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EXPERIMENTAL ARTICLES

Interaction of Chromosomal and Plasmid DNA in *Acidithiobacillus ferrooxidans* Strains Adapted to Different Oxidation Substrates

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Abstract—Restriction analysis of plasmids pTFK1 and pTFK2 of the *Acidithiobacillus ferrooxidans* strain TFBk was carried out, and the sizes of these plasmids were determined (13.5 and 30 kb, respectively). A macrorestriction map was built for plasmid pTFK1. DNA–DNA hybridization revealed that the plasmids contained homologous nucleotide sequences. Plasmid pTFK2 labeled with ^{32}P was used as a probe for Southern hybridization with blots of *Xba*I-generated fragments of the chromosomal DNA of *A. ferrooxidans* strains grown on a medium containing Fe^{2+} or adapted to different oxidation substrates. Low-intensity hybridization signals were observed for many fragments of the chromosomal DNA of the strains studied. In the process of adaptation to new oxidation substrates, the localization of bands producing the low-intensity hybridization signals changed in a number of cases. Certain fragments of the chromosomal DNA of the strains adapted to different oxidation substrates produced strong hybridization signals with pTFK2. The data obtained are discussed in terms of the possible role of IST elements and plasmids in the adaptation of *A. ferrooxidans* to new energy substrates, microevolution, and strain polymorphism.

Key words: *Acidithiobacillus ferrooxidans* strains, plasmids, chromosomal DNA, restriction patterns, Southern hybridization.

Strain polymorphism of the chromosomal DNA structure is characteristic of the acidophilic chemolithoautotrophic bacterium *Acidithiobacillus ferrooxidans*, which derives energy from the oxidation of Fe^{2+}, S^0/S^{2-}, or sulfide minerals. A great number of *A. ferrooxidans* strains have been isolated from different geographic areas and different substrates, including pulps of the concentrates produced during the enrichment of sulfide ores. These strains differed in the restriction profiles of the chromosomal DNA, analyzed by pulsed-field gel electrophoresis (PFGE) [1, 2]. The oxidation substrate is one of the major factors that determine structural changes in the chromosomal DNA [3–6]. It was shown that changes in the chromosomal DNA structure may appear as a result of the adaptation of *A. ferrooxidans* strains to new energy sources [3–5] or high concentrations of Fe^{3+} [6] or may accompany changes in ore characteristics (oxidation degree and antimony content) under natural conditions [5]. Possible mechanisms responsible for changes in the chromosomal DNA structure have been discussed [3, 4]. Changes in the number and size of the fragments generated by restriction of native chromosomal DNA may result from methylation of nucleotides at restriction sites, from intrachromosomal or plasmid–chromosome recombinations, or from changes in the location of IST elements. It was shown that the location of IST elements may change during prolonged cultivation in a medium containing enhanced concentrations of copper ions [7]. Most of the *A. ferrooxidans* strains studied by us contained plasmids [8]. In some of the strains, the adaptation to high concentrations of metals or to new oxidation substrates was demonstrated to be accompanied by changes in the chromosomal DNA structure and plasmid profiles [8–10]. This allows the possibility to be assumed of plasmid DNA excision from or integration into the chromosome. The exchange of genetic information between the plasmid and chromosomal DNA of *A. ferrooxidans* was shown to be the cause of the localization of the expressed genes of mercury resistance in the chromosomal DNA and of some of the genes of the impaired mercury operon in the plasmid pTF-FC2 [11], as well as of the duplication of some chromosomal genes in plasmid pTF5 [12].

The aims of the present work included the investigation of the structure of two plasmids of *A. ferrooxidans* strain TFBk and Southern hybridization of plasmid pTFK2 with blots of macrorestriction profiles of the chromosomal DNA of five *A. ferrooxidans* strains adapted to different oxidation substrates.

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MATERIALS AND METHODS

Strains and cultivation conditions. Three *A. ferrooxidans* strains were isolated from dense pulps of reactors during experimental biohydrometallurgical processes of gold recovery from concentrates of pyrite–arsenopyrite ores of the Nezhdaninskoe (strain TFN-d), Bakyrchikskoe (strain TFBk), and Olimpiadinskoe (strain TFO) deposits. Strain TFV-1 was isolated from the solution formed during dump leaching of copper from waste ore of the Volkovskoe deposit. Strain TFL-2 was isolated from the tail pulp during the obtaining of a copper–pyrite industrial product. DNA fragments of known size from *A. ferrooxidans* VKM B-458 served as molecular weight standards [1]. All the strains were adapted to elemental sulfur, pyrite, and ore concentrate from the Nezhdaninskoe deposit through no less than 20 sequential passages on media containing a given oxidation substrate. Sequential passages were repeated until maximal growth rate was attained and no further change in the growth rate occurred upon the following passages [9]. Media and cultivation conditions were described earlier [9].

Chromosomal DNA isolation and analysis. Chromosomal DNA was obtained as described by Kondratyeva *et al.* [1] and digested with *Xba*I restriction endonuclease. The fragments were separated by pulsed-field gel electrophoresis [1]. The gels were also used for hybridization with phosphor-labeled DNA of plasmid pTFK2 obtained from *A. ferrooxidans* strain TFBk (see below).

Plasmid DNA isolation. Plasmid DNA was extracted from *A. ferrooxidans* TFBk using a modified alkaline extraction procedure [13]. After neutralization of the alkaline lysate with potassium acetate, DNA was precipitated with 0.7 volume of isopropanol and dissolved in TE buffer. The resultant solution was treated with 1 M Tris–HCl in phenol (pH 8.0) and centrifuged for 3 min at 9000 *g*. An equal volume of chloroform was added to the water phase to remove phenol traces. The mixture was shaken for 1 min and centrifuged for 1 min at 9000 *g*. For DNA precipitation, 0.8 volume of isopropanol was added to the water phase; the mixture was shaken thoroughly, left for several minutes, and centrifuged at 9000 *g*. The supernatant was discarded, and the DNA-containing sediment was washed with 1 ml of 70% ethanol, dried, and dissolved in 50 μl of distilled water or TE buffer.

Restriction analysis of plasmid DNA. Fourteen restriction endonucleases were used throughout this study: *Eco*RI, *Eco*RV, *Xho*I, *Pst*I, *Hin*dIII, *Pvu*II, *Bam*HI (R + BSA buffer, Fermentas); *Sal*I, *Bgl*II (Promega D buffer); *Kpn*I, *Dra*I, *Hpa*I (B + BSA buffer, Fermentas); *Xba*I, and *Hin*dII (Y + BSA buffer, Fermentas). Endonuclease activity ranged from 5 to 10 U/μl. DNA restriction with a single restriction endonuclease was performed in the buffer solutions described above. Preliminary control of restriction activities was carried out using phage lambda DNA.

The conditions for plasmid DNA restriction with a single endonuclease were as follows. The reaction mixture contained 3 μl of plasmid DNA solution, 1 μl of a given buffer, 6 μl of water, and 1 μl of a given restriction endonuclease. The mixture was incubated for 2 h at 37°C. Then, 1.5 μl of a buffer containing bromophenol blue was added to stop the reaction. The mixture was transferred to 1% agarose gel for electrophoresis.

For double restriction of plasmid pTFK1 DNA, the following pairs of restriction endonucleases were used: *Sal*I + *Eco*RI, *Sal*I + *Pst*I, *Sal*I + *Bgl*II, *Sal*I + *Dra*I, *Sal*I + *Pvu*II, *Eco*RI + *Dra*I, *Pst*I + *Dra*I, *Bgl*II + *Dra*I (Promega D buffer); *Kpn*I + *Dra*I, *Dra*I + *Pvu*II (B + BSA buffer); *Xba*I + *Hin*dIII (Y + BSA buffer); *Xho*I + *Eco*RI, *Eco*RI + *Pst*I, *Eco*RI + *Bgl*II, *Eco*RI + *Pvu*II, *Pst*I + *Bgl*II, *Pst*I + *Pvu*II, and *Bgl*II + *Pvu*II (R + BSA buffer). The conditions for double restriction of plasmid DNA were as follows. The reaction mixture contained 3 μl of plasmid DNA solution, 1.1 μl of a given 10× buffer, 6 μl of water, 0.5 μl of enzyme 1, and 0.5 μl of enzyme 2. The mixture was incubated for 4 h at 37°C. Then, 1.5 μl of a buffer containing bromophenol blue was added to stop the reaction. The mixture was transferred to 1% agarose gel for electrophoresis. After electrophoresis, agarose gels were photographed using a Bio-Profol gel documentation system supplied with a Vilber Lourmat digital videocamera (USA).

Southern transfer and hybridization. Agarose gels with *Xba*I fragments (macrorestricts) of the chromosomal DNA from different *A. ferrooxidans* strains separated by PFGE were used for hybridization. Gels were maintained in 0.25 N HCl for 15 min before the transfer since the size of DNA fragments separated as stated above may reach hundreds of kilobases. Then gels were incubated in 0.4 N NaOH as described in [14]. After washing, DNA was blotted onto a nylon membrane (Gene Screen Plus, Du Pont, USA) by using a vacuum transfer apparatus (LKB). All the operations, including DNA transfer onto membranes, prehybridization and hybridization with a labeled DNA (probe), and membrane washing, were carried out as recommended by the membrane manufacturer. Hybridization was carried out for 5 h at 66–67°C in a Techne hybridization oven. X-ray films (NPO Tasma) were used for autoradiography.

Radioactive probe preparation. DNA of plasmid pTFK2 from *A. ferrooxidans* strain TFBk was used as a probe. As *A. ferrooxidans* TFBk harbors two plasmids simultaneously [8], they were separated as follows. Total DNA was treated with restriction endonuclease *Eco*RI. Plasmids pTFK1 and pTFK2 were digested by this enzyme into two and eight clearly visible fragments, respectively. The fragments were separated by electrophoresis in 1% agarose gel, the corresponding bands were cut out, and the DNA was eluted using the standard technique [14]. The separated fragments of the plasmid pTFK2 were mixed and labeled with a radioactive ^{32}P isotope [dATP], specific activity 5000 Ci/mM, using the Klenov fragment of *Escherichia coli* DNA

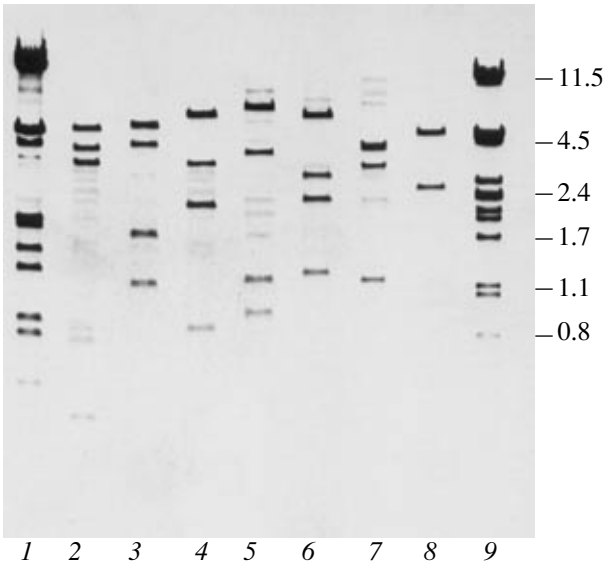

Fig. 1. Double restriction patterns generated from plasmid pTFK1 of *A. ferrooxidans* TFBk by treatment with restriction endonucleases (*2*) *Pst*I + *Bgl*II, (*3*) *Pst*I + *Dra*I, (*4*) *Pst*I + *Pvu*II, (*5*) *Bgl*II + *Dra*I, (*6*) *Bgl*II + *Pvu*II, (*7*) *Dra*I + *Pvu*II, and (*8*) *Eco*RI + *Pst*I. Molecular weight markers were generated from (*1, 9*) phage lambda DNA by digestion with *Hin*dIII + *Eco*RI and *Pst*I, respectively. Figures at the side of the panels are fragment sizes, kb (here and henceforth).

polymerase I, as described in [14]. For the experiments, 100–200 ng of DNA was taken. A 9-base random primer on a plasmid DNA was used to prime labeled DNA synthesis. Labeled DNA was separated by precipitation with 2.5 volumes of ethanol (96%) and 0.1 volume of 3 M CH_3COONa followed by washing with ethanol (80%).

RESULTS

Restriction analysis of plasmids from *A. ferrooxidans* TFBk. *A. ferrooxidans* TFBk harbors two plasmids, a smaller plasmid pTFK1 and a larger plasmid pTFK2, which differ in the copy number [8]. The smaller plasmid is present in a larger number of copies than the larger one. This follows from the differences in the luminescence intensity of the corresponding bands in the electrophoregrams formed by the restriction fragments of the two plasmids. The difference in the copy number of plasmids allowed us to carry out a restriction analysis without physical separation of plasmid replicons. Restriction fragments of the smaller plasmid pTFK1 were detected in the electrophoregram if small quantities of the plasmid DNA (200–300 ng) were taken for analysis. For the detection of restriction fragments of the larger plasmid pTFK2, the DNA quantity taken for restriction was tripled. In addition, we photographed gels at varying exposure time. The bands of high luminescence intensity were not taken into consideration.

For macrorestriction map creation, the plasmid pTFK1 DNA was digested with single restriction endonucleases and their pairs, using all the combinations possible. Some restriction patterns are presented in Fig. 1. The sizes of the generated fragments were estimated using known markers (restricts of phage lambda DNA obtained using restriction endonucleases *Pst*I, *Hin*dIII, etc.). The total size of all pTFK1 fragments generated by single restriction enzymes and their combinations amounted to 13.5 kb. A macrorestriction map of plasmid pTFK1 is presented in Fig. 2. Plasmid pTFK1 contained one *Sal*I restriction site and two restriction sites for *Eco*RI, *Bgl*II, *Pst*I, *Pvu*II and *Dra*I. However, the smaller plasmid did not have *Bam*HI, *Eco*RV, *Kpn*I, *Hin*dIII, *Xba*I, or *Xho*I recognition sites. At least three *Hpa*I restriction sites and four *Hin*dII restriction sites were present in plasmid pTFK1, but the position of these restriction sites was not determined.

The larger plasmid pTFK2 contained recognition sites of all the restriction endonucleases used throughout this study. Some restriction patterns of this plasmid are presented in Fig. 3. The restriction endonucleases *Kpn*I, *Xma*I, and *Bgl*II digested the plasmid pTFK2 DNA into seven clearly visible fragments. Thus, no less than seven *Bam*HI, *Hin*dIII, and *Sal*I restriction sites were present within this plasmid (small restricts of low luminescence intensity could stay undetected). Plasmid pTFK2 was digested into eight fragments by the restriction endonuclease *Eco*RI. The restriction endonuclease *Pst*I generated ten fragments. Four fragments were generated by the restriction endonucleases *Bam*HI, *Hin*dIII and *Sal*I. *Pvu*II and *Dra*I digested the plasmid pTFK2 DNA into three fragments. *Xba*I and *Eco*RV generated two fragments. pTFK2 had one *Xho*I restriction site. It should be noted that the presence and number of *Xba*I, *Eco*RV, and *Xho*I restriction sites within pTFK2 were determined via double restriction with *Xba*I + *Xho*I, *Xba*I + *Eco*RV, and *Xho*I + *Eco*RV. The size of plasmid pTFK2 was evaluated from the total size of *Pst*I, *Hin*dIII, and *Kpn*I restriction fragments as approximately 30 kb.

Hybridization of the plasmid pTFK2 DNA with the chromosomal DNA from *A. ferrooxidans* TFBk adapted to different oxidation substrates. We carried out Southern hybridization of phosphor-labeled plasmid pTFK2 DNA from strain TFBk with blots prepared from *Xba*I-generated fragments of the native chromosomal DNA from the cultures of this strain adapted to different oxidation substrates and separated by pulsed-field gel electrophoresis. The results are presented in Fig. 4. Strong hybridization of the pTFK2 DNA occurred with itself and with pTFK1 (Fig. 4b, lane *2*). Hybridization bands could be seen at the bottom of the gel in a lane that contained plasmid DNA. It is known that plasmids that are supercoiled, relaxed, or open with a single chain disruption migrate slower during PFGE than linear ones [15, 16]. Therefore, hybridization bands at the bottom of the gel related to linear plasmid forms. This is evidenced by Fig. 5. Fragments of the

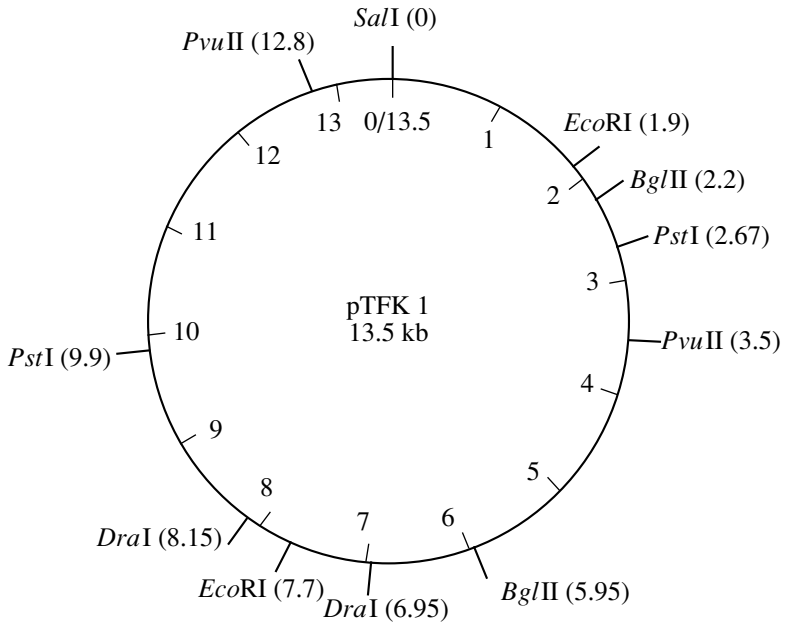

Fig. 2. Macrorestriction map of plasmid pTFK1 of *A. ferrooxidans* TFBk. Figures in parentheses are the distances from the zero point, kb.

plasmid DNA from strain TFBk generated with *Sal*I and *Xba*I (Fig. 5, lanes *3* and *5*) were localized in the same dimensional area as the bottom hybridization bands in Fig. 4, lane *2*.

Intense signals of hybridization with fragments of 328, 269, and 199 kb and two fragments of no more than 60 kb in size were detected in the autoradiograph of *Xba*I-generated fragments of the chromosomal DNA from strain TFBk grown on a medium containing Fe^{2+} or adapted to S^0, FeS^2, and ore concentrate from the Nezhdaninskoe deposit (henceforth, concentrate) (Fig. 4b, lanes *3–6*, indicated with arrows). Also, multiple low-intensity signals of hybridization of plasmid pTFK2 DNA with blots of restriction profiles of the chromosomal DNA from strain TFBk could be seen. If the culture was grown on media containing Fe^{2+} or S^0, hybridization occurred with a 214-kb fragment (Fig. 4b, lanes *3* and *4*, indicated with double arrows). However, if the culture was adapted to pyrite or sulfide concentrate, 214-kb fragments did not have nucleotide sequences complementary to pTFK2. Also, hybridization was observed with one of the fragments of the chromosomal DNA from strain TFBk at the bottom of the gel (Fig. 4b, lane *6*, indicated with a double arrow) if the culture was adapted to concentrate but was not if the culture was adapted to other substrates. The localization of low-intensity hybridization signals also varied for TFBk cultures adapted to different energy sources.

Hybridization of the plasmid pTFK2 DNA with the chromosomal DNA from other *A. ferrooxidans* strains adapted to different oxidation substrates. We performed Southern hybridization of phosphor-labeled pTFK2 DNA with blots prepared from fragments of the chromosomal DNA of four *A. ferrooxidans* strains (TFN-d, TFV-1, TFO, and TFL-2) grown on a medium containing Fe^{2+} or adapted to S^0, FeS_2, and concentrate (Figs. 6–8). We observed plural hybridization signals of low intensity in the autoradiographs of strains TFN-d, TFV-1, and TFO. Also, high-intensity signals were observed (indicated with arrows: TFV-1, Fig. 6b, lanes *2* and *3*; TFO, Fig. 8b, lane *1*; and TFN-d, Fig. 8b, lane *2*).

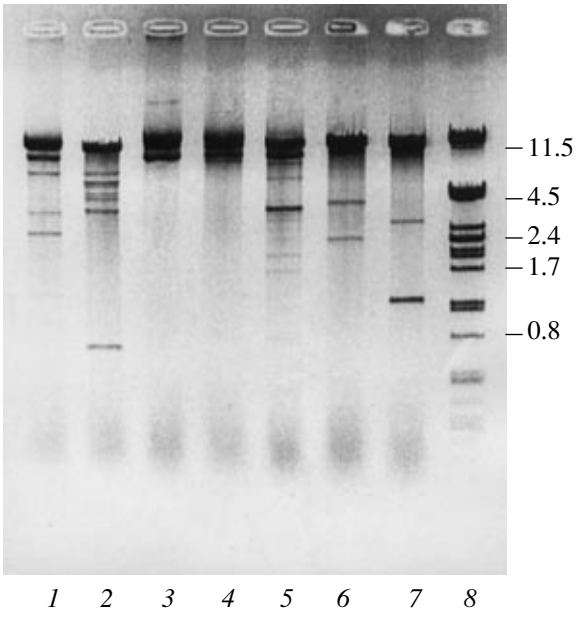

Fig. 3. Restriction patterns generated from plasmid pTFK2 of *A. ferrooxidans* TFBk with single restriction endonucleases: (*1*) *Kpn*I; (*2*) *Xma*I; (*3*) *Xho*I; (*4*) *Xba*I; (*5*) *Bgl*II; (*6*) *Sal*I; (*7*) *Dra*I. (*8*) Phage lambda DNA digested with *Pst*I.

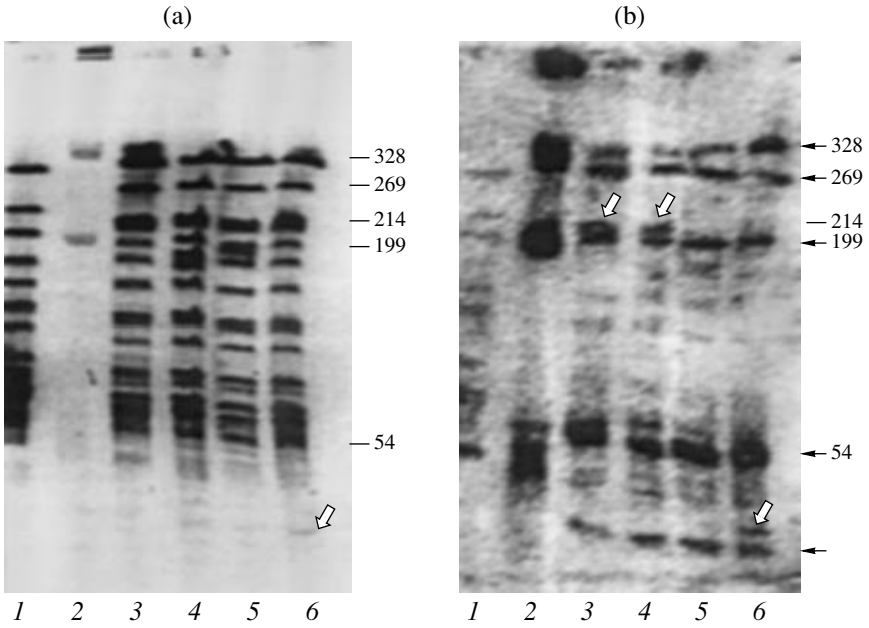

Fig. 4. (a) *Xba*I restriction patterns of chromosomal DNA from *A. ferrooxidans* TFBk adapted to different oxidation substrates: (*3*) Fe^{2+}; (*4*) S^0; (*5*) FeS_2; (*6*) ore concentrate. (*1*) Strain B-458; (*2*) plasmid DNA from strain TFBk. Pulsed-field gel electrophoresis (in this figure and Figs. 6 and 7) was run at 120 V and a 25-s pulse for 44 h at 13°C. (b) Autoradiograph of *Xba*I-generated fragments of chromosomal DNA from *A. ferrooxidans* TFBk hybridized with plasmid pTFK2 DNA from the same strain. Arrows indicate DNA fragments for which strong hybridization was observed with pTFK2; double arrows indicate chromosomal DNA fragments that hybridize not in all preparations (here and henceforth).

Strong hybridization was observed with certain fragments of DNA from strain TFV-1 grown on media containing Fe^{2+} and S^0 and strains TFO and TFN-d grown on a medium containing FeS_2. However, if strains TFO and TFN-d were adapted to Fe^{2+} (in the case of TFO, also to S^0), hybridization with these fragments did not occur. Changes in localization of hybridization signals for strain TFV-1 after switching from Fe^{2+} to S^0 were not observed.

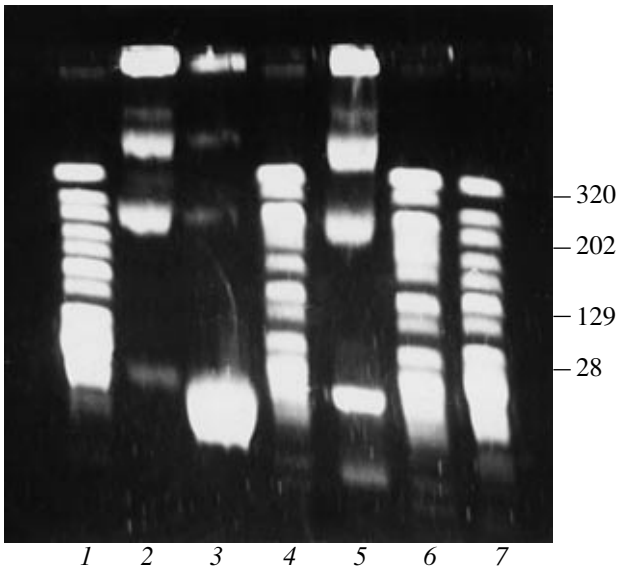

Fig. 5. Restriction patterns of chromosomal and plasmid DNA from *A. ferrooxidans* strains: (*1, 7*) *Xba*I-digested chromosomal DNA from strain VKM B-458; (*2*) native plasmid DNA from strain TFBk; (*3*) *Sal*I-digested DNA from strain TFBk; (*4, 6*) *Xba*I-digested chromosomal DNA from strain TFBk; (*5*) *Xba*I-digested plasmid DNA from strain TFBk.

In the upper part of the autoradiograph of fragments of the chromosomal DNA from strains TFO and TFN-d hybridized with pTFK2 (Fig. 8b, lanes *1* and *2*), we observed two weak bands for each strain not corresponding with the localization of fragments of the chromosomal DNA from these strains grown on FeS_2 (Fig. 8a, lanes *1* and *2*). One band not corresponding with the localization of fragments of the chromosomal DNA was also present in the autoradiograph of strain TFO grown on a medium containing Fe^{2+} (Fig. 6b, lane *5*). Also, one weak hybridization signal not coinciding with localization of the DNA fragments was present in the upper part of the autoradiograph of strain TFL-2 adapted to concentrate and, less pronouncedly, in the autoradiograph of the same strain adapted to FeS_2 (Fig. 7, lanes *1* and *2*). It was shown earlier [10] that strains TFO and TFN-d each harbor two plasmids of comparable sizes and strain TFL-2 carries one plasmid. The signals in question could result from hybridization of these plasmids with pTFK2. The high sensitivity of DNA–DNA hybridization methods allows revealing of much greater quantities of plasmid DNA not digested with *Xba*I as compared to PFGE followed by treatment of gels containing DNA fragments with ethidium bromide for DNA visualization.

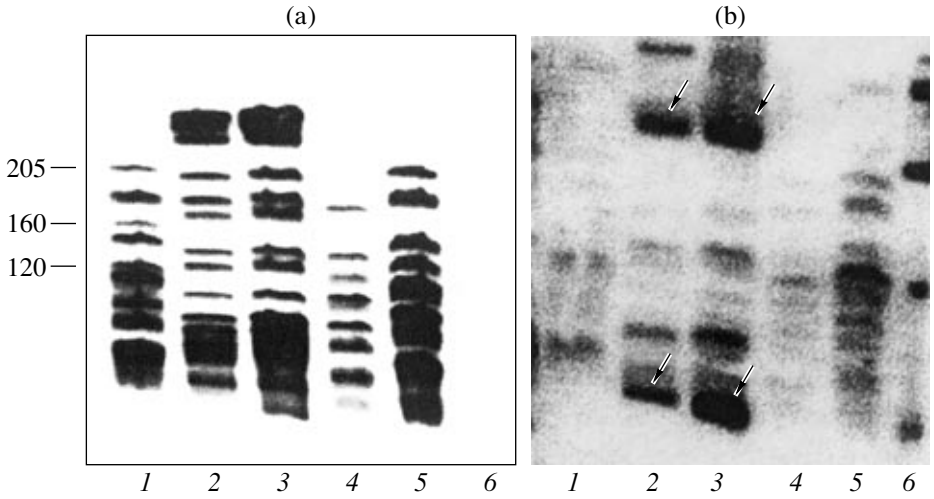

Fig 6. (a) *Xba*I restriction patterns of chromosomal DNA from *A. ferrooxidans* strains (*1*) TFN-d; (*2, 3*) TFV-1; (*4, 5*) TFO; (*6*) plasmid DNA from strain TFBk) adapted to different oxidation substrates: (*1, 3, 5*) Fe^{2+}; (*2, 4*) S^0. (b) Autoradiograph of *Xba*I-generated fragments of chromosomal DNA from *A. ferrooxidans* strains hybridized with plasmid pTFK2 DNA from strain TFBk.

DISCUSSION

Both the smaller plasmid pTFK1 and the larger plasmid pTFK2 of *A. ferrooxidans* TFBk were digested by the endonucleases *Sal*I, *Eco*RI, *Bgl*II, *Pst*I, *Dra*I, and *Pvu*II. However, they differed in the number of recognition sites of these restriction endonucleases. For example, the restriction endonuclease *Bgl*II digested pTFK1 and pTFK2 into two and seven fragments, respectively; *Eco*RI, two and eight; and *Pst*I, two and ten. The endonucleases *Bam*HI, *Eco*RV, *Kpn*I, *Hin*dIII, *Xba*I, and *Xho*I digested the plasmid pTFK2 DNA, but not pTFK1. The plasmids contained homologous nucleotide sequences, which resulted in strong hybridization. This allows the suggestion that pTFK1 originated from pTFK2 following a loss of some part of the genetic material.

Many *A. ferrooxidans* strains harbor a 20-kb plasmid pTFO [17]. Plasmids pTFK1 and pTFK2 differ substantially from pTFO in size and restriction sites.

The chromosomal DNA of the *A. ferrooxidans* strains studied contains a great number of nucleotide sequences complementary to those in plasmid pTFK2. Their small size (evaluated from the intensity of the hybridization signal), plurality, and localization on both plasmid and chromosomal DNA suggest they belong to IST elements found in the *A. ferrooxidans* genome by Holmes *et al.* [18]. It is possible that *A. ferrooxidans* strains differing in origin contain identical IST elements. In a number of cases, adaptation to a new oxidation substrate was accompanied by changes in location of these sequences in *A. ferrooxidans* TFBk, which resulted in the appearance of new fragments of the chromosomal DNA and weak hybridization of these fragments with the plasmid DNA.

The change in location of nucleotide sequences homologous to those of the plasmid DNA within the chromosomal DNA during adaptation to new oxidation substrates allows the suggestion that these sequences participate in regulation of the oxidation processes. Holmes and Haq [7] assumed that IST elements are involved in mechanisms of adaptation of *A. ferrooxidans* to changing environmental conditions. They studied the localization of IST elements belonging to the IST1 family in *A. ferrooxidans* before and after several months of passages on a medium containing increasing concentrations of copper (up to 40 g/l) and revealed changes in localization of one of the nucleotide sequences within the chromosomal DNA.

Migration of an IST1 element resulted in phenotypic switching of morphological types of *A. ferrooxidans* colonies and the ability to oxidize iron [19].

An IST1 element was found within a *res*B-like gene in *A. ferrooxidans* mutant ATCC19859, which is unable to oxidize iron [20]. The analogous gene of a wild strain did not contain this element. This gene encodes a cytochrome *c* protein necessary, in the authors' opinion, for the oxidation of iron but not sulfur.

In some cases, we observed more intensive signals of hybridization of pTFK2 with the fragments of the chromosomal DNA of this and other strains compared to plural hybridization signals of low intensity. This indicates that certain fragments of the chromosomal DNA contained nucleotide sequences of greater length homologous to those of plasmid pTFK2. Strong hybridization of plasmid pTFK2 from the strain TFBk occurred in lanes containing 199-kb fragments of the chromosomal DNA. This could result from hybridization of pTFK2 with pTFK1, which contains nucleotide sequences homologous to pTFK2, is not digested by *Xba*I, and is located in the same gel areas. Strong hybridization of pTFK2 from the strains TFO and TFN-d with one of the chromosomal DNA fragments occurred

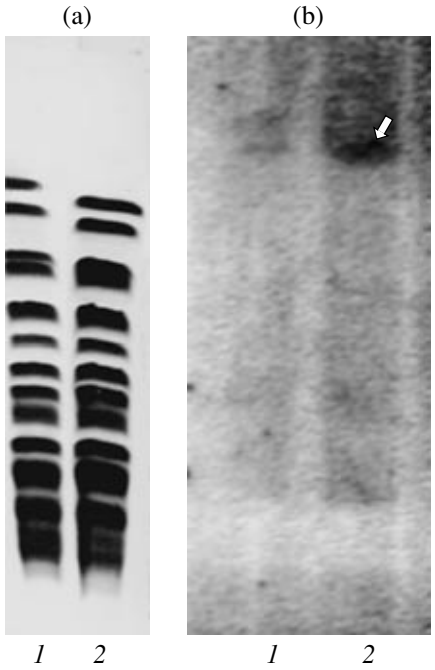

Fig. 7. (a) *Xba*I restriction patterns of chromosomal DNA from *A. ferrooxidans* TFL-2 adapted to (*1*) pyrite and (*2*) concentrate. (b) Autoradiograph of *Xba*I-generated fragments of chromosomal DNA from *A. ferrooxidans* TFL-2 hybridized with plasmid pTFK2 DNA from strain TFBk.

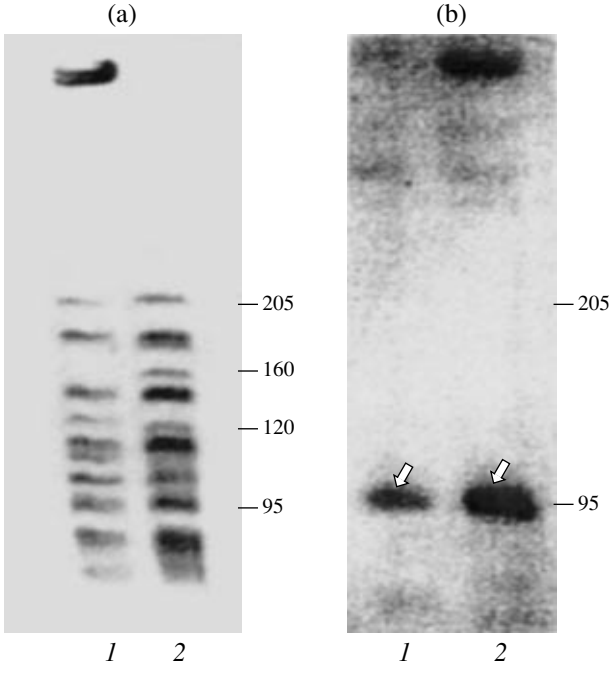

Fig. 8. (a) *Xba*I restriction patterns of chromosomal DNA from *A. ferrooxidans* strains (*1*) TFO and (*2*) TFN-d adapted to pyrite. (b) Autoradiograph of *Xba*I-generated fragments of chromosomal DNA from *A. ferrooxidans* strains (*1*) TFO and (*2*) TFN-d hybridized with plasmid pTFK2 DNA from strain TFBk.

only after adaptation of these strains to FeS_2. Plasmid pTFK2 and two plasmids of the strains TFO and TFN-d contained homologous sequences (Fig. 8b, lanes *1*, *2*). These data, as well as strong hybridization with one of the chromosomal DNA fragments after the adaptation to FeS_2, indicate that plasmid–chromosomal interactions in the process of adaptation of *A. ferrooxidans* to new energy substrates might take place. Strong hybridization could result from integration of the plasmid DNA into the chromosome.

Integration of the plasmid DNA into the chromosome is not only one of the ways of adaptation of *A. ferrooxidans* to changing environmental conditions [11, this paper]. In case of irreversibility, this process may be a mechanism of intraspecific variability, which is a basis of microevolution and strain genotypic polymorphism.

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