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EXPERIMENTAL ARTICLES

Carbon Metabolism in *Sulfobacillus thermosulfidooxidans* subsp. *asporogenes*, Strain 41

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Abstract—The activities of carbon metabolism enzymes were determined in cellular extracts of the moderately thermophilic, chemolithotrophic, acidophilic bacterium *Sulfobacillus thermosulfidooxidans* subsp. *asporogenes*, strain 41, grown either at an atmospheric content of CO_2 in the gas phase (autotrophically, heterotrophically, or mixotrophically) or autotrophically at a CO_2 content increased to 5–10%. Regardless of the growth conditions, all TCA cycle enzymes (except for 2-oxoglutarate dehydrogenase), one glyoxylate bypass enzyme (malate synthase), and some carboxylases (ribulose bisphosphate carboxylase, pyruvate carboxylase, and phosphoenolpyruvate carboxylase) were detected in the cell-free extracts of strain 41. During autotrophic cultivation of strains 41 and 1269, the increase in the CO_2 content of the supplied air to 5–10% resulted in the activation of growth and iron oxidation, a 20–30% increase in the cellular content of protein, enhanced activity of the key TCA enzymes (citrate synthase and aconitase), and, in strain 41, a decrease in the activity of carboxylases.

Key words: thermoacidophiles, growth, TCA cycle enzymes, carboxylases

Thermophilic facultatively autotrophic bacteria of the genus *Sulfobacillus*—*S. thermosulfidooxidans*, two its subspecies, *asporogenes* and *thermotolerans* [1], and *S. acidophilus* [2]—and the only known mesophilic representative of this genus, *S. disulfidooxidans* [3], are characterized by a versatile metabolism that allows them to grow hetero-, auto-, and mixotrophically.

The strains studied in the greatest detail in terms of their carbon metabolism under various cultivation conditions are strain ALV [4, 5], later named *S. acidophilus* [2], and the type strain *S. thermosulfidooxidans* VKM B-1269 [6]. Strain 41 (= INMIA B-6981, Armenia), described as an asporogenes subspecies of *S. thermosulfidooxidans* [7], has phenotypic properties close to those of the type strain of the species, VKM B-1269, and exhibits an 81% DNA homology with it. Strain 41 is characterized by an impaired sporulation process; it also differs from strain VKM B-1269 in the G+C content of DNA and the carbohydrate component of the S layer and by having a smaller genome and a lower optimal pH value during growth on iron-containing medium [8].

Strains 1269 and 41 show stable growth only on media containing 0.02% yeast extract in addition to the mineral energy source. For strain 1269, operation of the Calvin cycle was demonstrated in autotrophically and heterotrophically grown cells [9]. However, strain 1269, as well as strain 41, can withstand only a limited number of culture transfers under autotrophic or heterotrophic conditions. This may be due both to exogenous factors (low concentrations of dissolved atmospheric carbon dioxide and oxygen) and to the lack of a complete TCA cycle in cells of these strains [6]. It was established that carbon dioxide assimilation in strain 41 exhibits certain peculiarities as compared to other sulfobacilli [10]. The addition of 0.002% yeast extract to a Fe^{2+}-containing medium was shown to increase the rate of labeled CO_2 fixation by cells of strain 41 and to decrease the rate of this process in strain 1269 [6].

The aim of the present work was to study the activity of the TCA cycle enzymes and carboxylating enzymes in auto-, mixo-, and heterotrophically grown cells of strain 41 of *S. thermosulfidooxidans* subsp. *asporogenes* and the effect of an increased carbon dioxide concentration on the growth, ferrous iron oxidation, and activity of some carbon metabolism enzymes in this strain and in *S. thermosulfidooxidans* VKM B-1269.

MATERIALS AND METHODS

The main subject of investigation was strain *S. thermosulfidooxidans* subsp. *asporogenes* 41 (= INMIA B-6981, Armenia), isolated from mine waters emerging from a dump at the Armanis polymetallic sulfide deposit [7]. In the experiments set to study the effect of an increased carbon dioxide concentration, we also used strain *S. thermosulfidooxidans* VKM B-1269^T [1]. Strains 41 and 1269 were grown, respectively, on the modified media of Brierley [10] and Manning [6]. The Brierley medium contained (g/l) $(NH_4)_2SO_4$, 0.5; NaCl,

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0.2; KH_2PO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; $Ca(NO_3)_2$, 0.01; and 1 ml/l of trace element solution (which was composed of 3 g/l of $CuSO_4$; 3 g/l of $ZnSO_4$; 2 g/l of $CoSO_4$; 1 g/l of $NaMoO_4$; 0.1 g/l of H_3BO_3; traces of $MnSO_4$; and traces of KJ); the pH of the medium was 1.6–1.8. The manning medium contained (g/l) $(NH_4)_2SO_4$, 6; KCl, 0.2; K_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 1.0; and $Ca(NO_3)_2$, 0.02; the pH of this medium was 1.8–2.0. The media were supplemented with 20 g/l of $FeSO_4 \cdot 7H_2O$ as the carbon and energy source and 1–2 mM $Na_2S_2O_3$. The above media were used for autotrophic cultivation; for mixotrophic growth, the media were additionally supplemented with 0.02% yeast extract. Heterotrophic cultivation was performed on media with 0.05% yeast extract. The amount of the inoculum introduced was 10 vol % for hetero- and mixotrophic cultivation and 15–20% for autotrophic cultivation. The cultivation temperature was 48–50°C. Cultivation was performed in 5-l flasks bubbled through with air (2 l air/(l medium min)) or in 250-ml flasks with 100 ml of the medium (to obtain the inoculum). In the experiments set to study the effect of medium enrichment with carbon dioxide, CO_2 (5–10%) was added to the air bubbled through the medium. The concentration of dissolved CO_2 was not measured. Culture growth was monitored by determining the cell number and the protein content. Iron concentration, biomass protein, and the protein of cell-free extracts were determined as described earlier [6].

To determine the activity of the TCA cycle enzymes, mid-log-phase or late-log-phase cells were used. Cells were washed two times with 0.05 M Tris–HCl buffer (pH 7.2), resuspended in the same buffer, and ultrasonically disrupted (22 kHz, 3 × 1.5 min, under cooling). The supernatant obtained by centrifugation of the homogenate for 20 min at 40000 *g* was used to determine the enzymatic activities.

Citrate synthase (EC 4.1.3.7) and malate synthase (EC 4.1.3.2) were determined using 5,5'-dithiobis-(2-nitrobenzoate) at 412 nm [11, 12]. Isocitrate lyase (EC 4.1.3.1) was determined from the formation rate of glyoxylate from isocitrate [13]. Other enzymes of the TCA cycle and glyoxylate shunt (aconitase, EC 4.2.1.3; isocitrate dehydrogenase, EC 1.1.1.4; 2-oxoglutarate dehydrogenase, EC 1.2.4.2; succinate dehydrogenase, EC 1.3.99.1; fumarate hydratase, EC 4.2.1.2; and malate dehydrogenase, EC 1.1.1.37) were assayed as described earlier [14] on a model 200-20 spectrophotometer (Hitachi).

The activities of ribulose bisphosphate carboxylase (RuBPC, EC 4.1.1.39), pyruvate carboxylase (EC 6.4.1.1), phosphoenolpyruvate (PEP) carboxylase (EC 4.1.1.31), PEP carboxytransphosphorylase ((EC 4.1.1.38), and PEP carboxykinase (EC 4.1.1.32) were determined by the radioisotopic method [15] from the fixation rate of radiocarbon from [^{14}C]bicarbonate by cellular extracts containing 0.5–1.5 mg protein/ml; an LKB RacBeta 1127 scintillation counter and the ZhS-107 scintillation cocktail were used.

The activities of all enzymes were expressed in nmol/(min mg protein).

RESULTS AND DISCUSSION

1. Culture growth. Strain *Sulfobacillus thermosulfidooxidans* subsp. *asporogenes* 41 could grow under autotrophic and heterotrophic conditions over only one or two culture transfers and preferred mixotrophic conditions (Fig. 1a), which is typical for sulfobacilli. The specific growth rate under mixotrophic conditions was 0.332 h^{-1}, whereas under autotrophic and heterotrophic conditions, it was 0.087–0.090 h^{-1}. Cells of autotrophic and heterotrophic cultures were polymorphic. The highest rate of ferrous iron oxidation (0.15 g/(l h), or 2.7 mmol(l h)) was observed under mixotrophic conditions (Fig. 1b). Under autotrophic conditions, cells continued to oxidize the substrate for one day after the cessation of growth. The redox potential increased from 540 to 780–800 mV, reaching the highest values by the end of mixotrophic growth (Fig. 1b).

2. Enzymes of the TCA cycle and glyoxylate shunt. In cells of *S. thermosulfidooxidans* 41 grown under various conditions, all TCA cycle enzymes were revealed except for 2-oxoglutarate dehydrogenase (Table 1). One of the two enzymes of the glyoxylate shunt, malate synthase, was also revealed, whereas the other one, isocitrate lyase, could not be detected under any cultivation conditions. Thus, neither a complete TCA cycle nor the glyoxylate shunt can operate in strains 41 and 1269 [6]. The TCA cycle is represented by separate reactions apparently playing biosynthetic roles.

In cells of strain 41 grown under autotrophic and mixotrophic conditions (Table 1), the activities of citrate synthase (the key enzyme of the TCA cycle), as well as the activities of isocitrate dehydrogenase, were roughly the same. In cells of this strain grown heterotrophically, the levels of citrate synthase, aconitase, and isocitrate dehydrogenase were lower than in autotrophically grown cells. Conversely, the levels of succinate dehydrogenase, fumarate hydratase, and malate dehydrogenase in heterotrophically grown cells were higher than in cells grown autotrophically. In mixotrophically grown cells, the levels of the above enzymes were intermediate.

The data obtained with strain 41 can be compared with our earlier data on the activity of the TCA cycle enzymes in another *Sulfobacillus thermosulfidooxidans* strain, 1269. In the latter strain, the activity of all TCA cycle enzymes was higher after autotrophic growth; an exception was malate dehydrogenase, whose activity was higher after mixotrophic growth [6].

As follows from the data of the present paper, the cultivation conditions most greatly affect those TCA cycle enzymes that are involved in the initial reactions

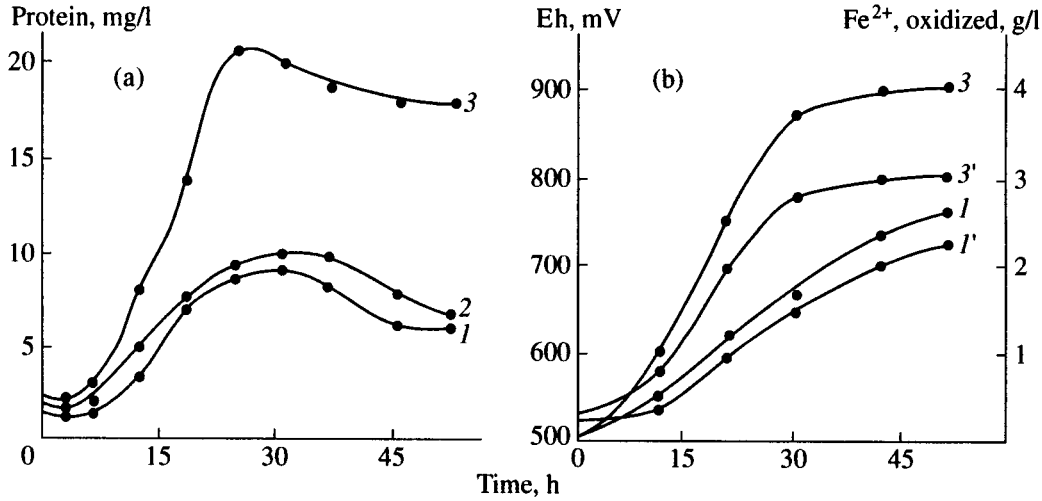

Fig. 1. (a) Biomass accumulation and (b) Fe^{2+} oxidation (curves *1*, *3*) and Eh changes (curves *1'*, *3'*) during the (*1*) autotrophic, (*2*) heterotrophic, and (*3*) mixotrophic growth of *S. thermosulfidooxidans* subsp. *asporogenes* 41.

of the cycle (citrate synthase, aconitase, and isocitrate dehydrogenase). Evidently, heterotrophically and mixotrophically grown cells are not as dependent on the initial reactions of the synthesis of organic compounds as autotrophically grown cells.

3. Carboxylases. The analyses of the carboxylating enzymes (Table 2) showed that, in the autotrophically grown cells of strain 41, the activity of RuBPC was rather high (44.6 nmol/(min mg protein)), exceeding that in the other sulfobacilli studied. Evidently, the Calvin cycle is the main mechanism of CO_2 fixation in strain 41. In cells grown under mixotrophic conditions, RuBPC activity was also high, whereas in heterotrophically grown cells, it was low (1.5 nmol/(min mg protein)).

It was established that CO_2 fixation by strain 41 cells grown under any of the conditions studied can also involve the reactions of pyruvate and PEP carboxylation. The activities of pyruvate and PEP carboxylases in autotrophically grown cells were 0.51 and 8.7 nmol/(min mg protein), respectively. The activity of PEP carboxylase considerably increased upon the addition of organic compounds to the medium, reaching 20.7 nmol/(min mg protein) in mixotrophically grown cells. Heterotrophically grown cells exhibited the lowest activities of all carboxylases. PEP carboxykinase and PEP carboxytransphosphorylase could not be detected in strain 41.

4. Effect of CO_2 concentration. An increase in the CO_2 content of the supplied air to 10% resulted in the activation of growth of both *Sulfobacillus thermosulfidooxidans* strains 1269 and 41. Although the cell concentration increased insignificantly as compared to the control, reaching 2–3 × 10^7 cells/ml, the cellular content of protein increased by 20–30% (Fig. 2a), and the rate of iron oxidation also increased (Fig. 2b). In cells of strain 1269, the activity of the TCA cycle enzymes

Table 1. Activities (nmol/(min mg protein)) of the enzymes of the TCA cycle and glyoxylate shunt in *S. thermosulfidooxidans* subsp. *asporogenes* strain 41 cells grown under various cultivation conditions

Enzyme	Cultivation conditions			
	autotrophic		heterotrophic	mixotrophic
	usual CO_2 concentration	increased CO_2 concentration		
Citrate synthase	13.6	17.7	8.7	19.8
Aconitase	146.8	323.0	17.6	18.8
Isocitrate dehydrogenase	24.4	14.6	2.2	17.6
2-Oxoglutarate dehydrogenase	0	0	0	0
Succinate dehydrogenase	46.7	17.2	64.0	56.1
Fumarate hydratase	30.0	17.8	105.0	58.2
Malate dehydrogenase	28.9	22.5	71.0	50.0
Isocitrate lyase	0	0	0	0
Malate synthase	6.4	5.4	9.6	5.2

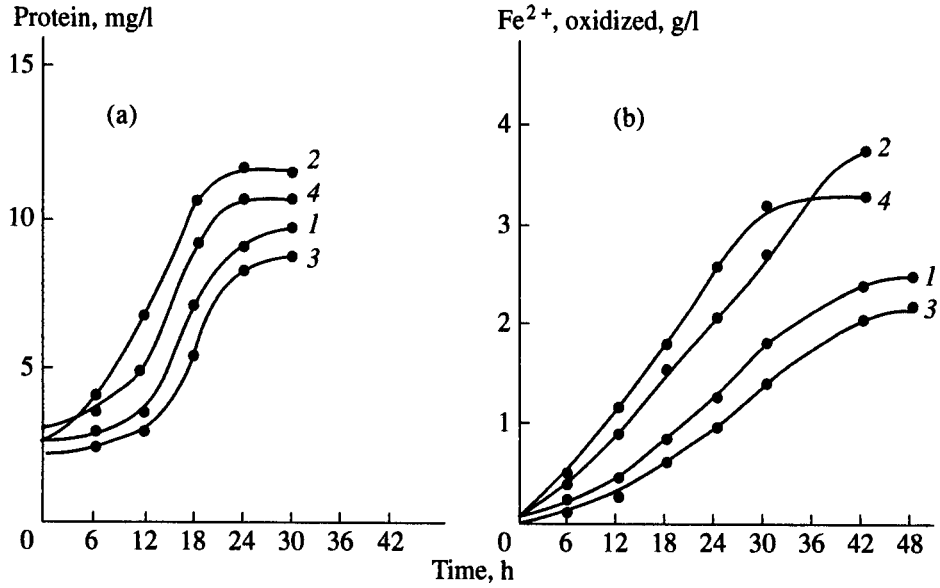

Fig. 2. (a) Biomass accumulation and (b) Fe^{2+} oxidation by (*1*, *2*) strain 41 and (*3*, *4*) strain 1269 during autotrophic growth at (*1*, *3*) normal and increased (*2*, *4*) CO_2 concentrations.

(isocitrate dehydrogenase and fumarate hydratase, see Table 3) increased as well. However, malate dehydrogenase activity decreased.

In the cells of strain 41, an increased CO_2 concentration in the medium caused a decrease in the activity of succinate dehydrogenase and fumarate hydratase. In both strains 41 and 1269, the increase in the CO_2 concentration led to an increase in the activity of citrate synthase and aconitase (and in strain 1269, of malate synthase as well); this provided for high rates of the initial reactions of the TCA cycle and oxalacetate regeneration and, consequently, for more stable autotrophic growth over three culture transfers.

In the cells of strain 41 grown autotrophically in the presence of an increased CO_2 concentration, the activities of RuBPC, PEP carboxylase, and pyruvate carboxylase (4.4, 0.6, and 0.31 nmol/(min mg protein)) were considerably lower than in cells grown in the presence of a normal CO_2 concentration (Table 2) but higher than in heterotrophically grown cells. In should be mentioned that all of the carboxylases studied retained their activity over three culture transfers under autotrophic conditions.

The data obtained in the present work allow us to state that, in *S. thermosulfidooxidans* subsp. *asporogenes* 41, separate reactions of the TCA cycle and glyoxylate shunt operate irrespective of the cultivation conditions; in this respect, strain 41 is similar to *S. thermosulfidooxidans* 1269, the only other sulfobacillus that has been the subject of a relevant study [6]. The activities of the TCA cycle enzymes in these two facultatively chemolithotrophic strains are low as compared to obligate heterotrophs. Sulfobacilli apparently use organic compounds mainly in biosynthetic reactions.

In strain 41, changes in the cultivation conditions mainly affect the initial oxidation stages of di- and tricarboxylic acids; e.g., under autotrophic conditions, the activity of the enzymes involved in these reactions increases (Table 1). In strain 1269 studied earlier, the activities of all TCA cycle enzymes except for malate dehydrogenase were higher under autotrophic conditions than under mixo- or heterotrophic conditions [6]. In both strains 41 (Table 1) and 1269 [6], under any

Table 2. Activities (nmol/(min mg protein)) of carboxylases in *S. thermosulfidooxidans* subsp. *asporogenes* strain 41 cells grown under various cultivation conditions

Enzyme	Cultivation conditions			
	autotrophic	autotrophic + CO_2	mixotrophic	heterotrophic
RuBPC	44.60	4.40	18.0	1.5
pyruvate carboxylase	0.51	0.31	0.26	0.21
PEP carboxylase	8.7	0.6	20.7	0.24
PEP carboxykinase	0	0	0	0
PEP carboxytransphosphorylase	0	0	0	0

growth conditions, the TCA is incomplete and operates as two inversely directed chains of reactions. Strain 41 displayed the activity of malate synthase (a glyoxylate cycle enzyme) irrespective of the growth medium composition, whereas strain 1269 exhibited this activity only after growth in the presence of an increased CO_2 concentration. (Table 3).

Due to the functioning of PEP and pyruvate carboxylases in strain 41 under any cultivation conditions, the ability of this strain to regenerate oxalacetate for biosynthetic purposes is greater than the ability of strain 1269 [9].

Based on our data and data available in the literature [5, 9], it may be concluded that, in all *Sulfobacillus* strains, CO_2 fixation occurs via the Calvin cycle. It should be mentioned that *S. thermosulfidooxidans* subsp. *asporogenes* 41 exhibits a higher specific activity of RuBPC than other strains of sulfobacilli [4, 6], especially during autotrophic growth. However, strains *S. thermosulfidooxidans* 41 and 1269 are close in their growth yield values (Fig. 2a).

In strain 1269, apart from the Calvin cycle reactions, only one additional reaction was found to be involved in CO_2 fixation; this was the reaction catalyzed by PEP carboxytransphosphorylase [9]; in strain 41 cells grown under various conditions, pyruvate carboxylase and a highly active PEP carboxylase were revealed (Table 2).

The activity of PEP carboxylase in strain 41 cells was the highest after mixotrophic growth, and this correlated with an increased biomass accumulated under mixotrophic conditions. In this strain, $^{14}CO_2$ fixation was earlier found to increase after the addition of 0.02% yeast extract to the medium [10]; this increase might be due to the operation of PEP carboxylase.

During the growth of strain 41 under autotrophic conditions at an atmospheric content of CO_2 in the supplied air, derepression of the syntheses of RuBPC, PEP carboxylase, and pyruvate carboxylase occurred. At a CO_2 content of the supplied air of 5–10%, the synthesis of the above enzymes diminished, probably due to the formation of repression-causing compounds such as PEP [16].

It was noted earlier that stable autotrophic growth of sulfobacilli [4, 17] and moderately thermophilic chemolithotrophs, e.g., *Thiobacillus caldus* [18], requires CO_2 concentrations that are higher than the atmospheric one. It was suggested that mechanisms of CO_2 fixation in *S. thermosulfidooxidans* are not efficient enough [19, 20]. Most probably, that is why sulfobacilli frequently occur in natural environments in tight symbiotic associations with other thermoacidophiles that possess efficient inducible mechanisms of CO_2 fixation (e.g., *Acidimicrobium ferrooxidans*) [21, 22]. This concomitant microflora may supply sulfobacilli with organic carbon [22].

In their natural ecotopes (dumps of polymetallic ores, sulfide mineral deposits, acidic hydrotherms), sulfobacilli and similar thermoacidophilic organisms suffer not only organic compound deficiency but also, at high temperatures, carbon dioxide and oxygen limitation. An unstable supply of organic compounds and a variable CCO_2 concentration have resulted in the development of adaptation mechanisms that provide for the survival of microorganisms under these conditions.

Table 3. Effect of CO_2 concentration on the activities (nmol/(min mg protein)) of the enzymes of the TCA cycle and glyoxylate shunt in *S. thermosulfidooxidans* strain 1269 cells grown autotrophically

Enzyme	Strain 1269	
	usual CO_2 concentration*	increased CO_2 concentration
Citrate synthase	3.8	13.2
Aconitase	7.8	51.6
Isocitrate dehydrogenase	16.0	28.7
2-Oxoglutarate dehydrogenase	0	0
Succinate dehydrogenase	–	40.0
Fumarate hydratase	62.0	115.0
Malate dehydrogenase	132.0	38.3
Isocitrate lyase	0	0
Malate synthase	0	18.3

Note: "–" means that a given experimental variant was not run.
*Data from [6].

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REFERENCES

1. Karavaiko, G.I., Golovacheva, R.S., Pivovarova, T.A., Tsaplina, I.A., and Vartanyan, N.S., Thermophilic Bacteria of the Genus *Sulfobacillus*, *Biohydrometallurgy: Proc. Int. Symp.*, Norris, P.R. and Kelly, D.P., Eds., Kew: Science and Technology Letters, 1988, pp. 29–41.
2. Norris, P.R., Clark, D.A., Owen, J.P., and Waterhouse, S., Characteristics of *Sulfobacillus acidophilus* sp. nov. and Other Moderately Thermophilic Mineral-Sulfide-oxidizing Bacteria, *Microbiology* (Reading, UK), 1996, vol. 142, pp. 775–783.
3. Dufresne, S., Bouquet, J., Boissinot, M., and Guay, R., *Sulfobacillus disulfidooxidans* sp. nov., a New Acidophilic, Disulfide-oxidizing, Gram-Positive, Spore-forming Bacterium, *Int. J. Syst. Bacteriol.*, 1996, vol. 46, no. 4, pp. 1056–1064.
4. Wood, A.P. and Kelly, D.P., Autotrophic and Mixotrophic Growth of Three Thermoacidophilic Iron-oxidizing Bacteria, *FEMS Microbiol. Lett.*, 1983, vol. 20, pp. 107–112.
5. Wood, A.P. and Kelly, D.P., Growth and Sugar Metabolism of a Thermoacidophilic 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. Vartanyan, N.S., Pivovarova, T.A., Tsaplina, I.A., Lysenko, A.M., and Karavaiko, G.I., A New Thermoacidophilic Bacterium of the Genus *Sulfobacillus, Mikrobiologiya*, 1988, vol. 57, no. 2, pp. 268–274.
8. Severina, L.O., Senyushkin, A.A., and Karavaiko, G.I., The Structure and Chemical Composition of the S-Layer in Representatives of the Genus *Sulfobacillus, Mikrobiologiya*, 1995, vol. 64, pp. 336–340.
9. 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.
10. Vartanyan, N.S., Karavaiko, G.I., and Pivovarova, T.A., Effect of Organic Compounds on the Growth and Oxidation of Inorganic Substrates by *Sulfobacillus thermosulfidooxidans* subsp. *asporogenes, Mikrobiologiya*, 1990, vol. 59, pp. 411–417.
11. Srere, P.A., Citrate Synthase, *Methods Enzymol.*, 1969, vol. 13, pp. 3–11.
12. Miernyk, J.A., Trelease, R.N., and Choinsky, G.S., Malate Synthase Activity in Cotton and Other Ungerminated Oilseeds, *Plant Physiol.*, 1979, vol. 63, no. 6, pp. 1068–1071.
13. Dixon, G.H. and Kornberg, H.L., Assay Methods for Key Enzymes of the Glyoxylate Cycle, *Biochem. J.*, 1959, vol. 72, no. 1, p. 3P.
14. Krasil'nikova, E.N., Pedan, L.V., Firsov, N.N., and Kondrat'eva, E.N., Tricarboxylic Acid Cycle Enzymes in Various Species of Phototrophic Bacteria, *Mikrobiologiya*, 1973, vol. 42, no. 6, pp. 995–1000.
15. Romanova, A.K., *Biokhimicheskie metody izucheniya avtotrofii u mikroorganizmov* (Biochemical Methods for Studying Autotrophy in Microorganisms), Moscow: Nauka, 1980, pp. 51–133.
16. Tabita, F.R., Molecular and Cellular Regulation of Autotrophic Carbon Dioxide Fixation in Microorganisms, *Microbiol. Rev.*, 1988, vol. 52, no. 2, pp. 155–189.
17. Marsh, R.M. and Norris, P.R., The Isolation of Some Thermophilic, Autotrophic, Iron- and Sulfur-oxidizing Bacteria, *FEMS Microbiol. Lett.*, 1983, vol. 17, pp. 311–315.
18. Dopson, M. and Lindström, E.B., Potential Role of *Thiobacillus caldus* in Arsenopyrite Bioleaching, *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 1, pp. 36–40.
19. Clark, D.A. and Norris, P.R., Acidophilic Bacteria and Their Activity in Mineral Sulfide Oxidation, *Microbial Mineral Recovery*, Ehrlich, H.L. and Brierley, C.L., Eds., New York: McGraw-Hill, 1996, pp. 3–27.
20. Clark, D.A. and Norris, P.R., *Acidimicrobium ferrooxidans* gen. nov., sp. nov.: Mixed-Culture Ferrous Iron Oxidation with *Sulfobacillus* Species, *Microbiology* (Reading, UK), 1996, vol. 142, pp. 785–790.
21. Johnson, D.B., Biodiversity and Ecology of Acidophilic Microorganisms, *FEMS Microbiol. Ecol.*, 1998, vol. 27, pp. 307–317.
22. Karavaiko, G.J., Kovalenko, T.V., and Golovacheva, R.S., Microbiological Aspects of Leaching Copper from Ores, *Proc. Int. Conf. on Use of Microorganisms in Hydrometallurgy*, Pecs (Hung.), 1980, pp. 95–107.