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EXPERIMENTAL ARTICLES

Phenotypic Properties of *Sulfobacillus thermotolerans*: Comparative Aspects

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Abstract—The phenotypic characteristics of the species *Sulfobacillus thermotolerans* Kr1^T, as dependent on the cultivation conditions, are described in detail. High growth rates (0.22–0.30 h^{-1}) and high oxidative activity were recorded under optimum mixotrophic conditions at 40°C on medium with inorganic (Fe(II), S^0, or pyrite–arsenopyrite concentrate) and organic (glucose and/or yeast extract) substrates. In cells grown under optimum conditions on medium with iron, hemes *a*, *b*, and, most probably, *c* were present, indicating the presence of the corresponding cytochromes. Peculiar extended structures in the form of cylindrical cords, never observed previously, were revealed; a mucous matrix, likely of polysaccharide nature, occurred around the cells. In the cells of sulfobacilli grown litho-, organo-, and mixotrophically at 40°C, the enzymes of the three main pathways of carbon utilization and some enzymes of the TCA cycle were revealed. The enzyme activity was maximum under mixotrophic growth conditions. The growth rate in the regions of limiting temperatures (55°C and 12–14°C) decreased two- and tenfold, respectively; no activity of 6-phosphogluconate dehydrogenase, one of the key enzymes of the oxidative pentose phosphate pathway, could be revealed; and a decrease in the activity of almost all enzymes of glucose metabolism and of the TCA cycle was observed. The rate of $^{14}CO_2$ fixation by cells under auto-, mixo-, and heterotrophic conditions constituted 31.8, 23.3, and 10.3 nmol/(h mg protein), respectively. The activities of RuBP carboxylase (it peaked during lithotrophic growth) and of carboxylases of heterotrophic carbon dioxide fixation were recorded. The physiological and biochemical peculiarities of the thermotolerant bacillus are compared versus moderately thermophilic sulfobacilli.

Key words: *Sulfobacillus thermotolerans* Kr1^T, substrate oxidation, cytochrome hemes, $^{14}CO_2$ fixation, cytology, limiting temperatures, carbon metabolism.

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The genus *Sulfobacillus*, which comprises chemolithotrophic thermoacidophilic spore-forming bacteria, includes the well-known validated moderately thermophilic species *S. thermosulfidooxidans* [1], *S. acidophilus* [2], and *S. sibiricus* [3], as well as *S. thermotolerans* Kr1^T (VKM B-2339^T = DSM 17362^T), the first thermotolerant species, which was recently given taxonomic status [4]. These bacteria utilize iron, sulfur and its reduced compounds, and some organic substances as electron donors and sources of energy; however, they can survive numerous passages only under mixotrophic conditions. The thermotolerant bacillus has larger cells and grows in the temperature range of 12–60°C with an optimum at 40°C. The culture was isolated from a dense pulp in the process of bacterial oxidation at 30°C of the gold-bearing sulfide concentrate of the Olimpiadinskoe deposit.

All the cultures of the genus *Sulfobacillus* possess versatile carbon metabolism and a variety of carbon dioxide fixation reactions [5–8]. Depending on the type of nutrition, the carbon metabolism pathways may change; however, intense growth and substrate oxidation, as well as high activity of the enzymes of carbon catabolism and of the TCA cycle, are characteristic of cells grown at optimum temperature under mixotrophic conditions, i.e., when the presence of a mineral source of energy in the medium is combined with the presence of yeast extract and/or glucose. The cytological characteristics of the sulfobacilli grown under these conditions have been studied in depth only for the moder-

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ately thermophilic bacterium *S. thermosulfidooxidans* 1269^T [9]. The cytological and the ultrastructural features of the novel thermotolerant species have not been described. The data on the electron transport chains in sulfobacilli are scarce [10, 11]; the hemes have not been identified.

The aim of this work was to study in detail the phenotypic characteristics of *S. thermotolerans* $Kr1^T$: the peculiarities of growth, cytology, and carbon metabolism (including study of these characteristics at limiting cultivation temperatures and after replacement of the energy source) and identification of hemes in this bacterium.

MATERIALS AND METHODS

The organism and the cultivation conditions. The subject of the study was the type strain *Sulfobacillus thermotolerans* Kr1 isolated from a pyrrhotite gold–pyrite–arsenic ore in eastern Siberia [4]. The culture was grown at 40°C in modified 9K medium [12] with Fe(II) (45 mM) under autotrophic conditions or with glucose (1.1 mM) and yeast extract (0.02%) under heterotrophic conditions, as well as mixotrophically in the presence of an Fe(II)/S^0/pyrite–arsenopyrite concentrate, yeast extract, and/or glucose. Elemental sulfur was introduced in an amount of 10 g/l; the gravitation sulfide concentrate (Nezhdaninskoe deposit), comminuted to particles of the size of –0.044 mm (80%), in an amount of 50 g/l; salts were added in the following amounts (g/l): $(NH_4)_2SO_4$, 3.0; KCl, 0.1; KH_2PO_4 – 0.5; $MgSO_4 \cdot 7H_2O$ – 0.5; $Ca(NO_3)_2 \cdot 4H_2O$, 0.02. The initial pH value of media with iron was 1.8; of medium with sulfur, 2.5; of medium with sulfide concentrate, 2.2. Cells of a culture grown on medium with iron and yeast extract served as inoculation material, introduced in an amount of 10 vol %. The organism was cultivated in 250-ml Erlenmeyer flasks with 100 ml of medium on a shaker (180 rpm).

For assaying the enzymes, the culture was grown under forced aeration in 2.5-l conical flasks and with 1.8 l of medium at temperatures of 12–14, 40, and 55°C for 21–24 days, 16 h, and 10 h, respectively.

In order to carry out the experiments with elemental sulfur, the latter was washed with distilled water, sterilized with ethanol at 55°C, and introduced into the medium together with 10 mg/l of $FeSO_4 \cdot 7H_2O$ as a trace element. The sulfide concentrate, which contained 40% pyrite and 35% arsenopyrite as the main components and sphalerite, galenite, and chalcopyrite as the minor components, was sterilized at 0.5 atm.

Electron microscopy. Negative staining of whole cell specimens and cell preparation for obtaining ultrathin cell sections were carried out according to the techniques described in [9, 13]. The negatively stained specimens and ultrathin sections were examined under a JEM 100B electron microscope at an accelerating voltage of 80 kV.

Heme identification. To identify hemes, differential spectrophotometry methods and reversed-phase HPLC were used according to the method described in [14]. The cells at the end of the exponential growth phase were harvested by centrifugation at 10000 *g* for 40 min, washed with a medium free from the source of energy, and resuspended in 0.1 M Tris–HCl buffer, pH 7.4. Cell disruption was carried out with chicken egg lysozyme (Fluka, Belgium) followed by treatment in a UZDN-1 ultrasound disintegrator at 22 kHz for 5–7 min with 1-min intervals for cooling. To solubilize membrane proteins and to decrease light scattering by the samples, the cell membrane preparation in 0.01 M Tris–glycine buffer, pH 7.2, was lysed with Triton X-100 for 1 h. Then 1.5 ml of a mixture containing 0.133 M NaOH and 33% pyridine was added to each of the two 1-ml aliquots of a sample in 1-cm optic cuvettes. The oxidized sample in one cuvette contained 25 μl of 0.2 M ferricyanide solution; the reduced sample in the other cuvette contained 3 mg of sodium dithionite. Another method of identification of hemes noncovalently bound with apoproteins was as follows. The hemes were extracted from the cell membrane suspension with an acetone–HCl mixture (19 : 1 vol/vol); the extract was transferred to an aqueous ethyl acetate solution, which was evaporated to redissolve the residue in acetonitrile. The mixture was applied onto a 3.9×150 mm C-18 column (Waters, United States) connected to a PU 4110 chromatograph (Philips, United States). The hemes were eluted with an increasing (30–100%) gradient of an aqueous solution of acetonitrile acidified with 0.5% trifluoroacetic acid. The chromatographic peaks were recorded at 405 nm; the elution rate was 1 ml/min. The extract of the bacterium *Bacillus* sp. FTU was used as the standard containing hemes *a* and *b* with known retention.

Determination of $^{14}CO_2$ fixation by cell suspensions. The cells of a mixotrophic culture at the end of the exponential growth phase were concentrated by centrifugation at 10000 *g* for 40 min. The precipitate was resuspended in a medium free from an energy source; the insoluble iron compounds were removed by precipitating them at 2000 *g* for 1–2 min. The cells (1–3 mg by protein) were then resuspended in 10 ml of 9K medium that contained no energy source. The experiments on the fixation of carbon dioxide were carried out in hermetically closed 100-ml flasks. $^{14}CO_2$ fixation was studied under auto-, mixo-, and heterotrophic conditions. The variant in which the cell suspension was introduced into the medium free from sources of energy and organic carbon sources served as the control. The radioactivity of the preparations was determined using an LKB RacBeta 1127 liquid scintillation counter (Sweden). $^{14}CO_2$ fixation by the cells was expressed in nanomoles of $^{14}CO_2$ per 1 mg of cell protein. The rate of carbon dioxide fixation by cells was calculated in the linear section of the $^{14}CO_2$ fixation time curve and expressed in nmol/(h mg protein) [15]. When the experiment was completed, the purity of the

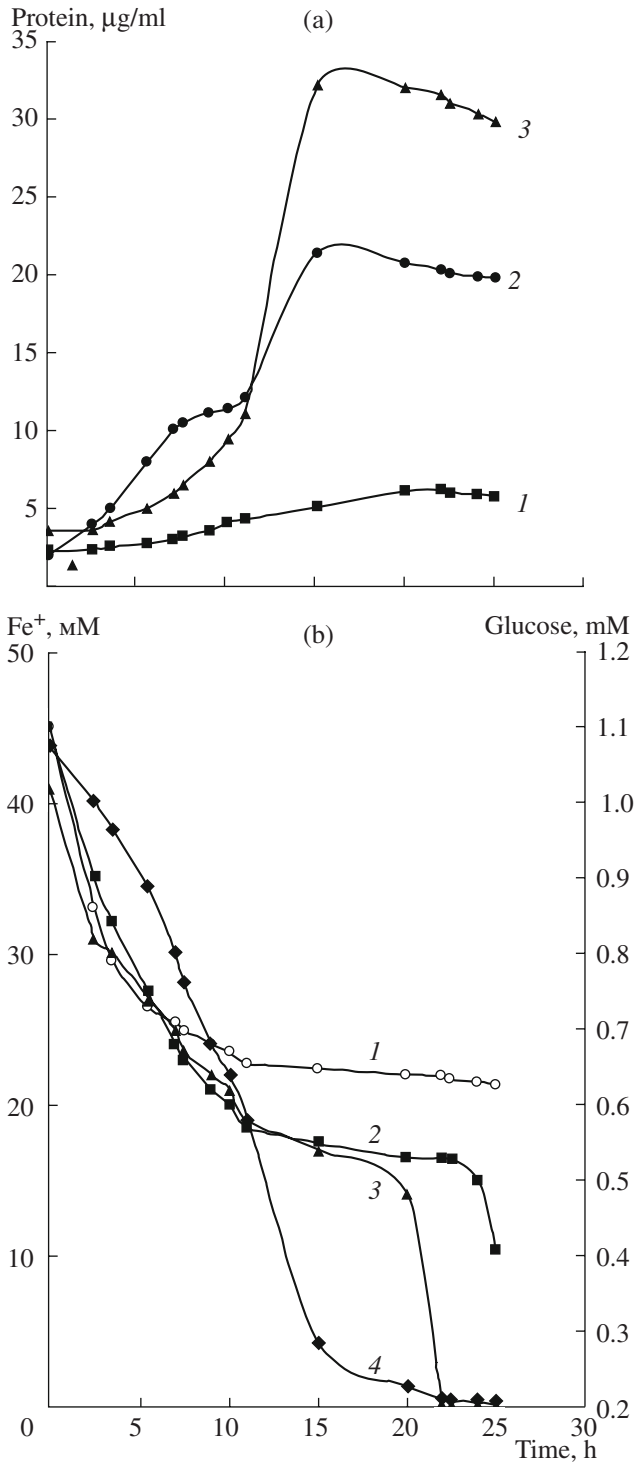

Fig. 1. Growth of *S. thermotolerans* Kr1 and substrate consumption under different cultivation conditions. (a) Culture growth under (*1*) autotrophic (Fe(II)), (*2*) heterotrophic (glucose and yeast extract), and (*3*) mixotrophic (Fe(II), glucose, and yeast extract) conditions. (b) Consumption of (*1*) glucose under heterotrophic conditions, (*2*) glucose under mixotrophic conditions, (*3*) Fe(II) under mixotrophic conditions, and (*4*) Fe(II) under autotrophic conditions.

culture was tested by inoculation of media of different compositions according to [7].

Enzyme activity assays. After centrifugation, exponential-phase cells were resuspended in 0.1 M Tris–HCl buffer, pH 7.4, and disrupted in a UZDN-1 ultrasound disintegrator as described above. The homogenate obtained was centrifuged for 20–30 min at 40000 *g*. The supernatant fluid was used for determining the enzyme activities.

The activity of the carboxylating enzymes was determined by the radioisotopic method [16, 17]. The activity of the enzymes of the TCA cycle, glyoxylate bypass, and carbon metabolism pathways was determined spectrophotometrically, except for isocitrate lyase and fructose-1,6-bisphosphate aldolase, whose activities were determined colorimetrically [6]. The enzyme activity was expressed in nmol/(min mg protein).

The analysis of growth and substrate utilization. The cell mass was determined by protein using the Lowry method; the culture growth was assessed by direct count of the cell number in a Goryaev chamber under a Lyumam I1 phase-contrast microscope. The pH and *Eh* values were recorded with a pH-150m pH meter–millivoltmeter (Belarus); the redox potential was expressed in mV (in relation to the normal hydrogen electrode). The Fe(II) and Fe(III) content was determined by complexonometric titration with Trilon B [18]; the content of glucose in the medium was determined by the anthrone reaction [19]; the content of sulfate ions was measured as described earlier [20]; the total arsenic concentration in the liquid phase of the pulp was determined by iodometric titration [21].

The experiments were done in two or three parallels, with three to five replicates; the significance of the results was assessed using the Student's *t*-test at the significance level $P \le 0.005$.

RESULTS

The growth of *S. thermotolerans* Kr1 under different conditions. Figure 1a shows the typical curves of the litho-, organo-, and mixotrophic growth of the culture at the optimum temperature of 40°C. The cell yield and growth rate under mixotrophic conditions were higher than in the other experimental variants. Thus, under mixotrophic conditions (curve *3*), the cell yield was approximately 1.5 and 5 times higher than in chemoorganotrophic (curve *2*) and chemolithotrophic (curve *1*) metabolisms. The specific growth rate of 0.27–0.30 h^{-1} under mixotrophic conditions was also 1.5–1.8 times higher than during cultivation in the absence of a mineral substrate (0.16–0.17 h^{-1}) and 2.2–2.5 times higher than during growth on medium that contained only a mineral source of energy (0.11–0.12 h^{-1}).

The analysis of the consumption of the substrates by the sulfobacillus (Fig. 1b) also demonstrates the highest

Table 1. Parameters of growth and substrate oxidation by *S. thermotolerans* Kr1

Substrate	Growth, days	Cell number, $\times 10^8$/ml	pH	*Eh*, mV	SO_4^{2-}, g/l	Fe(II)/Fe(III), g/l	As_{tot}, g/l
Pyrite-arsenopyrite concentrate	1	1.6	2.45	733	0.42	0.56/0.14	0.81
	2	1.7	2.42	768	0.48	0.60/0.28	1.57
	3	3.2	2.02	751	0.56	0.70/0.14	2.43
	4	2.6	1.83	749	1.16	1.12/0	3.10
	7	1.7	1.50	768	1.70	2.80/0.28	4.90
S^0	1	4.3	1.91	–	0.48	–	–
	2	4.2	1.66	–	0.50	–	–
	3	5.5	1.62	–	0.64	–	–
	5	8.6	1.56	–	0.91	–	–

Note: The strain was cultivated on 9K medium with 50 g/l of the sulfide mineral concentrate or 10 g/l of S^0 in the presence of 0.2 g/l of yeast extract.

rates of glucose utilization and ferrous iron oxidation (curves *2* and *3*, respectively) under mixotrophic conditions. Thus, the greatest biomass yield was connected with more complete glucose utilization under mixotrophic conditions as compared to heterotrophic conditions (curve *1*). Ferrous iron was oxidized completely under all growth conditions tested (curves *3, 4*); what differed (approximately twofold) was the oxidation rate.

When we studied the growth of *S. thermotolerans* Kr1 and the oxidation of other inorganic energy sources—elemental sulfur and sulfide minerals of the pyrite-arsenopyrite concentrate—the following dynamics of parameter changes was revealed in the presence of yeast extract (Table 1). After five days of cultivation on medium with sulfur, the pH value decreased from 2.5 to 1.56; this, together with the increase in the concentration of sulfate ion (the end oxidation product) to a level of 0.91 g/l and a considerable cell yield value of 8.6×10^8 cells/ml, was indicative of a high oxidative activity and the capacity for intense and stable growth on the not easily oxidizable substrate. The specific growth rate was 0.28 h^{-1}.

When the sulfide minerals of the pyrite-arsenopyrite concentrate were the main source of energy, the culture attained its maximum cell yield of 3.2×10^8 cells/ml after three days of growth. After seven days, the cell number decreased twofold, likely due to the deficiency of easily utilizable substrates and in connection with the accumulation in the liquid phase of arsenic ions (4.9 g/l) and ferric iron ions (2.8 g/l). In these experiments, the highest specific growth rate recorded was 0.21–0.23 h^{-1}. At the beginning of the process of bacterial sulfide oxidation, after 24 h of growth, alkalization of the pulp liquid phase from pH 2.2 to pH 2.45 was observed; at the end of the process, on the fifth day, a significant pH value decrease (to pH 1.50) occurred, which gave evidence of the actively proceeding processes of oxidation of reduced sulfur compounds; the sulfate ion concentration in the pulp liquid phase constituted 1.7 g/l. A significant increase in the redox potential was recorded: the *Eh* value increased from 603 (the initial value) to 768 mV, which also confirmed the high oxidative activity of the thermotolerant sulfobacillus.

Thus, depending on the source of energy and the electron donor, the *S. thermotolerans* Kr1 generation time varied from 2.6 h during mixotrophic cultivation on medium with Fe(II) and 2.8 h on medium with S^0 to 8 h on medium with the sulfide ore concentrate. In chemoorganotrophic metabolism, the generation time was equal to 4.4 h; in lithoautotrophic metabolism, 6.3 h.

The growth of *S. thermotolerans* Kr1 was also studied under mixotrophic conditions on medium with iron, glucose, and yeast extract at a limiting low cultivation temperature (12–14°C) and at a temperature close to the limiting maximum for growth (55°C, the maximum is 60°C). At low temperatures, the growth rate decreased by an order of magnitude, to 0.015–0.02 h^{-1}; at a high temperature, it decreased only twofold, to 0.13–0.14 h^{-1}. At 12–14°C, the cell yield constituted 6.31×10^7 cells/ml; at 55°C – 2.7×10^7 cells/ml. Many cells in chains were noted; in addition, at a high temperature, elongated and curved filamentous cells were observed. Under nonoptimum temperature conditions of cultivation, the bacterial cells did not completely oxidize the substrates introduced. Up to 15 and 25% of iron and up to 50 and 40% of glucose remained unutilized at the high and low growth temperatures, respectively.

Heme identification. The differential absorption spectrum of alkaline pyridine hemochromes (Fig. 2a) had two bands: at 550–560 and 585 nm. As judged from their location in the spectrum, the latter band belongs to heme *a*. The former band may belong either to heme *b* or to heme *c* or to both these hemes.

HPLC resulted in two peaks with the retention times on the column of 13.3 and 17.5 min (Fig. 2b). The first

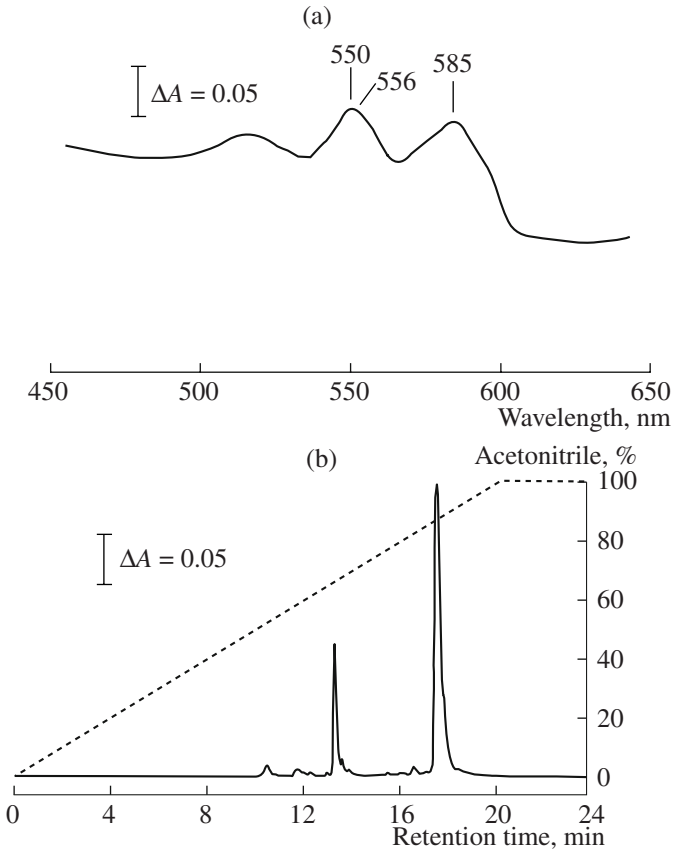

Fig. 2. Identification of *S. thermotolerans* Kr1 hemes: (a) the difference absorption spectrum (dithionite-reduced versus ferricyanide-oxidized) of a mixture of pyridine hemochromes and (b) elution profile of extracted hemes upon reversed-phase HPLC.

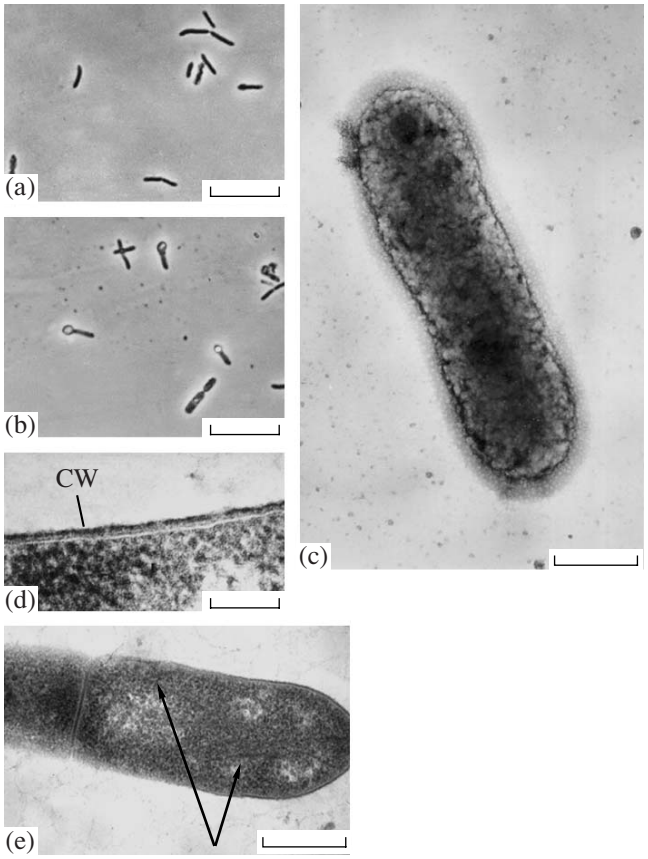

Fig. 3. Morphology and ultrastructure organization of *S. thermotolerans* Kr1 cells. Phase-contrast micrographs of the cells grown mixotrophically (a) on medium with iron and (b) on medium with the pyrite-arsenopyrite concentrate and electron micrographs of (c) negatively stained cell specimen, (d) a fragment of ultrathin section, and (e) longitudinal section of cells with internal cylindrical structures in the form of cords. Designation: CW, cell wall; C, cords. Bars: (a, b) 10 μm, (c) 1 μm, (d) 0.20 μm, and (e) 0.5 μm.

peak, according to the comparison with the *Bacillus* sp. FTU heme peaks (data not shown), belongs to heme *b*, and the second peak, to heme *a*.

Cytological peculiarities of *S. thermotolerans* Kr1. Figures 3a and 3b show photomicrographs of cells grown on media with iron and sulfide ore concentrate (mixotrophic conditions). The bacterial population is represented by rods, 0.8–1.2 × 1.5–4.5 μm, single or in chains; the spores are terminal, oval, or spherical. A mucous capsule (Fig. 3c), likely of polysaccharide nature, whose size, depending on the source of energy, varies between 0.12 and 0.25 μm, is seen outside the cells. It was especially pronounced when the bacterium grew on the sulfide concentrate. A thin cell wall with the murein layer 130 Å (Fig. 3d), to which the capsule adjoins very closely, is seen in the ultrathin sections of the strain Kr1 cells. In the cell cytoplasm, cords (from 1 to 5), previously unknown extended intracellular structures of regular cylindrical form having a small diameter (Fig. 3e), were revealed; polyphosphate granules were also present.

$^{14}CO_2$ assimilation by cell suspensions. Figure 4 shows the results of analysis of carbon dioxide fixation by cell suspensions of *S. thermotolerans* Kr1 upon introduction of organic and inorganic compounds into the incubation medium. The cells begin to assimilate carbon dioxide immediately after the substrate is introduced. The $^{14}CO_2$ fixation curves attain a plateau after 15–24 h of incubation. The lowest level of carbon dioxide fixation (134.7 nmol CO_2/mg protein) was noted in the variant where glucose and yeast extract were introduced into the medium (curve *3*). In the presence of ferrous iron in the incubation medium, the highest rate of carbon dioxide fixation (558.9 nmol CO_2/mg protein, curve *1*) was recorded. Under mixotrophic conditions in the presence of Fe(II), glucose, and yeast extract in the incubation medium, the maximum level of carbon dioxide fixation was slightly lower than in the presence of iron alone and constituted 431.0 nmol CO_2/mg protein (curve *2*). The analyses showed complete oxidation of ferrous iron by the cells in the autotrophic variant with Fe(II) and in the mixotrophic variant with Fe(II) and organic compounds. In the process, 70% of glucose

was metabolized. In the heterotrophic variant, the value of glucose consumption by the cells constituted no more than 40% of the amount introduced into the incubation medium.

The activity of carboxylases. RuBP carboxylase/oxygenase (RuBPC/O), the key enzyme of the Calvin cycle, was revealed in the cell extracts of *S. thermotolerans* Kr1 (Table 2). The highest activity—7.2 nmol CO_2/(min mg protein)—was revealed in extracts of cells grown under autotrophic conditions on medium with ferrous iron. The RuBPC activity in Kr1 cells decreased under mixotrophic conditions when organic substances were introduced into the Fe(II)-containing medium. In extracts of cells grown in the presence of Fe(II), glucose, and yeast extract, the RuBPC activity constituted 4.9 nmol CO_2/(min mg protein). In the organotrophic growth of the *S. thermotolerans* Kr1 culture in the presence of glucose and yeast extract, the RuBPC activity was equal to 1.1 nmol CO_2/(min mg protein).

The activities of several carboxylases of heterotrophic CO_2 fixation were revealed in the Kr1 cell extracts (Table 2). The most active was PEP carboxylase, whose maximum level equal to 1.1 nmol CO_2/(min mg protein) was recorded in organotrophically grown cells and constituted 0.9 and 0.7 nmol CO_2/(min mg protein) in cells grown litho- and mixotrophically, respectively.

Two more carboxylases, PEP carboxytransphosphorylase and pyruvate carboxylase, were also revealed. The activity of PEP carboxytransphosphorylase was at its maximum in cells grown under mixotrophic conditions. The level of pyruvate carboxylase activity was lower than that of PEP carboxytransphosphorylase and was virtually independent of the cultivation conditions.

The activity of the carbohydrate metabolism enzymes. As judged from the presence and activity of the enzymes involved in carbohydrate utilization, carbohydrate metabolism of *S. thermotolerans* Kr1 may be mediated by three main pathways: fructose bisphosphate, pentose phosphate, and Entner–Doudoroff (Table 3). When the strain grew at 40°C under the optimum mixotrophic conditions in the presence of yeast extract and glucose, the activity of all the enzymes studied, except aldolase, involved in the Entner–Doudoroff pathway, was higher than in cells grown organotrophically. The total activity of 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase was higher when the bacteria grew under heterotrophic conditions, compared to the activity in cells grown mixotrophically. One of the enzymes of the pentose phosphate pathway, 6-phosphogluconate dehydrogenase, had by far a lower activity. These data give evidence of the increasing role of the Entner–Doudoroff pathway in chemoorganoheterotrophic glucose metabolism in the Kr1 cells.

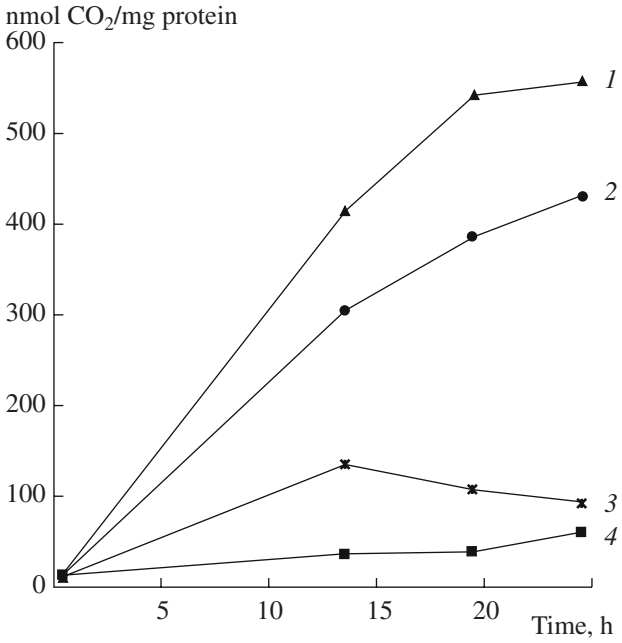

Fig. 4. $^{14}CO_2$ fixation by the cell suspension of *S. thermotolerans* Kr1 upon addition to the incubation medium of (*1*) Fe(II); (*2*) Fe(II), glucose, and yeast extract; or (*3*) glucose and yeast extract and (*4*) in the control.

The temperature at which the strain was grown under mixotrophic conditions influenced the activity of the enzymes involved in carbohydrate metabolism. At

Table 2. Activity of carboxylating enzymes (nmol/(min mg protein)) in the cells of *S. thermotolerans* Kr1 grown under different conditions

Enzyme	Culture growth conditions		
	Autotrophic	Mixotrophic	Heterotrophic
RuBP carboxylase (EC 4.1.1.39)	7.2	4.9	1.1
Pyruvate carboxylase (EC 6.4.1.1)	0.2	0.3	0.3
PEP carboxylase (EC 4.1.1.31)	0.9	0.7	1.1
PEP carboxykinase (EC 4.1.1.49)	ND	ND	ND
PEP carboxytransphosphorylase (EC 4.1.1.38)	0.3	0.8	0.6

Note: The abbreviation ND in this and the following tables means "not detected under the experimental conditions used."

Table 3. Activity of the carbohydrate metabolism enzymes (nmol/(min mg protein)) in *S. thermotolerans* Kr1 cells grown under different conditions

Enzyme	Mixotrophic conditions			Autotrophic conditions	Heterotrophic conditions
	12–14°C	40°C	55°C		
Hexokinase (EC 2.7.1.1)	22.3	24.0	3.1	7.1	10.2
Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)	27.8	70.1	4.5	28.1	31.0
6-Phosphogluconate dehydrogenase (EC 1.1.1.43)	ND	9.6	ND	0.6	0.5
6-Phosphogluconate dehydratase (EC 4.2.1.12) + 2-keto-3-deoxy-6-phosphogluconate aldolase (EC 4.1.2.14)	6.7	9.3	1.8	1.2	24.0
6-Phosphofructokinase (EC 2.7.1.11)	16.0	28.1	10.9	26.2	10.6
Fructose-1,6-bisphosphate aldolase (EC 4.1.2.13)	147.0	132.8	37.5	100.2	157.5
3-Phosphoglyceraldehyde dehydrogenase (EC 1.2.1.12)	30.6	72.4	10.2	52.3	32.5

Table 4. Activity of the TCA cycle enzymes (nmol/(min mg protein)) in *S. thermotolerans* Kr1 cells grown under different conditions

Enzyme	Mixotrophic conditions			Autotrophic conditions	Heterotrophic conditions
	12–14°C	40°C	55°C		
Citrate synthase (EC 4.1.3.7)	14.4	21.0	4.7	3.1	5.8
Aconitate hydratase (EC 4.2.1.3)	ND	9.3	ND	2.2	4.2
Isocitrate dehydrogenase (EC 1.1.1.42)	3.7	70.2	6.7	35.8	15.5
2-Oxoglutarate dehydrogenase (EC 1.2.4.2)	ND	ND	ND	ND	ND
Succinate dehydrogenase (EC 1.3.99.1)	20.0	39.0	5.3	42.3	31.8
Fumarate hydratase (EC 4.2.1.2)	106.5	118.65	45.5	52.3	ND
Malate dehydrogenase (EC 1.1.1.37)	87.4	96.7	8.9	57.5	13.3

low temperatures (12–14°C), the growth of the bacterium was suppressed, and the activity of certain enzymes decreased 1.5- to 2-fold compared to the variants of the strain growth at the optimum temperature. The hexokinase and fructose-1,6-bisphosphate aldolase activities appeared to be at the same level as in cells grown at the optimum temperature. 6-Phosphogluconate dehydrogenase, one of the key enzymes of the pentose phosphate pathway, was mostly influenced by temperature: its activity was not revealed at either a low or increased temperature of culture growth. It should be noted that, at a high temperature (55°C) of growth, a noticeable decrease in the activity of all the enzymes involved in glucose utilization was observed, compared to their activity in the cells grown at the optimum temperature.

Activity of the TCA cycle enzymes. *S. thermotolerans* Kr1, as other known representatives of this genus, contain all the TCA cycle enzymes except 2-oxoglutarate dehydrogenase (Table 4). The highest enzyme activity was revealed in cells grown under mixotrophic conditions in the presence of Fe(II), glucose, and yeast extract. The growth of the strain under autotrophic or heterotrophic conditions was accompanied by a considerable decrease in the activity of the key enzyme of the TCA cycle, citrate synthase, compared to its activity in cells growing under mixotrophic conditions. In addition, we did not succeed in revealing fumarate hydratase in the bacteria grown under heterotrophic conditions. The glyoxylate bypass enzymes, isocitrate lyase and malate synthase, were not revealed in Kr1 cells. A decrease or an increase in the growth temperature of the bacterium (compared to the optimum 40°C) influenced the TCA cycle enzyme activity. At a low (12–14°C) or higher growth temperature (55°C), the activity of all the enzymes decreased. Aconitase was not revealed, and the isocitrate dehydrogenase activity decreased more than tenfold. It was noted that low temperatures were more favorable for growth and manifestation of the enzyme activity than

high temperatures: in the extracts of cells grown at 55°C, almost all the TCA cycle enzymes had a lower activity than in the cells grown at 12–14°C.

DISCUSSION

The type strain of the novel species of facultatively chemolithotrophic sulfobacilli *S. thermotolerans* Kr1^T [4] and isolates of thermotolerant/mesophilic bacteria phylogenetically close to it were isolated in different geographic regions of the world, mainly as components of the microbial populations oxidizing sulfide minerals. These are *S. thermotolerans* clones D3-31, D1-45, and DY-6 [22]; strains L15 and RIV14 [23]; strain Y0017 [24]; and the uncultivated forms of *Sulfobacillus* spp.: P3-5 (GenBank AF460985), D3-5, and D3-28 [22]. Thus, at present, the species *S. thermotolerans* includes ten close isolates.

The first reports on strain Kr1 contained characteristics of its high leaching activity as the predominant culture in the bacterial association involved in the process of sulfide concentrate oxidation [25–27]. As a thermotolerant organism, strain Kr1 grows in a wide range of temperatures, from 12 to 60°C. However, no data are available in the literature on the metabolism of thermotolerant or moderately thermophilic sulfobacilli cultivated at temperatures close to growth-limiting. The cytology and ultrastructural organization of Kr1 cells were not studied in detail either. The data on the electron-transport system of sulfobacilli are also very scarce.

As a result of our experiments, the minimum generation time of cells grown at 40°C under different conditions was determined: under mixotrophic conditions, the generation time was 2.6 h when cultivation was carried out on medium with Fe(II); 2.8 h, on medium with S^0; and 8h, on medium with sulfide ore concentrate; under heterotrophic conditions, the generation time was 4.4 h; under autotrophic conditions, it was 6.3 h.

Comparative analysis showed that the generation time of *S. thermotolerans* Kr1 bacteria grown under different nutrition and temperature conditions, as well as the generation time of the moderately thermophilic sulfobacilli *S. sibiricus* N1^T, *S. thermosulfidooxidans* 1269^T, and *S. thermosulfidooxidans* subsp. *asporogenes* 41^T [6–8], is comparable with the growth parameters of *S. sibiricus* N1^T [28, 29], despite the differences in the optimum temperatures of their growth. This may be linked to close technogenic conditions of the habitat of strains Kr1 and N1, which were isolated from the dense pulp of reactors with a continuous process of biological oxidation of pyrite- and arsenopyrite-containing concentrates proceeding under mesophilic conditions. Other sulfobacilli, *S. thermosulfidooxidans* 1269 and *S. thermosulfidooxidans* subsp. *asporogenes* 41, were isolated from natural ecological niches [1, 7].

The range of temperatures for growth of different species of moderately thermophilic bacteria of the genus *Sulfobacillus* is within 17–60°C [4]. In the thermotolerant strain Kr1, the lower limit is 5–8°C lower than in moderate thermophiles, whereas the upper temperature limit is the same. At limiting (or close to limiting) temperatures of *S. thermotolerans* Kr1 cultivation, an increase in the generation time to 35 h at 12–14°C and to 5.2 h at 55°C was recorded. Under nonoptimal temperature conditions, the cell morphology of the thermotolerant bacillus also underwent some changes. Low temperatures influenced cells less negatively, which was likely connected with the origin of strain Kr1 as a component of an aboriginal microbial community adapted to mediate the process of metal sulfide leaching under mesophilic conditions.

The sulfide minerals of the pyrite-arsenopyrite concentrate of the Nezhdaninskoe deposit appeared to be good substrates for *S. thermotolerans* Kr1. This was evidenced by the high oxidative capacity and sufficient stability of the cell population developing in the pulp (with the ratio S/L = 1/20, where S and L stand for the pulp solid and the liquid phase, respectively) and the minimum generation time of 8 h. The Fe(III)/Fe(II) ratio was high, equaling 10; the concentration of the arsenic sulfide (III and V) leached constituted no less than 65.2% in the liquid phase (the amount of arsenic sulfide in the concentrate introduced into the medium was 15.03%). Strain Kr1 differed little from the moderately thermophilic sulfobacilli in the capacity for active oxidation of elemental sulfur and sulfide sulfur and, as judged from the accumulation of sulfate ion, the end oxidation product of reduced sulfur, and from a considerable decrease in the pH of the culture fluid, it was inferior only to certain strains of *S. sibiricus* [29].

At present, the respiratory chain in sulfobacilli is poorly studied. Thus, in *S. thermosulfidooxidans* BC1, a soluble protein with an absorption maximum of 458 nm in the oxidized state, which is characteristic of flavoproteins, was revealed in the cell-free extract [10]. In strain SSO of *S. sibiricus*, the presence of type aa_3 and *b* cytochromes was shown. Earlier, the presence of menaquinones, predominantly those with seven isoprenoid units, which is a typical taxonomic characteristic of the bacteria of the family *Alicyclobacillaceae*, was shown by us in *S. thermotolerans* Kr1 [4]. As the first step in determining the cytochrome composition, the identification of hemes as the prosthetic groups of cytochromes was attempted in this work. It was shown that Kr1 cells contain hemes *a*, *b*, and, probably, *c* (Fig. 2a), which are most typical of eubacteria. The contribution of heme *b* absorption to the heme *c* band at 550 nm and the contribution of heme *c* absorption to the heme *b* band at 556 nm may be evidence of this band being determined by both hemes. The determined heme composition corresponds to the membrane cytochromes. In the respiratory chain of certain aerobic microorganisms, cytochrome *c* is known to function as an intermediate carrier between the bc_1 complex and terminal oxidases. Therefore, the presence of hemes *b* and *c* may indicate the presence of this complex. Heme *a*

serves as an indication of the presence of the cytochrome oxidase caa_3 or aa_3. The study of the respiratory chains of this bacterium will be continued with the aim of revealing these cytochrome complexes.

When studying the morphocytological peculiarities of *S. thermotolerans* Kr1 cells, we observed cell motility in the absence of flagella or pili. The discovery in the cells of cylindrical cords extending across the cell cytoplasm and likely related to the cytoskeleton suggests that there may exist a motility mechanism determined by contraction of these structures. In fact, what we observed was not an orderly movement but a directed oscillatory movement from the tip to the middle of the cell. No such structures were revealed earlier in either sulfobacilli or any other bacteria. Apparently, the thick capsular structures that we revealed promote cell homeostasis, affording selective permeability and resistance to unfavorable environmental factors: high pH and temperature and high concentrations of heavy metals.

Strain *S. thermotolerans* Kr1, as the moderate thermophile *S. sibiricus* N1, is known for its capacity for more active organotrophic growth compared to other sulfobacilli studied [1, 3–5, 7]. These strains grow at the expense of organic substances more quickly, tolerate a large number of culture passages under these conditions, and have a higher intracellular ATP pool compared to other sulfobacilli [30–32]. These regularities are probably the result of adaptation to the organic substances formed during the autolysis of microorganisms in the dense pulps of industrial reactors, as well as to the presence of organic carbon in sulfide concentrates.

It should be pointed out that, by intensity of carbon dioxide fixation, the thermotolerant strain Kr1 is also very close to *S. sibiricus* N1 (Pivovarova and Melamud, unpublished data). Strains Kr1 and N1 possess a wide spectrum of carboxylating enzymes, which are active during litho-, organo-, and mixotrophic growth of the cultures. RuBP carboxylase, PEP carboxylase, and pyruvate carboxylase provide organic acids for the unclosed TCA cycle, which performs biosynthetic functions. The activity of anaplerotic carboxylases in strain Kr1 depends little on the type of nutrition.

The differences of the thermotolerant sulfobacillus from the moderately thermophilic strains are also connected with the possibility of functioning of the three main pathways of carbohydrate catabolism in all types of metabolism: chemolithoautotrophic, chemoorganoheterotrophic, and mixotrophic. Judging from the values of the enzyme activity, the primary role in mixotrophic metabolism belongs to the fructose bisphosphate pathway. When switching from mixotrophic to autotrophic metabolism, the activity of the enzymes of the Entner–Doudoroff pathway and oxidative pentose phosphate pathway decreases, but the priority role of the Embden–Meyerhof pathway is retained. Under heterotrophic conditions, in addition to the glycolytic pathway, the utilization of carbohydrates via the Entner–Doudoroff pathway becomes a priority. In some sulfobacilli, *S. sibiricus* and *S. acidophilus*, the latter pathway does not function at all because of the absence of activity of the corresponding enzymes [5, 8]. The weak activity of the enzymes of the oxidative pentose phosphate pathway under autotrophic and heterotrophic conditions is a characteristic feature of the sulfobacilli studied except *S. sibiricus* N1 [8].

The TCA cycle enzymes in the thermotolerant sulfobacillus retain their activity with a change in the source of energy, except for fumarate hydratase, whose activity was not revealed during organotrophic growth, and aconitate hydratase in the variant where the bacteria grew at limiting temperatures. In the cells of *S. sibiricus* N1, aconitate hydratase was not revealed under auto- or heterotrophic conditions; in cells of *S. thermosulfidooxidans* 1269, citrate synthase and aconitate hydratase were not found under heterotrophic conditions.

Thus, we can note the tolerance of the novel sulfobacillus *S. thermotolerans* $Kr1^T$ not only to the cultivation temperature but also to changes in the type of nutrition. This tolerance is afforded by functioning of different hexose catabolism pathways, the Calvin cycle, and reactions of anaplerotic carboxylation of PEP and pyruvate. These mechanisms allow *S. thermotolerans* Kr1 to switch, as a result of regulatory rearrangements, from the optimal mixotrophic type of nutrition to lithoautotrophic or organoheterotrophic types of nutrition and to retain viability under changing conditions in technogenic econiches.

REFERENCES

1. Golovacheva, R.S. and Karavaiko, G.I., A New Genus of Thermophilic Sporeforming Bacteria, *Sulfobacillus* gen. nov., *Mikrobiologiya*, 1978, vol. 47, no. 5, pp. 815–822.
2. Norris, P.A., Clark, D.A., Owen, J.P., and Waterhouse, S., Characteristics of *Sulfobacillus acidophilus* sp. nov. and Other Moderately Thermophilic Mineral-Sulphide-Oxidizing Bacteria, *Microbiology* (UK), 1996, vol. 142, pp. 775–783.
3. Melamud, V.S., Pivovarova, T.A., Tourova, T.P., Kolganova, T.V., Osipov, G.A., Lysenko, A.M., Kondrat'eva, T.F., and Karavaiko, G.I., *Sulfobacillus sibiricus* sp. nov., a New Moderately Thermophilic Bacterium, *Mikrobiologiya*, 2003, vol. 72, no. 5, pp. 681–688 [*Microbiology* (Engl. Transl.), vol. 72, no. 5, pp. 605–612].
4. Bogdanova, T.I., Tsaplina, I.A., Kondrat'eva, T.F., Duda, V.I., Suzina, N.E., Melamud, V.S., Tourova, T.P., and Karavaiko G.I., *Sulfobacillus thermotolerans* sp. nov., a Thermotolerant, Chemolithotrophic Bacterium, *Int. J. Syst. Evol. Microbiol.*, 2006, vol. 56, pp. 1039–1042.
5. Wood, A.P. and Kelly, D.P., Growth and Sugar Metabolism of a Thermophilic Iron-Oxidizing Mixotrophic Bacterium, *J. Gen. Microbiol.*, 1984, vol. 130, pp. 1337–1349.
6. Zakharchuk, L.M., Tsaplina, I.A., Krasil'nikova, E.N., Bogdanova, T.I., and Karavaiko, G.I., Carbon Metabolism in *Sulfobacillus thermosulfidooxidans, Mikrobiologiya*, 1994, vol. 63, no. 4, pp. 573–580.

7. Tsaplina, I.A., Krasil'nikova, E.N., Zakharchuk, L.M., Egorova, M.A., Bogdanova, T.I., and Karavaiko, G.I., Carbon Metabolism in *Sulfobacillus thermosulfidooxidans* subsp. asporogenes, Strain 41, *Mikrobiologiya,* 2000, vol. 69, no. 3, pp. 334–340 [*Microbiology* (Engl. Transl.), vol. 69, no. 3, pp. 271–276].
8. Zakharchuk, L.M., Egorova, M.A., Tsaplina, I.A., Bogdanova, T.I., Krasil'nikova, E.N, Melamud, V.S., and Karavaiko, G.I., Activity of the Enzymes of Carbon Metabolism in *Sulfobacillus sibiricus* under Various Conditions of Cultivation, *Mikrobiologiya*, 2003, vol. 72, no. 5, pp. 621–626 [*Microbiology* (Engl. Transl.), vol. 72, no. 5, pp. 553–557].
9. Suzina, N.E., Severina, L.O., Senyushkin, A.A., Karavaiko, G.I., and Duda, V.I., Ultrastructural Organization of Membrane System in *Sulfobacillus thermosulfidooxidans*, *Mikrobiologiya*, 1999, vol. 68, no. 4, pp. 491–500 [*Microbiology* (Engl. Transl.), vol. 68, no. 4, pp. 429–436].
10. Blake, R.C., Shute, E.A., Greenwood, M.M., Spencer, G.H., and Ingledew, W.J., Enzymes of Aerobic Respiration on Iron, *FEMS Microbiol. Rev.*, 1993, vol. 11, pp. 9–18.
11. Dinarieva, T.Yu., Zakharchuk, L.M., Tsaplina, I.A., Bogdanova, T.I., and Karavaiko, G.I., Cytochromes of a Bacterium of the Genus *Sulfobacillus*, *Materialy vserossiiskogo simpoziuma "Avtotrofnye mikroorganizmy"* (Proc. All-Russia Symp. "Autotrophic Microorganisms"), Moscow: MAKS Press, 2005.
12. Melamud, V.S. and Pivovarova, T.A., Specific Features of the Growth of the Type Strain of *Sulfobacillus thermosulfidooxidans* in Medium 9K, *Prikl. Biokhim. Mikrobiol.*, 1998, vol. 34, no. 3, pp. 309–315. [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 34, no. 3, pp. 281–286].
13. Reynolds, E.S., The Use of Lead Citrate at High pH as an Electron-Opaque Stain in Electron Microscopy, *J. Cell Biol.*, 1963, vol. 17, pp. 208–213.
14. Sone, N. and Fujiwara, Y., Effects of Aeration during Growth of *Bacillus stearothermophilus* on Proton Pumping Activity and Change of Terminal Oxidases, *J. Biochem.*, 1991, vol. 239, pp. 1024–1031.
15. Krasil'nikova, E.N., Pedan, L.V., Firsov, N.N., and Kondrat'eva, E.N., Tricarboxylic Cycle Enzymes in Different Species of Phototrophic Bacteria, *Mikrobiologiya*, 1973, vol. 42, no. 6, pp. 995–1000.
16. Romanova, A.K., *Biokhimicheskie metody izucheniya avtotrofii u mikroorganizmov* (Biochemical Methods for Studying Autotrophy in Microorganisms), Moscow: Nauka, 1980.
17. Ivanovsky, R.N., Fal, Y.I., Berg, I.A., Ugolkova, N.V., Krasil'nikova, E.N., Keppen, O.I., Zakharchuk, L.M., and Zyakun, A.M., Evidence for the Presence of the Reductive Pentose Phosphate Cycle in a Filamentous Anoxygenic Photosynthetic Bacterium *Oscillochloris trichoides* Strain DG-6, *Microbiology*, 1999, vol. 145, pp. 1743–1748.
18. Reznikov, A.A., Mulikovskaya, E.P., and Sokolov, I.Yu., *Metody analiza prirodnykh vod* (Analytical Methods for Natural Waters), Moscow: Nedra, 1970.
19. Ashwell, G., Colorimetric Analysis of Sugars. Anthrone Reaction, *Methods Enzymol.,* 1957, vol. 3, pp. 84–85.
20. Krasil'nikova, E.N., Bogdanova, T.I., Zakharchuk, L.M., Tsaplina, I.A., and Karavaiko, G.I., Metabolism of Reduced Sulfur Compounds in *Sulfobacillus thermosulfidooxidans*, Strain 1269, *Mikrobiologiya*, 1998, vol. 67, no. 2, pp. 156–164 [*Microbiology* (Engl. Transl.), vol. 67, no. 2, pp. 125-132].
21. Suvorovskaya, I.A., Titov, V.I., Brodskaya, V.M., Vasil'ev, P.I., Lipshchits, B.M., and Elentur, M.P., Determination of Arsenic, *Tekhnicheskii analiz tsvetnoi metallurgii* (Technical Analysis in Nonferrous Metallurgy), Moscow: Metallurgizdat, 1957, pp. 182–164.
22. Shiguo He, Shegmu Xiao, and Xuehui Xie, Microbial Diversity in Acid Mineral Bioleaching Systems of Dongxiang Copper Mine and Yinshan Lead–Zinc Mine, *Extremophiles*, 2007, Springer Japan, 2007, DOI 10.1007/S00792-007-0130-x.
23. Yahya, A., Roberto, F.F., and Johnson, D.B., Novel Mineral-Oxidizing Bacteria from Montserrat (W.I.): Physiological and Phylogenetic Characteristics *Biohydrometallurgy and the Environment: Toward the Mining of the 21th Century, Process Metallurgy 9A*, Amils, R. and Ballaster, A., Eds., Amsterdam: Elsevier, 1999, pp. 729–740.
24. Johnson, D.B., Okibe, N., and Roberto, F.F., Novel Thermoacidophilic Bacteria Isolated from Geothermal Sites in Yellowstone National Park: Physiological and Phylogenetic Characteristics, *Arch. Microbiol.*, 2003, vol. 180, pp. 60–68.
25. Kondrat'eva, T.F., Melamud, V.S, Aslanukov, R.Ya, Voronina, O.B, and Karavaiko, G.I, Chemolithotrophic Bacterial Community in the Pulp of a Pilot Plant during Gold-Arsenic Pyrrhotite Concentrate Processing, *Biotechnology and Environment Including Biogeotechnology*, Zaikov, G.E., Ed., New York: Nova Science Publishers, 2004, pp. 147–160.
26. Tsaplina, I.A., Bogdanova, T.I., Krasil'nikova, E.N., Egorova, M.A., Zakharchuk, M.A., and Karavaiko, G.I., Physiological and Biochemical Characteristics of Chemolithotrophic Bacteria of the Genus *Sulfobacillus, Mater. 3-go Mezhd. Kongressa "Biotekhnologiya—sostoyanie i perspektivy razvitiya," Moskva 14–16 marta 2005* (Proc. 3rd Int. Conf. "Biotechnology: Current State and Development Prospects," Moscow, March 14–16, 2005), part 2, pp. 251–252.
27. Tsaplina, I.A., Bogdanova, T.I., Savari, E.E., Sergeeva, T.V., Kondrat'eva, T.F., Zhuravleva, A.E., Sedel'nikova, G.V., and Karavaiko, G.I., A Study of a Community of Moderately Thermophilic Sulfobacilli in the Process of Oxidation of Pyrite–Arsenopyrite Concentrate, *Mater. 4-go Mezhd. Kongressa "Biotekhnologiya—sostoyanie i perspektivy razvitiya", Moskva 124–16 marta 2007* (Proc. 4th Int. Conf. "Biotechnology: Current State and Development Prospects," Moscow, March 12–16, 2007), Moscow: "ZAO Ekspo-Biokhim-Tekhnologii," Mendeleev Russ. Chem. Technol. Univ., part 2, p. 325.
28. Tsaplina, I.A., Zhuravleva, A.E., Bogdanova, T.I., and Kondrat'eva, T.F., Biotechnological Potential of the Thermoacidophilic Chemolithotrophic Bacterium *Sulfobacillus sibiricus, Mater. mezhd. nauchn. konf. "Mikroorganizmy i biosfera," Moskva 19–20 noyabrya 2007* (Proc. Int. Conf. "Microorganisms and the Biosphere," Moscow, Nov. 19–20, 2007), Moscow: Makspress, 2007, pp. 140-141.
29. Tsaplina, I.A., Bogdanova, T.I., Melamud, V.S., Kondrat'eva, T.F., and Karavaiko, G.I., Genotypic and Phenotypic Polymorphism of Environmental Strains of the

Moderately Thermophilic Bacterium *Sulfobacillus sibiricus, Mikrobiologiya*, 2008, vol. 77, no. 2, pp. 178–188 [*Microbiology* (Engl. Transl.), vol. 77, no. 2, pp. 151–158].

30. Tsaplina, I.A., Zhuravleva, A.E., Ismailov, A.D., Zakharchuk, L.M., Krasil'nikova, E.N., Bogdanova, T.I., and Karavaiko, G.I., The Dependence of Intracellular ATP Level on the Nutrition Mode of the Acidophilic Bacteria *Sulfobacillus thermotolerans* and *Alicyclobacillus tolerans, Mikrobiologiya*, 2007, vol. 76, no. 6, pp. 742–751 [*Microbiology* (Engl. Transl.), vol. 76, no. 6, pp. 654–662].
31. Zhuravleva, A.E, Tsaplina, I.A, Ismailov, A.D, Zakharchuk, L.M, and Karavaiko, G.I, Metabolism peculiarities of bacteria of the genus *Sulfobacillus, Advanced Material Research*, Schippers, A., Sand, W., Glombitza, F., and Willscher, S., Eds., Switzerland–UK–USA: Trans Tech Publications LTD, 2007, vol. 20–21, pp. 469–472.
32. Zhuravleva, A.E., Ismailov, A.D., Zakharchuk, L.N., Krasil'nikova, E.N., and Tsaplina, I.A., Bioenergetics of the Growth Processes in the Moderately Thermophilic Chemolithotrophic Bacteria of the Genus *Sulfobacillus Mikrobiologiya*, 2008, vol. 77, no. 5, pp. 709–712 [*Microbiology* (Engl. Transl.), vol. 77, no. 5].