	0.45	0.12	ND	0.29	ND	ND	ND
PbS	0.15	–	0.22	0.18	–	0.25	0.14	–	0.20	0.18	–	0.39	0.20	–	0.50
ZnS	0.13	–	0.15	0.11	–	0.20	0.13	–	0.10	0.18	–	0.22	0.21	–	0.95
AsS	0.20	–	0.40	0.17	–	0.35	0.19	–	0.50	0.23	–	0.40	ND	ND	ND
As_2S_3	0.20	–	0.70	0.20	–	0.50	0.18	–	0.60	0.28	–	0.40	ND	ND	ND.
MoS_2	0.16	–	0.20	0.17	–	0.30	0.20	–	0.50	0.24	–	0.45	ND	ND	ND
Pyrrhotite pyrite-arsenopyrite concentrate	0.25	0.80	1.20	0.20	0.60	1.40	0.26	0.80	1.00	0.22	0.40	1.40	0.16	0.35	0.50
Pyrite-arsenopyrite concentrate	0.20	0.40	0.68	0.25	0.25	0.55	0.21	0.30	0.68	0.19	0.16	0.35	0.25	0.43	0.40

Note: The strains were grown for two days on the medium with yeast extract (0.2 g l^{-1}) and Fe(II); on other substrates, the strains were grown for four days at optimal pH and temperature. Initial concentrations of sulfide minerals or sulfide ore concentrates were 10 g l^{-1}; those of Fe(II) or S^0 were 5 g l^{-1}. Maximal rates of formation of terminal oxidation products:

* Fe(III), g l^{-1} h^{-1} for experimental variants with Fe(II) as an energy substrate and Fe(III), g l^{-1} day^{-1} for all other iron-containing substrates;

** $(SO_4)^{2-}$, g l^{-1} day^{-1}, for all the substrates containing S^0 or S^{2-}; ND, not determined. The average values of at least three independent experiments in three repeats are presented.

and 0.95 g l^{-1} day^{-1}, respectively; the growth rates were 0.2 and 0.21 h^{-1}, respectively.

DISCUSSION

Strain polymorphism plays an important part in the rates of chemical processes of bacterial leaching of nonferrous metals and in extraction of precious metals from sulfide ores and concentrates. Strains of acidophilic chemolithotrophic bacteria (*S. thermosulfidooxidans*, *S. acidophilus*, and *Acidithiobacillus ferrooxidans*) isolated from various environments differ in their genomic characteristics, phenotypic features, and biotechnological potential [1, 15, 26, 27]. Knowledge of their characteristic properties, including their preferred oxidation substrates and growth conditions formed in the course of colonization of their environments, will improve our understanding and control of complex multifactor biohydrometallurgical processes under acceptable values of temperature, pH, and composition of sulfide ore concentrates and requirements to the composition of the liquid phase of the pulp.

We have obtained pure cultures of four strains of moderately thermophilic chemolithotrophic bacteria isolated from topographically different technogenic niches with active exothermic processes of sulfide ore oxidation; the genotypic and phenotypic characteristics of the isolates supported their affiliation with the species *S. sibiricus*. Their physiological properties are characteristic of the cultures adapted to oxidation of sulfide minerals. Both the new isolates and the *S. sibiricus* type strain $N1^T$ (isolated from pyrrhotite-free sulfide ores of a different Siberian region) possess flexible carbon metabolism [15, 16]. The sulfur metabolism of these strains is of a similar type; its oxidoreductase spectrum is different from that of other sulfobacilli species [28]. Both high temperatures resulting from the exothermic oxidative reactions and the adaptation of strains to specific ores in their native habitats provide for the differences in the rates of growth and mineral component oxidation. Genetic divergence of the *S. sibiricus* strains may be also explained by the chemical composition of the ore (depending on the mineral ratio) and electrophysical characteristics of the energy substrates under environmental conditions, localization of a strain close to a specific compound, or preference for certain substrates and growth conditions. Strains B1 and B2 with different rates of oxidation of the iron and sulfur components in the minerals were isolated from a single microniche. Another microniche yielded strains OFO and SSO; the first had a higher rate of iron oxidation, and the second of sulfur oxidation. The highest oxidation rate did not always coincide with the highest growth rate. Uncoupling of growth and substrate oxidation can result in increased heat production [29] and thus enhance the net thermogenesis in the course of sulfide ore oxidation. Thermogenesis can possibly stimulate the evolutionary processes leading to phenotypic strain diversity, along with such factors as substrate properties, pH, and metal ion concentrations, as well as spontaneous mutagenesis, irreversible intrachromosomal and plasmid–chromosomal recombinations, and changes in localization of IS elements.

The differences between *S. sibiricus* strains enabled their application for efficient oxidation of ore concentrates with different qualitative and quantitative mineral composition, at 45–55°C, and at high pulp densities (the S : L ratio varied from 1 : 10 to 1 : 5). The gravitation concentrate from the Nezhdaninskaya processing plant (with predominance of pyrite and arsenopyrite) at 10% pulp density was practically oxidized by an association of *S. sibiricus* strains after four days at 45–47°C; the degree of FeAsS and FeS_2 oxidation was 96 and 92%, respectively [30]. The differences in the biotechnological potential of *S. sibiricus* strains were also used to intensify the oxidation of the Olimpiadinskaya gold-containing flotation concentrate including pyrrhotite, arsenopyrite, and antimonite as the major minerals [31]. At the high pulp density of 20%, after three days of processing at 50°C the degree of oxidation was 96.44, 98.48, and 85.72% for sulfide iron, sulfide arsenic, and sulfide antimony, respectively.

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