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EXPERIMENTAL ARTICLES

Patterns of Growth and Oxidation of Natural Pyrites by the Representatives of Acidophilic Chemolithotrophic Microorganisms

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Abstract—The patterns of the growth and oxidation of different types of natural pyrites were studied for the three microbial species adapted to these substrates and belonging to phylogenetically remote groups: gram-negative bacterium *Acidithiobacillus ferrooxidans*, gram-positive bacterium *Sulfobacillus thermotolerans*, and the archaeon *Ferroplasma acidiphilum.* For both *A. ferrooxidans* strains, TFV-1 and TFBk, pyrite 4 appeared to be the most difficult to oxidize and grow; pyrite 5 was oxidized by both strains at an average rate, and pyrite 3 was the most readily oxidized. On each of the three pyrites, growth and oxidation by TFBk were more active than by TFV-1. The effectiveness of the adaptation of *S. thermotolerans* Kr1^T was low compared to the *A. ferrooxidans* strains; however, the adapted strain Kr1^T showed the highest growth rate on pyrite 3 among all the cultures studied. No adaptation of strain Kr1^T to pyrite 5 was observed; the rates of growth and pyrite oxidation in the third transfer were lower than in the first transfer. The strain *F. acidiphilum* Y^T was not adapted to pyrites 3 and 5; the rates of growth and pyrite oxidation were the same in the first five transfers. The strains of three species of the microorganisms studied, *A. ferrooxidans, S. thermotolerans*, and *F. acidiphilum*, grew on pyrite 3 (hole-type (p) conductivity) and oxidized it better than pyrite 5 (mixed-type (n-p) conductivity). The most readily oxidized were the pyrites with a density of 5.6–5.7 g/cm^3 and high resistance values (ln R = 8.8). The pyrite oxidation rate did not depend on the type of conductivity. Changes in the chromosomal DNA structure were revealed in strain TFBk on adaptation to pyrites 3 and 4 and in the TFV-1 plasmid profile on adaptation to pyrite 3. Correlation between genetic variability and adaptive capabilities was shown for *A. ferrooxidans.* No changes in the chromosomal DNA structure were found in *S. thermotolerans* Kr1^T and *F. acidiphilum* Y^T on adaptation to pyrites 3 and 5. Plasmids were absent in the cells of these cultures.

Key words: Acidithiobacillus ferrooxidans, Sulfobacillus thermotolerans, Ferroplasma acidiphilum, characteristics of pyrites, adaptation, rate of growth and oxidation, chromosomal DNA structure, plasmid profiles.

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Attention to the kinetics and mechanisms of microbial oxidation of sulfide minerals, mainly pyrite, is explained by the economic significance of biohydrometallurgic technologies of leaching nonferrous metals and extracting noble metals from sulfide ores and concentrates and by the environmental damage caused by microbial oxidation of pyrite-containing ores with the formation of acidic drain waters. For a long time, the attention of scientists has mainly been focused on studying such mesophilic representatives of the acidophilic chemolithotrophs as *Acidithiobacillus ferrooxidans*, *A. thiooxidans*, and *Leptospirillum ferrooxidans*. However, the tasks of intensification of the process of bacterial-chemical oxidation of sulfide substrates stimulated the study of moderately thermophilic and thermophilic communities and the isolation and description of new species of acidophilic chemolithotrophic microorganisms.

Earlier, we studied the parameters of adaptation and of oxidation of two pyrite types differing in physical, chemical, and electrophysical properties (type 1 (n-type) conductivity pyrite and type 2 (p-type) conductivity pyrite) for two *Acidithiobacillus ferrooxidans* strains, TFV-1 and TFBk [1, 2]. This work is a continuation of these investigations and is dedicated to the study of the characteristics of the representatives of phylogenetically remote groups of acidophilic chemolithotrophic microorganisms (*A. ferrooxidans, Sulfobacillus thermotolerans*, and *Ferroplasma acidiphilum*) on oxidation of three pyrite samples, designated as 3, 4, and 5, under the conditions optimal for the growth of each strain.

The aim of the work was to study the adaptation of three species of microorganisms, *A. ferrooxidans,*

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S. thermotolerans, and *F. acidiphilum*, to three types of natural pyrites, the patterns of growth and pyrite oxidation, as well as the genetic characteristics.

MATERIALS AND METHODS

Microbial strains and growth conditions. The work was carried out using the following strains: *A. ferrooxidans* TFV-1 and TFBk, *S. thermotolerans* Kr1^T, and *F. acidiphilum* Y^T from the culture collection of the Laboratory of Chemolithotrophic Microorganisms of the Winogradsky Institute of Microbiology, Russian Academy of Sciences. The microorganisms were grown in 500-ml Erlenmeyer flasks with 200 ml of medium on a shaker at 150 rpm. *A. ferrooxidans* TFV-1 and TFBk were grown on Silverman and Lundgren's medium [3] with Fe^{2+} at 28 ± 2°C. *F. acidiphilum* Y^T was cultivated at 35 ± 0.5°C on modified Silverman and Lundgren's medium [4]. *S. thermotolerans* Kr1^T was cultivated as described in [5] at 40 ± 0.5°C. Exponential phase cultures grown on the same medium with Fe^{2+} were used as inocula. The inoculum was introduced at a rate of 10% by volume. Strain adaptation to the pyrites was carried out at a pulp density of 1% as described in [1] by sequential transfers on the mineral basis of the media indicated above for each species, with the pyrite samples instead of ferrous iron. The number of *A. ferrooxidans* cells was determined by direct counting under an Olympus CX41 phase contrast microscope (Japan). The Fe^{3+}/Fe^{2+} ion concentration in the liquid phase was determined by the spectrophotometric thiocyanate method according to the modified technique [6]. The pH value was determined using an EKSPERT-001 pH-meter-ionometer (Ekoniks-Ekspert, Russia); *Eh*, with a pH150M pH-meter (Eksport, Belarus).

Analysis of the genotypic characteristics. The conditions of precipitation, biomass washing, the methods for isolation of native chromosomal DNA, and its restriction analysis were described earlier [7]. Three restriction endonucleases were used for cutting native chromosomal DNA: for *A. ferrooxidans Xba*I (40 U/30 μl), the restriction site T↓CTAGA; for *S. thermotolerans Not*I (30 U/30 μl), the restriction site GC↓GGCCGC; for *F. acidiphilum Xho*I (40 U/30 μl), the restriction site C↓TCGAG. The chromosomal DNA structure was analyzed by pulsed-field electrophoresis. Fragment separation was carried out in 0.5× TBE buffer (1× TBE: 90 mM Tris, 90 mM boric acid, 2.5 mM EDTA, pH 8.0) in a gel (1% agarose solution for electrophoresis (Lachema, Czech Republic) in 0.5× TBE) at 120 V and a pulse time of 25 s for large fragments (more than 140 kb) or at 130 V and a pulse time of 10 s for small fragments (less than 140 kb). The *A. ferrooxidans* DNA fragments obtained on cleavage with *Xba*I restriction endonuclease served as the size markers.

The isolation of plasmid DNA from the cells of *A. ferrooxidans*, *S. thermotolerans*, and *F. acidiphilum* was carried out as described in [8]. The plasmid profiles of microbial strains were analyzed according to the standard technique by electrophoresis in 1% agarose gel in TAE buffer (40 mM Tris, 2mM EDTA, 20 mM sodium acetate, pH 8.0) at a constant voltage of 90 V. The fragments of the λ phage DNA cleaved with *Hind*III restriction endonuclease served as the standards of molecular mass.

Analysis of the pyrite characteristics The analysis of the pyrite characteristics is described in [1].

RESULTS AND DISCUSSION

Characterization of the pyrites. Table 1 shows the characteristics of the pyrites used in the experiments. The pyrites were of different origin: pyrite 3 with the hole-type (p) conductivity was collected at the ore deposit of the Tetyukhinsk complex; type p pyrite 4 was taken from the Soimanovskaya Valley, Perm oblast; pyrite 5 with mixed-type (n-p) conductivity, from the sulfide deposit area of the Gaiskii ore mining and processing enterprise, Orenburg oblast. The pyrites differed in density, microhardness, the electrophysical characteristics, the sulfur and iron content, and the composition of trace admixtures.

Adaptation and growth patterns of *A. ferrooxidans* TFV-1 and TFBk on pyrites 3, 4, and 5. As it was shown earlier, the strains *A. ferrooxidans* TFV-1 and TFBk differed in the rate of oxidation of such energy substrates as ferrous iron, elemental sulfur, the concentrates of sulfide ores of different composition [9], and pyrites 1 and 2 [1]. The dynamics of their adaptation to pyrites 3, 4, and 5 was studied.

In the process of adaptation of *A. ferrooxidans* TFV-1 to pyrites 3 and 5, the duration of the lag phase did not change; it was two and five days, respectively. The duration of the exponential growth phase decreased from 10 to 5 days on pyrite 3 and from 8 to 16 days on pyrite 5. Strain TFV-1 grew on pyrite 4 during two transfers only, the duration of the lag phase in the first transfer was 38 days; of the exponential phase, 12 days. In the process of adaptation of *A. ferrooxidans* TFBk to all types of pyrites, shortening of the duration of the lag phase from 2, 3, and 1.5 days on pyrites 3, 4, and 5 to 0, 1, and 1 day, respectively, and of the exponential phase from 6, 9, and 4 days to 5, 4, and 3.5 days, respectively, was observed.

All the cultures continued active pyrite oxidation after transition to the stationary growth phase. The growth rate in the first transfer was comparatively low (Fig. 1) and increased with every transfer. The strains differed in the growth rate on different pyrites. The initial and adapted cultures of both strains accumulated a high biomass on pyrite 3 and the least biomass on pyrite 4. As with pyrites 1 and 2, the adapted TFBk culture grew more actively than TFV-1 on all the pyrites. In the process of adaptation, the maximum specific growth rate increased and the generation time decreased for both *A. ferrooxidans* strains (Tables 2, 3). No significant

Table 1. Main physical and electrophysical parameters of pyrites from the samples studied

Sample no.	Density, g/cm^3	Microhardness, kgf/mm^2	Electric properties			Trace admixtures	Content**, %	
			Type of conductivity	$K_{thermoEMF}$, $\mu V\ K^{-1}$	lnR		Sulfur	Iron
3*	5.20	635–861 (810) 1482–1690 (1612)	p	112–508 (347 ± 107)	7–9.5 (8.3 ± 0.6)	Co	48.80–58.71 (55.57) 54.24–54.72 (54.40)	40.22–51.35 (43.48) 45.15–45.57 (45.37)
4	6.10	1362–1434 (1386)	p	275–480 (399 ± 49)	5.3 –9.2 (6.8 ± 1.1)	Ni, Co	50.27–58.92 (57.55) 54.85–55.16 (55.01)	40.46–50.83 (43.79) 44.38–45.32 (44.97)
5*	5.26	824–910(874)	n-p	–148–240 (–57 ± 74)	4.6–7.7 (5.6 ± 0.8)	Cu, Co, Sn, Zn, As, Cr, Au	52.99–55.95 (54.20) 53.44–54.28 (53.76)	43.34–47.20 (44.75) 45.20– 46.56 (45.88)

In brackets, the means are shown.

Notes: * The pyrites are heterogeneous in microhardness; they are represented by two generations.

** The results of the measurements made on the natural on and polished samples are given.

change in the activity of medium acidification by the initial and adapted cultures was observed, compared to pyrites 1 and 2 [1].

At all the adaptation stages, pyrite 3 was oxidized more actively by both strains than pyrites 4 and 5 (Fig. 2). Pyrite 4 was oxidized by the adapted TFBk culture at the slowest rate.

Strain TFBk adapted both to pyrites 1 and 2 and pyrites 3, 4, and 5 grew and oxidized all the three pyrites more actively than strain TFV-1. In terms of difficulty of growth and oxidation, the pyrites may be arranged in the following order: type p pyrite 4, type n-p pyrite 5, type p pyrite 3, type n pyrite 1, and type p pyrite 2.

Higher effectiveness of adaptation to different pyrite types in strain TFBk than in TFV-1 can be explained by the prehistory of its existence on the gold-arsenic concentrate of a more complex composition, which contributed to the development of a labile system of regulation of the processes of oxidation of different types of substrates.

Adaptation and the growth patterns of strain *S. thermotolerans* Kr1^T on pyrites 3 and 5. Among the sulfobacilli, which are moderately thermophilic organisms, the optimal growth temperature for *S. thermotolerans* Kr1^T (40°C) was the closest to the mesophilic bacterium *A. ferrooxidans.* It was chosen for the comparative studies of the dynamics of adaptation and growth patterns on type p pyrite 3 and type n-p pyrite 5 in order to decrease the role of the temperature factor in these processes.

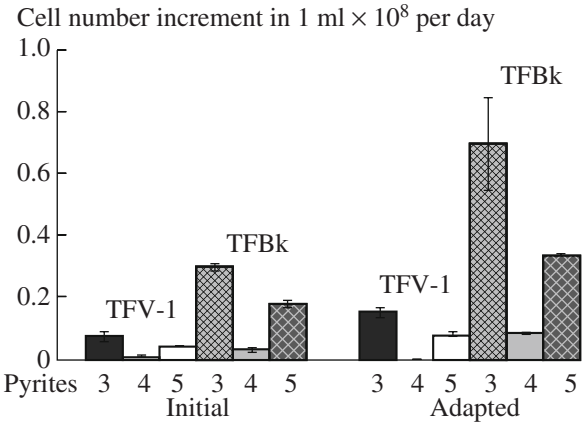

Fig. 1. Average growth rate of *A. ferrooxidans* strains TFV-1 and TFBk on pyrites 3, 4, and 5.

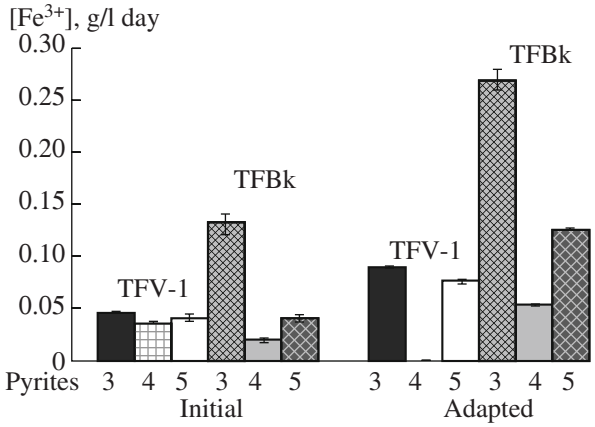

Fig. 2. Maximum oxidation rate of pyrites 3, 4, and 5 by *A. ferrooxidans* TFV-1 and TFBk.

Table 2. Parameters of growth and pyrite oxidation in the initial and adapted cultures of strain *A. ferrooxidans* TFV-1

Pyrite	Culture	Growth and pyrite oxidation parameters			
		Specific growth rate (μ_{max}), h^{-1}	Cell duplication time (t_d), h	Initial and finite values	
				pH	*Eh*
3	Initial	0.009 ± 0.0004	75.3 ± 2.8	1.98	710
				1.84	760
	Adapted	0.028 ± 0.005	24.8 ± 5.3	1.96	622
				1.86	690
4	Initial	0.007 ± 0.0003	100.4 ± 5.2	1.96	724
				1.80	766
	Adapted	–	–	–	–
5	Initial	0.009 ± 0.0002	79.7 ± 4.4	1.90	658
				1.90	777
	Adapted	0.021 ± 0.002	33.4 ± 2.7	1.99	660
				1.80	779

Table 3. Parameters of growth and pyrite oxidation in the initial and adapted cultures of strain *A. ferrooxidans* TFBk

Pyrite	Culture	Growth and pyrite oxidation parameters			
		Specific growth rate (μ_{max}), h^{-1}	Cell duplication time (t_d), h	Initial and finite values	
				pH	*Eh*
3	Initial	0.018 ± 0.001	38.1 ± 1.7	1.94	712
				1.82	800
	Adapted	0.030 ± 0.002	23.1 ± 1.5	1.99	645
				1.89	810
4	Initial	0.010 ± 0.0005	67.3 ± 7.2	1.94	727
				1.90	830
	Adapted	0.023 ± 0.005	30.7 ± 7.8	2.13	768
				1.86	808
5	Initial	0.025 ± 0.002	27.7 ± 2.0	1.95	732
				1.78	801
	Adapted	0.037 ± 0.001	18.8 ± 0.5	1.92	700
				1.77	799

The duration of the lag period and of the exponential phase in strain $Kr1^T$ grown on a medium with 2 g/l Fe^{2+} were 2 and 8 h, respectively. The maximal activity of Fe(II) oxidation is 0.22 g/(l h). In the initial and adapted $Kr1^T$ cultures, the lag period on pyrite 3 lasted 3 h; the exponential phase, 10 and 13 h, respectively. The specific growth rate on the pyrites was lower, while the generation time was longer than on ferrous iron (Table 4). However, both the original and adapted cultures of strain $Kr1^T$ grown on pyrite 3 accumulated more biomass than on Fe^{2+} (iron content, 2.0 g/l) (Fig. 3). The peak rate of iron oxidation on pyrite 3 was significantly lower than on ferrous iron (Fig. 4). The increment in cell number per 1 ml × 10^8 in 24 h in the pyrite 3 adapted culture was only 15% higher than in the initial culture (Fig. 3); the maximal pyrite oxidation rate increased by 26% (Fig. 4). For *A. ferrooxidans* TFV-1 and TFBk grown on the same pyrite 3, the same characteristics increased approximately by 50% in both cases (Figs. 3, 4). Thus, the effectiveness of $Kr1^T$ adaptation to pyrite 3 was not high. However, the adapted $Kr1^T$ culture oxidized pyrite 3 more actively (0.34 g/l day) than TFV-1 (0.091 g/l day) and TFBk (0.27 g/l day). This might result, among other things, from more active chemical processes at 40°C than at 28°C.

We did not observe the process of adaptation to pyrite 5 (Table 4). In the third transfer, the growth rate and the activity of pyrite oxidation were lower than in the first transfer (Figs. 3, 4). However, strain $Kr1^T$ was

Table 4. Parameters of growth and oxidation of pyrites and ferrous iron in the initial and adapted cultures of strain *S. thermotolerans* Kr1^T

Substrate	Culture	Growth and oxidation parameters			
		Specific growth rate (μ_{max}), h^{-1}	Cell duplication time (t_d), h	Initial and finite values	
				pH	*Eh*
Fe^{2+}		0.32 ± 0.01	2.2 ± 0.1	1.77	580
				1.97	733
3	Initial	0.19 ± 0.01	3.6 ± 0.2	1.87	651
				1.80	788
	Adapted	0.25 ± 0.03	2.8 ± 0.3	1.99	618
				1.90	796
5	Initial	0.15 ± 0.01	4.2 ± 0.1	1.96	691
				1.87	790
	The third transfer	0.09 ± 0.01	7.7 ± 0.8	1.99	630
				1.93	756

capable of growth on pyrite 5 for a number of transfers (more than 10).

Strain Kr1^T grew and oxidized pyrite 3 more actively than pyrite 5. Thus, type p pyrite 3 was a more readily oxidized substrate than type n-p pyrite 5 for both *S. thermotolerans* Kr1^T and *A. ferrooxidans*.

Adaptation and growth patterns of strain *F. acidiphilum* Y^T on pyrites 3 and 5. The mesophilic autotrophic archaeon *F. acidiphilum* Y^T (the optimum growth temperature 35°C), which requires yeast extract for growth and is unable to oxidize sulfur compounds, was the third microorganism chosen for these investigations [4]. An attempt was made to adapt strain Y^T to pyrites 3 and 5; however, no adaptation was observed (Table 5, Figs. 5, 6). The rates of growth and pyrite oxidation on respective pyrites remained the same for the first five transfers. It may be concluded that this microorganism has no mechanisms of regulation of metabolic processes similar to those in *A. ferrooxidans*, when a transfer from an iron-containing medium into the pyrite-containing one results in the activation of the enzyme systems related to the oxidation of sulfur compounds and in an increase in the growth and oxidation characteristics.

Strain Y^T grew and oxidized pyrite 5 worse than pyrite 3. Thus, the strains of the three microorganisms studied, *A. ferrooxidans*, *S. thermotolerans*, and *F. acidiphilum*, oxidized type p pyrite 3 better than type n-p pyrite 5.

The influence of the characteristics of the pyrites on the capacity of microorganisms for their oxidation. We did not reveal any relationship between the type of the pyrite and its ability to be oxidized by the microorganisms; however, some regularities were noted. Pyrite 4, the hardest to oxidize, had the highest density (6.10 g/cm^3) of all the five pyrite types studied [1, this work]. However, the least dense pyrites 3 and 5

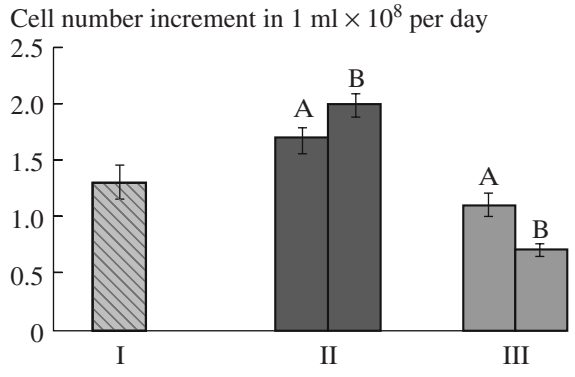

Fig. 3. Average growth rate of *S. thermotolerans* Kr1^T grown on medium with ferrous iron (I); of the initial (A) and adapted (B) culture grown on pyrite 3 (II) and in the first (A) and third (B) transfers on pyrite 5 (III).

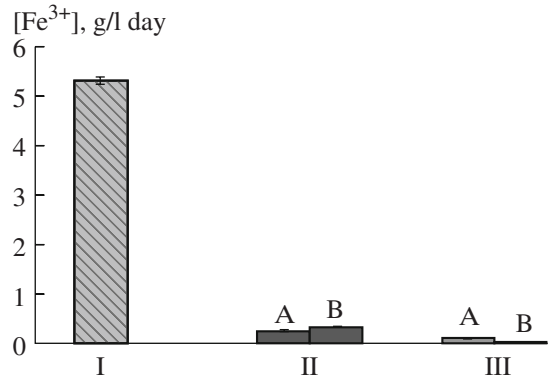

Fig. 4. Maximum oxidation rate of ferrous iron (I) and pyrite 3 (II) by the initial (A) and adapted (B) cultures and on pyrite 5 (III) in the first (A) and third (B) transfer by strain *S. thermotolerans* Kr1^T.

Table 5. Parameters of growth and oxidation of pyrites and ferrous iron in the cultures of strain *F. acidiphilum* Y^T

Substrate	Growth and oxidation parameters			
	Specific growth rate (μ_{max}), h^{-1}	Cell duplication time (t_d), h	Initial and finite values	
			pH	*Eh*
Fe^{2+}	0.0140 ± 0.0010	50.6 ± 4.4	1.68 2.05	614 758
Pyrite 3	0.0100 ± 0.001	67.8 ± 4.8	1.71 1.64	697 711
Pyrite 5	0.0075 ± 0.0005	92.4 ± 5.8	1.71 1.62	700 734

(5.20 and 5.26 g/cm^3) were not the most readily oxidized. The most readily oxidized pyrites belonged to different conductivity types, type n pyrite 1 and type p pyrite 2, had an average density of 5.60 and 5.72 g/cm^3, respectively. Thus, it is suggested that the density parameter can be used to predict the capacity of different types of microorganisms to oxidize pyrites. The most readily oxidized pyrites have the density values close to 5.6–5.7 g/cm^3. Deviations in both directions lead to a decreased capacity of the microorganisms for pyrite oxidation.

No relationship between the microhardness of the pyrites and the rate of their microbial oxidation was found.

The composition of trace admixtures in all the pyrite samples studied was different. It is difficult to judge about their role in pyrite oxidation.

An important characteristic is the content of the major elements in pyrite, sulfur and iron. The series in order of increasing sulfur content is as follows: pyrite 5 (53.76%) ⟶ pyrite 1 (54.04%) ⟶ pyrite 3 (54.40%) ⟶ pyrite 2 (54.66%) ⟶ pyrite 4 (55.01%). The pyrite series in order of decreasing iron content is as follows: pyrite 5 (45.88%) ⟶ pyrite 1 (45.59%) ⟶ pyrite 3 (45.37%) ⟶ pyrite 4 (44.97%) ⟶ pyrite 2 (44.7%). Type n-p pyrite 5 has the lowest sulfur content and the highest iron content. Further, type n pyrite 1 ranks second in the decreasing iron content series followed by three type p pyrites (3, 4, and 2). Pyrite 2, the most readily oxidized by the *A. ferrooxidans* strains, had average sulfur content and the lowest iron content. No relationship was noted between the iron and sulfur content in the pyrites and the rates of their microbial oxidation.

Pyrite 3 was the most heterogeneous of all the five pyrites in terms of the $K_{thermoEMF}$ value (Table 1). Most of the pyrite 3 and 4 grains had $K_{thermoEMF}$ of 400–500 μV K^{-1}. The maximum number of pyrite 2 grains

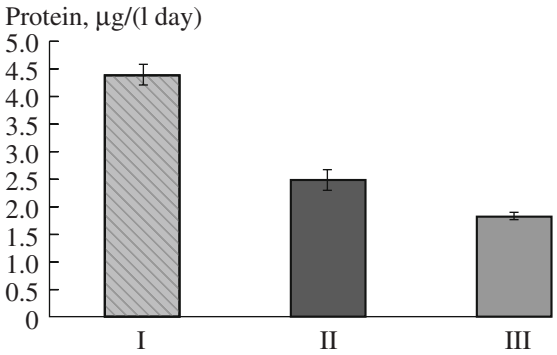

Fig. 5. Average growth rate of *F. acidiphilum* Y^T on medium with ferrous iron (I), on pyrite 3 (II), and on pyrite 5 (III).

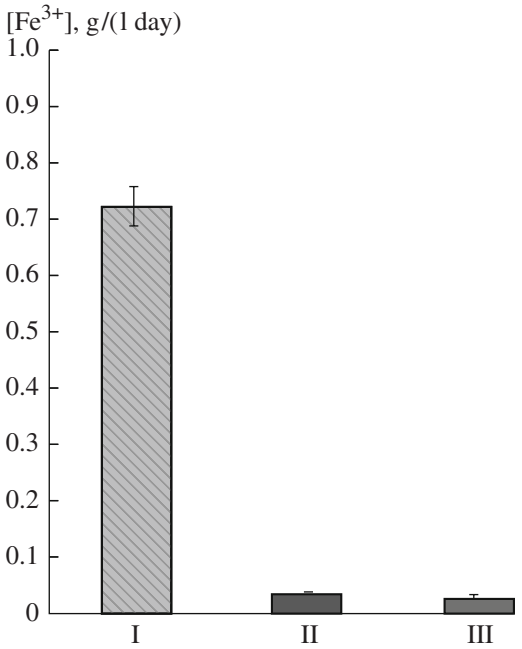

Fig. 6. Maximum oxidation rate of ferrous iron (I), pyrite 3 (II), and pyrite 5 (III) by *F. acidiphilum* Y^T.

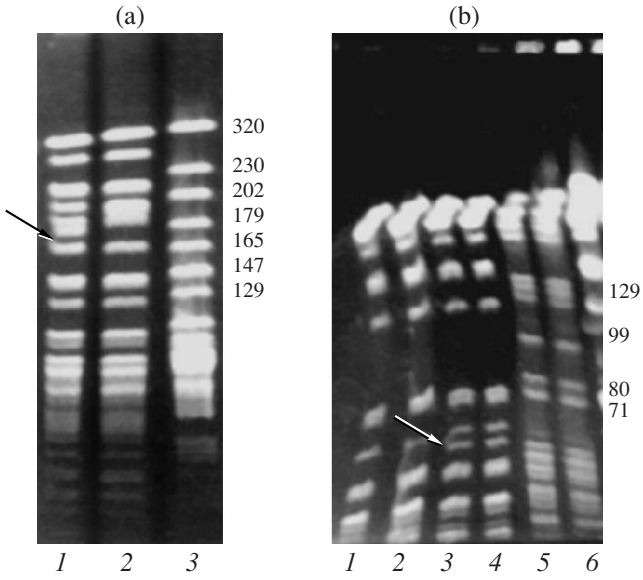

Fig. 7. Samples of *Xba*I restriction of the chromosomal DNA: (a) strain *A. ferrooxidans* TFBk grown on medium with pyrite 3 (lane *1*), on medium with Fe^{2+} (lane *2*); and strain *A. ferrooxidans* VKM B-458 grown on medium with Fe^{2+} (lane *3*); PF conditions: voltage 120 V, pulse time 25 s, temperature 15°C, duration 44 h; (b) strain *A. ferrooxidans* TFBk grown on medium with Fe^{2+} (lanes *1, 2*), on medium with pyrite 4 (lanes *3, 4*), and strain VKM B-458 grown on medium with Fe^{2+} (lanes *5, 6*); PF conditions: voltage 130 V, pulse time 10 s, temperature15°C, duration 64 h. The arrows show new DNA fragments. At the side of the panels, the DNA fragment sizes in kb are indicated.

fitted the range of the $K_{thermoEMF}$ values about 200 μV K^{-1}. Pyrite 2 was the most readily oxidized. It may be suggested that pyrites with such a $K_{thermoEMF}$ value will be oxidized more readily than those with higher $K_{thermoEMF}$ values, e.g., pyrites 3 and 4. In pyrite 1 with the electronic type of conductivity, most of the grains had $K_{thermoEMF} = -100$ μV K^{-1}. The pyrites with such $K_{thermoEMF}$ values were readily oxidized. The maximum number of pyrite 5 grains had $K_{thermoEMF}$ values of about 0 μV K^{-1} with the predominance of grains with negative values. Pyrites with such a $K_{thermoEMF}$ value were harder to oxidize. Thus, the pyrites with the absolute $K_{thermoEMF}$ values in the region of 200–100 μV K^{-1} were more readily oxidized than those whose values were 400–500 μV K^{-1} or close to 0. To support this hypothesis, it would have been interesting to study a pyrite sample with $K_{thermoEMF} \approx -400$ μV K^{-1} for comparing it with the already-studied pyrites.

By the ln R value, type p pyrites may be arranged in the following series: pyrite 4 (6.8 ± 1.1), pyrite 3 (8.3 ± 0.6), pyrite 2 (8.8 ± 0.8); in this series, the logarithm of resistance increased and the conductivity decreased. Type n pyrite 1 had the highest ln R value, similar to that of type p pyrite 2, and the widest range (8.8 ± 1.9). Type n-p pyrite 5 had the lowest ln R value (5.6 ± 0.8) of all the five pyrites studied. Pyrites 4 and 5 were the hardest to oxidize, and pyrites 1 and 2 were most readily oxidized by the microorganisms. Irrespective of the type of conductivity, the logarithm of resistance of a pyrite sample is a characteristic related to the ease of its oxidation. The pyrites with higher ln R values were oxidized more readily than those with lower values. In the pyrites of the same conductivity type (type p in our work), the ease-of-oxidation and the ln R series coincided.

In order to predict the ease of microbial pyrite oxidation, a complex approach should be used, including the analysis of all the characteristics, most importantly density, the sulfur and iron content, the type of conductivity, the $K_{thermoEMF}$ and ln R values.

The peculiarities of the chromosomal DNA structure in the pyrite-adapted strains. Adaptation to new energy substrates may be accompanied by changes in the chromosomal DNA structure [10, 11]. Growth on pyrite 3 did not result in any changes in the chromosomal DNA structure of *A. ferrooxidans* TFV-1 after 10 transfers. On pyrite 4, strain TFV-1 died at the third transfer; on pyrite 5, it accumulated very little biomass, which was insufficient for isolating the chromosomal DNA for analysis by this method.

The absence of changes in the chromosomal DNA structure in TFV-1 grown on pyrite 3 supports the earlier data that TFV-1 has a relatively stable genome structure [11, 12]. The TFV-1 genome contained a small number of copies of IS elements, the transposition of which causes the changes in the DNA nucleotide sequences [13]. Changes in the chromosomal DNA structure in strain TFV-1 were observed only once on adaptation to pyrite 1 [2].

Strain TFBk exhibited changes in the chromosomal DNA structure in the region of large fragments after more than 10 transfers on pyrite 3 (Fig. 7a, lane *1*). In the *Xba*I restriction samples, a new band consisting of 177-kb fragments is seen. The emergence of fragments of a similar size was noted on adaptation of strain TFBk to the cyanided ore concentrate from the Nezhdaninsk deposit (in this case, another band containing 158-kb fragments was also observed) [11] and on adaptation to the mineralogically pure pyrite from the Akchatau deposit [10].

In pyrite 4-adapted strain TFBk, changes in the chromosomal DNA structure were found among small fragments after more than 10 transfers. In the *Xba*I restriction samples, a new band consisting of fragments about 67 Kb in size is seen (Fig. 7b, lanes *3*, *4*). The fragment was in the nonequimolar amount with the fragments invariably present. We observed the same picture upon adaptation of strain TFBk to pyrites 1 and 2: with an increase in the number of transfers, the number of fragments constituting new bands increased accordingly, which determined the intense brightness of luminosity of the new bands [2]. Based on this, we can speak about a gradual accumulation of clones with changes in the chromosomal DNA structure in the bacterial population of TFBk adapted to a new substrate.

No changes in the chromosomal DNA structure were revealed in *A. ferrooxidans* TFBk after adaptation to pyrite 5.

These results confirm our earlier findings that the adaptation of strain TFBk to the pyrites from different deposits is accompanied by an ambiguous genomic response [10, 11, 14]. In these studies, mineralogically pure pyrites from the Akchatau, Angren, and Tulun deposits were used. Adaptation to the pyrites of different origin in some cases (pyrite from the Achkatau deposit [10], pyrites 1, 2 [2], pyrites 3, 4 [this work]) was accompanied by the appearance of new fragments in the DNA chromosomal *Xba*I restriction samples; in other cases (Angren, Tulun [11, 14], and pyrite 5 [this work]) it was not. The physical, chemical, and electrophysical characteristics of the pyrites as an energy substrate could have influenced the genomic changes in the same *A. ferrooxidans* strain TFBk. It should be note once more that, in this work, the changes in the chromosomal DNA structure of *A. ferrooxidans* TFBk were revealed only after 10 transfers on a new substrate. This may be the consequence of the fact that they did not appear in all the cells within the population. The accumulation in the population of the cells with a changed structure of the chromosomal DNA, as the number of transfers increased, suggests their advantage in the rates of growth and oxidation of a new substrate.

Figure 8 shows the DNA restriction profiles of *S. thermotolerans* $Kr1^T$ (on cleavage with the *Not*I restriction endonuclease) and *F. acidiphilum* Y^T (on cleavage with the *Xho*I restriction endonuclease) cells grown on ferrous iron. Analysis of the chromosomal DNA restricts with pulsed-field electrophoresis did not reveal any changes in the chromosomal DNA structure of these cultures after more than 10 transfers of the bacteria on pyrites 3 and 5. Note that the study of the changes in the chromosomal DNA structure of *F. acidiphilum* under replacement of the energy substrate was carried out for the first time. Earlier, the genome structure under a change in the source of energy (yeast extract, Fe^{2+}, S^0, sulfide minerals, ore concentrates) was studied in three *S. thermosulfidooxidans* strains: VKM B-1269; subsp. *asporogenes*, strain 41; and subsp. *thermotolerans* strain K1 [15]. Changes were only noted in the genome of the asporogenic subspecies *S. thermosulfidooxidans* subsp. *asporogenes*, strain 41, when metabolism was switched from using the yeast extract to the oxidation of elemental sulfur. In 2005, strain *S. thermosulfidooxidans* subsp. *thermotolerans* K1 was reclassified as *Alicyclobacillus tolerans* and is no longer identified with sulfobacilli [16]. Adaptation of *S. thermosulfidooxidans* strains to pyrites was not studied. Our results did not show any changes in the DNA restriction profiles in the strains of two studied species of microorganisms, *S. thermotolerans* and *F. acidiphilum*, during the process of their adaptation to pyrites 3 and 5. This may be evidence of the stable structure of their chromosomal DNA.

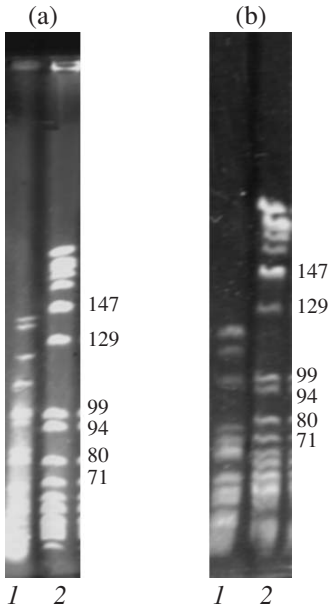

Fig. 8. Samples of *Not*I restriction of the chromosomal DNA of strain *S. thermotolerans* $Kr1^T$ (a, lane *1*); *Xho*I restriction of the chromosomal DNA of strain *F. acidiphilum* Y^T (b, lane *1*); *Xba*I restriction of chromosomal DNA of strain *A. ferrooxidans* VKM B-458 grown on medium with Fe^{2+} (a, b, lanes *2*). PF conditions: voltage 130 V, pulse time 10 s, temperature 15°C, duration 64 h. At the side of the panels, the DNA fragment sizes in kb are indicated.

Thus, different cultures of chemolithotrophic microorganisms (gram-negative bacteria *A. ferrooxidans*, gram-positive bacteria *S. thermotolerans*, and the archaeon *F. acidiphilum*) behave differently when their metabolism is switched to the oxidation of a new energy substrate. Of the four cultures studied, *A. ferrooxidans* TFBk had the most labile genome. Its response to adaptation to pyrite as a source of energy was variable: in some cases, we observed changes in the chromosomal DNA structure; in others, we did not. This may be related to the diversity of the characteristics of natural pyrites, to the peculiarities of their physical, chemical, and electrophysical characteristics.

Plasmid profiles in pyrite-adapted strains. Another mechanism of adaptation to new environmental conditions may be related to plasmids [17, 18]. Both *A. ferrooxidans* strains studied contain plasmids: in strain TFV-1, from two to six plasmids were revealed on different substrates (Fe^{2+}, S^0, FeS_2, concentrate), while in strain TFBk, invariably two plasmids were revealed when it grew on the same substrates [18]. Plasmids in *F. acidiphilum* Y^T and *S. thermotolerans* $Kr1^T$ were not studied earlier. We did not reveal any plasmids in Y^T and $Kr1^T$ grown on medium with ferrous iron.

Nor did we reveal any changes in the plasmid composition of *A. ferrooxidans* TFBk adapted to either pyrites 3, 4, and 5 or to pyrites 1 and 2 [2]. Changes in the plasmid profile of TFV-1 were noted in the course of adaptation to pyrite 3 (Fig. 9). The strain grown on

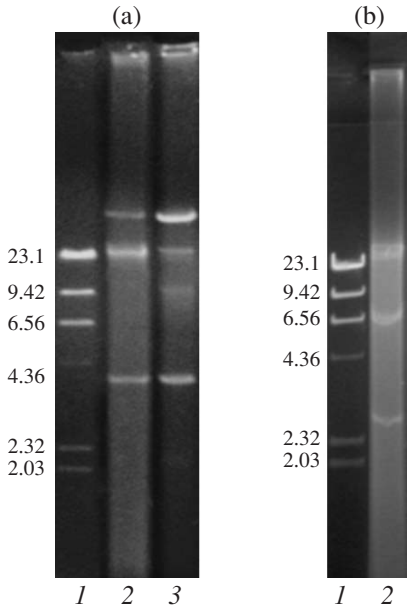

Fig. 9. Plasmid profiles of strain *A. ferrooxidans* TFV-1 grown on medium with Fe^{2+} (a, lane *2*) or adapted to pyrite 3 (a, lane *3*) and strain *S. thermotolerans* $Kr1^T$ grown on the ore concentrate from the Nezhdaninsk deposit (b, lane *2*). Phage λ DNA cleaved with *Hind*III restriction endonuclease (a, b, lanes *1*). At the side of the panels, the DNA fragment sizes in kb are indicated.

pyrite 4 died during the third transfer. When TFV-1 grew on pyrite 5, the biomass it accumulated was not sufficient for the isolation and analysis of the plasmid DNA. After 10 transfers of TFV-1 on pyrite 3, four plasmids were revealed; three of these were similar to those revealed in ferrous iron-grown bacteria, and one plasmid was different (Fig. 9a, lanes *2*, *3*). It may be suggested that, in this case, we observed either a change in the number of copies of different plasmids or a change in the content of clones within the population with a different set of plasmids in the cells adapted to different energy substrates.

After 10 transfers on pyrites 3 and 5, no plasmids were revealed in the cells of *S. thermotolerans* $Kr1^T$. However, after 10 transfers on the ore concentrate from the Nezhdaninsk deposit, three plasmids were revealed in $Kr1^T$ (Fig. 9b, lane *2*). Note that pyrite is the main sulfide mineral of the Nezhdaninsk ore deposit (its characteristics have not been studied so far). The ore contains such heavy metals as zinc, copper, lead, and silver. The presence of plasmids in the cells of the *S. thermotolerans* $Kr1^T$ culture adapted to this substrate might be linked to increasing resistance to heavy metals in the process of adaptation.

No plasmids were found in strain *F. acidiphilum* Y^T when it was grown on pyrites 3, 5, and the ore concentrate from the Nezhdaninsk deposit (the data are not shown).

The phenotype of the *A. ferrooxidans* plasmids has not been determined yet (they do not carry the genes of resistance to heavy metals and antibiotics as most of the plasmids), and they are considered to be cryptic. Nevertheless, they are retained in the cells of most *A. ferrooxidans* strains despite the additional energy expenditure required for their sustenance. It is not impossible that plasmid-containing *A ferrooxidans* cells have selective advantage over those which have none. A number of researchers tend to believe that the plasmids in the cells of *A. ferrooxidans* perform the regulatory function and are important for the adaptation to the ever changing environmental factors [19]. The possibility for the plasmid DNA to be integrated into the chromosomal DNA and cleaved out was shown; this may result in changing the amount of plasmid DNA [20]. An interesting fact is that, in strain TFBk, with a large number of copies of the IS elements and the constant plasmid composition, alterations in the chromosomal DNA structure were observed when the energy substrate was changed, while in strain TFV-1, with the genome relatively stable from the point of view of its chromosomal DNA structure and a low IS content, changes in the plasmid composition were observed with a change in the source of energy. Thus, on adaptation to a new source of energy, the two *A. ferrooxidans* strains exhibited changes either in the chromosomal DNA structure or in the plasmid profiles. The functions of plasmids and IS elements in *A. ferrooxidans* are not clear. Both the former and the latter are supposedly involved in the regulation of the genome, which is manifested as a change in the phenotypic properties. Insertions or excisions of IS elements from certain chromosomal DNA sites may, for example, initiate or repress the functions of the regulator genes of certain operons [13, 20].

Thus, on a broad set of pyrites and pyrite-oxidizing microorganisms, the physical, chemical, and electrophysical characteristics of pyrites were shown to influence the rates of growth and pyrite oxidation in acidophilic chemolithotrophs. The work showed that, based on the analysis of the aggregate of the pyrite characteristics, it is possible to predict their oxidation rate.

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