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EXPERIMENTAL ARTICLES

Dependence of the Genotypic Characteristics of *Acidithiobacillus ferrooxidans* on the Physical, Chemical, and Electrophysical Properties of Pyrites

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Abstract—This study focused on the effect of physical, chemical, and electrophysical properties of two pyrites, pyrite 1, which had electron-type (*n*-type) conductivity, and pyrite 2, with hole-type (*p*-type) conductivity, on the genotypic characteristics of *Acidithiobacillus ferrooxidans* strains TFV-1 and TFBk, which were isolated from different substrates. After the adaptation of the strains to the pyrites at a pulp density of 1%, pulsed-field electrophoresis revealed changes in the chromosomal DNA of strain TFV-1 adapted to pyrite 1, and strain TFBk adapted to either of the pyrite types. In pyrite-adapted strain TFBk, the plasmid composition was the same as after growth on a medium containing ferrous iron, whereas, in strain TFV-1, changes in plasmid sizes or both in plasmid sizes and plasmid number occurred. After an increase in the density of the pyrite 2 pulp from 1 to 10%, the plasmid number increased from three to four, and, after an increase in the density of the pyrite 1 pulp from 1 to 7%, the plasmid number increased from two to six.

Key words: properties of pyrites, *Acidithiobacillus ferrooxidans* strains, structure of chromosomal DNA, plasmid profiles.

The adaptation of the acidophilic chemolithotrophic bacterium *Acidithiobacillus ferrooxidans* to new energy substrates or high concentrations of metal ions is often accompanied by changes in the structure of its chromosomal DNA, which can be revealed by pulsed-field electrophoresis [1–3]. During the adaptation of strain TFBk to pyrites taken from different ore deposits, the *Xba*I restriction pattern of its DNA was found to change in the case of one of the pyrites and remain unaltered in the case of the other. These results allowed us to assume that the genotypic variability of *A. ferrooxidans* strains is influenced not only by the chemical nature of the substrate but also by its physical, chemical, and electrophysical properties.

The present study focused on the influence of the physical, chemical, and electrophysical properties of two types of pyrite on the structure of the chromosomal DNA of two *A. ferrooxidans* strains, TFV-1 and TFBk, and on their plasmid profiles.

MATERIALS AND METHODS

Bacterial strains and cultivation conditions. Experiments were conducted with two *A. ferrooxidans* strains, TFV-1 and TFBk, stored in the culture collection of the Laboratory of Chemolithotrophic Microorganisms, Winogradsky Institute of Microbiology, Russian Academy of Sciences. Strain TFBk was isolated from the pulp at the plant that processes high-sulfur gold–arsenic ore concentrate from the Bakyrchikskoe deposit (this concentrate mainly contains pyrite and arsenopyrite and provides for high concentrations of iron and arsenic in the solutions). Strain TFV-1 was isolated from the poor copper ore of the Volkovskoe deposit; this ore is relatively simple in its mineralogical composition. Cultivation of the strains and their adaptation to pyrites of different types were performed as described in [4].

Analysis of genotypic characteristics. Harvesting and washing of the biomass, isolation of native genomic DNA, and its restriction analysis were performed as described in [5]. Isolation of plasmid DNA and its electrophoretic analysis were performed as described in [6].

Analysis of the properties of the pyrites is described in [4].

RESULTS AND DISCUSSION

Characteristics of the pyrites. Pyrite 1 and pyrite 2 exhibited certain differences in their densities (5.6 and 5.72 g/cm^3, respectively); microsolidity (pyrite 2 particles were more homogeneous in terms of their microsolidity (1119–1233 kG/mm^2) than pyrite 1 particles, whose microsolidities fell into the ranges 794–824 or

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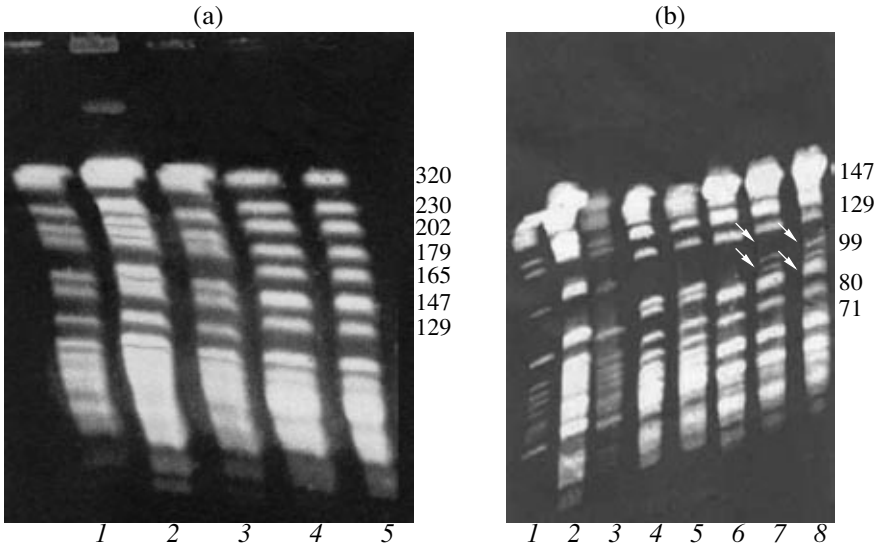

Fig. 1. *Xba*I-restriction patterns of the chromosomal DNA of *A. ferrooxidans* strains: strain TFV-1 grown on media containing (*1*) Fe^{2+}, (*2*) pyrite 1, and (*3*) pyrite 2; (*4, 5*) strain VKM B-458 grown on a medium containing Fe^{2+}; and strain TFBk grown on media containing (*6*) Fe^{2+}, (*7*) pyrite 1, and (*8*) pyrite 2. Pulsed-field electrophoresis was run (a) for 44 h at 120 V, 13°C, and an impulse time of 25 s and (b) for 68 h at 130 V, 13°C, and an impulse time of 10 s. DNA fragment sizes are indicated (in kb) on the right-hand side of the panels.

1360–1780 kG/mm^2); content of microadmixtures (Ni, V, and Sb in pyrite 1 and Co, Ni, As, and Ag in pyrite 2); the properties of their surface, which was more uneven in pyrite 2; the structure of their crystals; solubility in nitric acid, which was greater for pyrite 1; and electrophysical properties. According to data from a K_{TEMF} analysis, pyrite 1 exhibited electron-type (*n*-type) conductivity, whereas pyrite 2 was characterized by hole-type (*p*-type) conductivity.

Specific features of chromosomal DNA structure in the *A. ferrooxidans* strains adapted to pyrites. After five passages on pyrites 1 and 2 at a 1% pulp density, no changes in the structure of the chromosomal DNA could be revealed either in strain TFV-1 or strain TFBk by treatment with the *Xba*I restriction endonuclease (data not shown). In strain TFV-1, isolated from the poor ore of the Volkovskoe deposit, changes in the structure of its chromosomal DNA were revealed after ten passages on pyrite 1 (Fig. 1a, lane *2*). In the *Xba*I restriction pattern, a new 202-bp band appeared, which was absent from the restriction pattern of the original strain grown on Fe^{2+}. On pyrite 2, no changes in the chromosomal DNA of this strain occurred. Earlier, we performed the adaptation of strain TFV-1 to different substrates, including two pyrites, and analyzed the chromosomal DNA structure of the adapted cultures with the use of three restriction nucleases (*Xba*I, *Bcu*I, and *Smi*I), but no changes were revealed. It was concluded that strain TFV-1 has a stable genome structure [3]. In the present study, however, we did reveal changes in the chromosomal DNA of this strain after its adaptation to pyrite 1. It is noteworthy that, during the adaptation to this substrate, the rates of growth and substrate oxidation were the lowest [4].

In strain TFBk, isolated from a mineralogically complex pyrite–arsenopyrite concentrate, changes in the chromosomal DNA structure were revealed after ten passages on pyrites 1 and 2 at a 1% pulp density (Fig. 1b, lanes *7, 8*). In the *Xba*I restriction patterns of both of the strains, two new bands appeared representing 114- and 96.5-kb fragments. Earlier, we showed that the adaptation of strain TFBk to pyrites from different deposits was accompanied by different reactions of its genome. Table 1 shows the sizes of the fragments formed upon *Xba*I restriction of the native DNA of strain TFBk when grown on a medium containing ferrous iron or adapted to different pyrites (published data [1, 3] and results of the present study), specifically, mineralogically pure pyrite from the Angren deposit (Angren 1 and Angren 2, pyrite 2) and pyrites from the Tulun and Karpushikha deposits (pyrite 1). It can be seen that the adaptation to different pyrites led to different reactions of the genome. In the case of the pyrites from the Akchatau, Karpushikha, and Angren 2 deposits, new fragments appeared in the *Xba*I restriction patterns of the chromosomal DNA, whereas, in the case of the pyrites from the Angren 1 and Tulun deposits, the corresponding restriction patterns lacked the fragments that were present after growth on a medium containing ferrous iron in nonequimolar amounts, i.e., were characteristic only of part of the population. Thus, the reaction of the genome of *A. ferrooxidans* strain TFBk was influenced by the physical, chemical, and electrophysical properties of the energy substrate. During the adaptation of strain TFBk to pyrites 1 and 2, which differ in

Sizes (kb) of the *Xba*I-restriction fragments of the chromosomal DNA of strain *A. ferrooxidans* TFBk after adaptation to pyrites of different origins

Band no. from the start line	Energy substrate					
	Ferrous iron	Pyrite from the deposit				
		Angren 1	Tulun	Akchatau	Karpushikha	Angren 2
1	328	328	328	328	328	328
2	269	269	269	269	269	269
3	214	214	214	214	214	214
4	199	199	199	199	199	199
5	186	186	186	186	186	186
6	169	169	169	177**	169	169
7	158*	147	147	169	147	147
8	147	129	129	147	129	129
9	129	88	88	129	114**	114**
10	115*	77	77	88	96**	96**
11	88	65	65	77	88	88
12	77	60	60	65	77	77
13	65	54	54	60	65	65
14	60	50	50	54	60	60
15	54	46	46	50	54	54
16	50	36	36	46	50	50
17	46			36	46	46
18	36				36	36

* The amount of the fragment is lower than the equimolecular one.
** A new fragment.

their properties, the identical changes in the chromosomal DNA structure that we observed could be a reaction to the composition of the energy source. The fact that, in the present study, changes in the chromosomal DNA structure of the *A. ferrooxidans* strains could be detected only after ten culture passages on a new substrate suggests that they occurred only in part of the cell population. The accumulation of cells with an altered chromosomal DNA structure in the population with culture passages indicates that these cells have a higher rate of growth and higher rate of oxidation of the new substrate. The changes in the structure of the chromosomal DNA that appeared after the adaptation to pyrites 1 and 2 at a pulp density of 1% persisted after experiments that employed a denser pulp [4].

Plasmid profiles in the *A. ferrooxidans* strains adapted to pyrites. Both of the strains under study harbor plasmids. Two to six plasmids can be revealed after the growth of strain TFV-1 on different substrates. Two plasmids were invariably revealed in strain TFBk on the earlier studied substrates [7].

In the present study, no changes were revealed in the plasmid composition of strain TFBk adapted to pyrites 1 and 2 (Fig. 2b). Only in strain TFV-1 were changes in the plasmid profile recorded after the adaptation to these pyrites at a pulp density of 1% (Fig. 2a). Earlier, we revealed two plasmids in strain TFV-1 when grown on a medium containing Fe^{2+} [6, 7]. After the adaptation of this strain to pyrite 1 at a pulp density of 1% (ten culture passages), we again revealed two plasmids. One of them was of the same size as the plasmid found after growth on Fe^{2+} or pyrite 1, while the other one was larger (Fig. 2a, lane *3*). In strain TFV-1 adapted to pyrite 2 (ten culture passages), three plasmids were

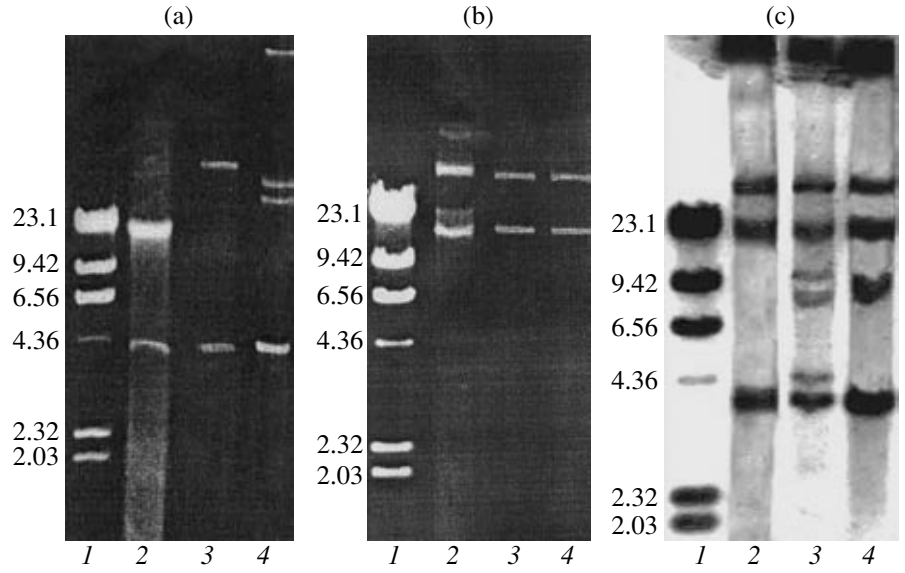

Fig. 2. Plasmid profiles of *A. ferrooxidans* strains (a, c) TFV-1 and (b) TFBk (*2*) grown on a medium containing Fe^{2+} or adapted to (*3*) pyrite 1 or (*4*) pyrite 2. Lanes *1* represent phage lambda DNA digested with *Hin*dIII restriction endonuclease. DNA fragment sizes are indicated (in kb) on the left-hand side of the panels.

revealed. One of them was of the same size as the plasmids found in cells grown on Fe^{2+} or pyrite, and the two other plasmids corresponded to the plasmids occurring in this strain after its adaptation to pyrite–arsenopyrite concentrate (Fig. 2a, lane *4*) [7].

After a year of cultivation of strain TFV-1 on medium 9K with Fe^{2+}, three plasmids were revealed, two of which corresponded to the earlier detected ones while the third had a larger size (Fig. 2c, lane *2*). After using a denser pulp (7% for pyrite 1 and 10% for pyrite 2), a greater number of plasmids was detected. On pyrite 1, a substrate that proved to be a more difficult to oxidize for strain TFV-1, six plasmids were revealed (Fig. 2c, lane *3*), while, on pyrite 2, only four plasmids were found (Fig. 2c, lane *4*).

The spontaneous change in the number of detectable plasmids occurring during the maintenance of strain TFV-1 on a medium containing divalent iron is difficult to explain. The *A. ferrooxidans* plasmids are cryptic, i.e., their phenotype is as yet undetermined. As distinct from most plasmids, they do not carry genes for resistance to heavy metals or antibiotics. Nevertheless, plasmids persist in most *A. ferrooxidans* strains, despite the additional energy expenditures needed for their maintenance. It is possible that *A. ferrooxidans* cells harboring plasmids have certain selective advantages over plasmidless cells. Some researchers are inclined to believe that *A. ferrooxidans* plasmids perform a regulatory function and are important during adaptation to changing environmental conditions [8]. The possibility of integration of plasmid DNA into the chromosome and of its excision has been shown; these processes result in changes in plasmid detectability [9].

Thus, changes in the chromosomal DNA structure and plasmid profiles of *A. ferrooxidans* strains occur not only upon switching of the metabolism to oxidation of a new energy substrate but also as a reaction to the physical, chemical, and electrophysical properties of chemically similar substrates.

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