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REVIEW

Diversity of the Communities of Acidophilic Chemolithotrophic Microorganisms in Natural and Technogenic Ecosystems

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Abstract—The main representatives of acidophilic chemolithotrophs oxidizing sulfide minerals, ferrous iron, elemental sulfur, and reduced sulfur compounds and forming microbial communities in the natural and technogenic ecosystems with low pH values and high concentrations of heavy metal ions are listed. The species and strain diversity of the communities and environmental factors affecting their composition (temperature, pH value, energy substrate, mineralogical composition of sulfide ore concentrates, the presence of organic substances, and level of aeration) are analyzed. Involvement of mobile genetic elements (IS elements and plasmids) in the structural changes of the chromosomal DNA in the course of switching microbial metabolism to the oxidation of new energy substrates or under increased concentrations of metal ions is shown to be a probable mechanism responsible for the intraspecific genetic heterogeneity of the populations. Importance of determination of the dominant strains of different microbial species in the communities and of their physiological peculiarities for stabilization, optimization, and enhancement of efficiency of biotechnological processes for sulfide mineral oxidation is stressed.

Keywords: acidophilic chemolithotrophs, microbial communities, temperature, energy substrate, aeration level, technological conditions, chromosomal DNA structure, IS elements, plasmids, genetic intraspecific heterogeneity.

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ACIDOPHILIC CHEMOLITHOTROPHIC MICROORGANISMS

The communities of acidophilic chemolithotrophic microorganisms (ACM) are formed in sulfide ore deposits, mine waters, pyrite coals, the wastes of ore mining and processing industry in different geographical and climatic zones, as well as in the pulps of reactors for the technologies of bacterial–chemical oxidation of sulfide ore concentrates, i.e., in the presence of sulfide minerals and their oxidation products, elemental sulfur, reduced sulfur compounds, and ferrous iron. As a result of their activity, the medium is acidified, sometimes to pH 0.5 or less, and the concentrations of heavy metals and toxic elements (copper, zinc, nickel, cobalt, arsenic, etc.) increase. These microorganisms therefore belong to extreme chemolithotrophic acidophiles. They include representatives of bacteria (both gram-negative and gram-positive) and archaea.

Tables 1–3 present the best-studied and most frequently occurring ACM, the substrates they oxidize, and the ranges and optimal values of pH and temperature for their growth [1–38]. It can be seen that some microorganisms can oxidize all inorganic substrates mentioned above, e.g., gram-negative bacteria of the genus *Acidithiobacillus* (*At. ferrooxidans, At. ferrivorans*), gram-positive bacteria of the genus *Sulfobacillus* (*S. thermosulfidooxidans, S. thermotolerans, S. benefaciens*), and the archaeon *Acidianus* (*Ac.*) *brierleyi*. Ferrous iron, elemental sulfur and sulfide minerals are used as energy sources by *Metallosphaera sedula* and *M. prunae*. Other microorganisms can obtain energy by oxidizing only ferrous iron or pyrite: *Leptospirillum ferrooxidans, L. ferriphilum,* and *Ferroplasma acidiphilum*. Most of the microorganisms (*At. thiooxidans, At. caldus*, and archaea) use elemental sulfur and reduced sulfur compounds as an energy substrate. The pH optima for the growth of these microorganisms are different, but all of them are in the range of acidic values. ACM live in a broad temperature range of 4 to 90°C. They include mesophiles with the optimal temperature for growth and oxidation of the energy substrate in the range of 28–35°C, moderate thermophiles with the temperature optimum at 40 to 55°C, and thermophiles with the temperature optimum above 60°C. The latter include mainly archaea. For instance, *Ac. infernus* has a temperature optimum for growth at 90°C [20].

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Table 1. Diversity of acidophilic chemolithotrophic gram-negative bacteria

Microorganisms	Inorganic energy sources	Optimal pH and temperature values, °C/limits of activity	References
At. ferrooxidans	Fe^{2+}, S^0 (S^{2-}), sulfide minerals	pH 1.7–2.5/1.0–4.5; *T*°C 28–30/2–37	[1, 2]
At. ferrivorans	Fe^{2+}, S^0 (S^{2-}), sulfide minerals	pH 2.5/1.9–3.4; *T*°C 28–33/4–37	[3]
At. thiooxidans	S^0 (S^{2-})	pH 2.0–3.0/0.5–5.5; *T*°C 28–30/2–37	[2, 4]
At. caldus	S^0 (S^{2-})	pH 2.0–2.5/1.0–3.5; *T*°C 45/32–52	[2, 5]
L. ferrooxidans	Fe^{2+}, FeS_2	pH 1.8–2.2/1.5–5.0; *T*°C 30–45/2–50	[6]
L. thermoferrooxidans	Fe^{2+}	pH 1.65–1.9/1.3–4.0; *T*°C 45–50/30–60	[7, 8]
L. ferriphilum	Fe^{2+}, FeS_2	pH 1.4–1.8; *T*°C 30–37	[9]
L. ferrodiazotrophum	Fe^{2+}	pH 1.2; *T*°C 37	[10]

Table 2. Diversity of acidophilic chemolithotrophic gram-positive bacteria

Microorganisms	Inorganic energy sources	Optimal pH and temperature values, °C/limits of activity	References
S. thermosulfidooxidans	Fe^{2+}, S^0 (S^{2-}), sulfide minerals	pH 1.7–2.4/1.5–5.5; *T*°C 50–55/20–60	[11]
S. acidophilus	Fe^{2+}, S^0, sulfide minerals	pH 2.0; *T*°C 45–50	[12]
S. sibiricus	Fe^{2+}, S^0, sulfide minerals	pH 2.0/1.1–2.6; *T*°C 55/17–60	[13]
S. thermotolerans	Fe^{2+}, S^0, $S_4O_6^{2-}$, sulfide minerals	pH 2.0/1.2–2.0; *T*°C 40/20–60	[14]
S. olympiadicus	Fe^{2+}, S^0, sulfide minerals	pH 1.9/0.9–2.2; *T*°C 42–45/15–55	[15]
S. benefaciens	Fe^{2+}, S^0, $S_4O_6^{2-}$, sulfide minerals	pH 1.5/0.8–2.2; *T*°C 38.5/30–47	[16]
Alicyclobacillus disulfidooxidans	Fe^{2+}, S^0, $S_2O_3^{2-}$, FeS_2	pH 1.5–2.5/0.5–6.0; *T*°C 35/4–40	[17, 18]
Al. tolerans	Fe^{2+}, S^0, sulfide minerals	pH 2.0–2.7/1.5–5.0; *T*°C 28–42/20–55	[18, 19]

THE SPECIES AND STRAIN DIVERSITY OF ACIDOPHILIC CHEMOLITHOTROPHIC MICROORGANISMS

In the 21st century, the attention of ACM researchers has shifted from isolation and description of pure cultures and investigation of the peculiarities of their physiology to the isolation of microbial communities from natural and technogenic ecosystems and investigation of their species composition. These microorganisms are involved either in the competition for common substrates or in cooperation in utilization of the latter. It is manifested by successive degradation of a complex substrate such as sulfide minerals, where each component of the medium which is formed as a result of oxidation is utilized by certain microorganisms, and the products of their metabolism or cell lysis, in turn, are utilized by other microorganisms. For example, the ACM community includes not only autotrophic and mixotrophic microorganisms, but also acidophilic heterotrophs (fungi and bacteria). Autotrophs provide organic substances for mixotrophs and heterotrophs.

ACM communities from different natural and technogenic ecosystems vary in the species composition of constituent microorganisms (Tables 4, 5) [39–68]. Tables 4 and 5 generalize the results of investigation of the compositions of microbial communities isolated from habitats with low pH values: the Tinto River, mine waters, sulfide ores, and from the technological processes of sulfide ore biooxidation. Mesophilic communities are the richest in species composition. High temperatures limit the range of microorganisms that form a community. At above 70°C, inorganic substrates are oxidized at low pH values only by archaea.

Research results (Tables 4 and 5) show that the communities of acidophilic chemolithotrophic microorganisms detected in different habitats by cultural or molecular biological methods vary in the species composition. The representatives of mesophilic ACM most frequently occurring in acidic waters, sulfide ore fields, or coals belong to the genera *Acidithiobacillus* and *Leptospirillum*, to acidophilic heterotrophs *Acidiphilium, Acidocella, Acidospora,* and the *Ferroplasma* archaea [39–53]. In biogeotechnological processes, the associations of acidophilic chemolithotrophic microorganisms have different predominant species depending on the characteristics of the

Table 3. Diversity of acidophilic chemolithotrophic archaea

Microorganisms	Inorganic energy sources	Optimal pH and temperature values, °C/limits of activity	References
Acidianus brierleyi	Fe^{2+}, S^0 (S^{2-}), sulfide minerals	pH 1.5–2.0/1.0–6.0; *T*°C 70/45–76	[20]
Ac. infernus	S^0	pH 1.5–2.0/1.0–5.5; *T*°C 90/65–96	[20]
Ac. ambivalens	S^0	pH 2.5/1.0–3.5; *T*°C 80/55–87	[21, 22]
Ac. tengchongensis	S^0	pH 2.5/1.5–5.0; *T*°C 70/55–80	[23]
Ac. manzaensis	S^0	pH 1.2–1.5/1.0–5.0; *T*°C 80/60–90	[24]
Metallosphaera sedula	Fe^{2+}, S^0, sulfide minerals	pH 1.5–2.0; *T*°C 50–80	[25]
M. prunae	Fe^{2+}, S^0, sulfide minerals	pH 3.0/1.0–5.0; *T*°C 70/55–80	[26]
M. hakonensis	S^0, (S^{2-}), $S_4O_6^{2-}$	pH 3.0/1.0–4.0; *T*°C 70/50–80	[27, 28]
Sulfolobus acidocaldarius	S^0, (S^{2-}), $S_4O_6^{2-}$	pH 2.5/2.0–4.0; *T*°C 70/55–85	[29]
Sul. solfataricus	S^0, (S^{2-}), $S_4O_6^{2-}$	pH 4.5/3.5–5.0; *T*°C 70/55–85	[30]
Sul. shibatae	S^0	pH 3.0; *T*°C 81	[31]
Sul. metallicus	S^0	pH 3.0/1.0–3.5; *T*°C 65/50–75	[32]
Sul. yangmingensis	S^0, (S^{2-}), $S_4O_6^{2-}$	pH 4.0/2.0–6.0; *T*°C 80/65–90	[33]
Sul. tokodaii	S^0	pH 2.5–3.0/2.0–5.0; *T*°C 80/70–85	[34]
Sul. tengchongensis	S^0	pH 3.5/2.5–4.5; *T*°C 85–90/65–95	[35]
Ferroplasma acidiphilum	Fe^{2+}, FeS_2	pH 1.7–1.8/1.3–2.2; *T*°C 35/15–45	[36]
F. acidarmanus	Fe^{2+}	pH 1.2/0–1.5; *T*°C 42/23–46	[37]
F. cupricumulans	Fe^{2+}	pH 1.0–1.2; *T*°C 53/22–63	[38]

oxidized substrate and temperature conditions [54–68]. For instance, *L. ferrooxidans* was dominant during continuous leaching of the zinc ore concentrate [54], while *L. ferriphilum* and *At. caldus* predominated during the leaching of the cobalt–iron concentrate [55]. The archaea *Ac. brierleyi* and *Metallosphaera sedula* predominated in the community of microorganisms during bioleaching of the chalcopyrite-containing concentrate at 70°C [57]. *At. caldus* strains and the novel archaeal species '*Ferroplasma cyprexacervatum*' were isolated during heap leaching of chalcocite from the samples of solid and liquid phases [62].

The species composition and cell number in the communities of microorganisms oxidizing the concentrates of gold–arsenic ores of different mineralogical composition from different geographical and climatic zones were different (Tables 6, 7) [69]. Thus, the communities of microorganisms involved in the two technological processes with ore concentrates from the Albazinskoye and Kokpatasskoye ore deposits were not shown to contain leptospirilla, while archaea were not found in the processes with ore concentrates from the Natalkinskoye and Maiskoe deposits. Other microorganisms in each process showed similar species composition but different quantitative ratios.

However, microbial communities varied not only in the species composition. The same species of microorganisms in the communities isolated from different natural and technogenic ecosystems were represented by different strains [70–78]. This conclusion was a result of using the method of strain identification of ACM based on the analysis of the structure of their chromosomal DNA cleaved by restriction endonucleases. The same species of microorganisms isolated from different sulfide ores varied in the structure of chromosomal DNA, indicating that they belonged to different strains. At the same time, several strains of the same species could be present within a community. For instance, two strains of *F. acidiphilum*, Y-9 and Y-10, were isolated from the ACM community from the pulp of bioreactors during bacterial–chemical oxidation of the floatation ore concentrate from the Olympiadinskoe deposit, illustrating strain heterogeneity of a species in the same microbial community.

In our studies we have applied, for the first time, the comparative approach to investigation of the species and strain diversity of the communities of acidophilic chemolithotrophic microorganisms, depending on the geographical and climatic conditions of sulfide ore deposits, peculiarities of their mineralogical composition, and the composition of concentrates in the biohydrometallurgic technologies, as well as temperature conditions of the processes of bacterial–chemical oxidation of sulfide mineral concentrates, ratio of various

Table 4. Species composition of the communities of acidophilic chemolithotrophic microorganisms in natural ecosystems

Ecosystem	Isolation site	Species composition	Literature cited
The Tinto River, Spain	The Tinto River, pH 2.3, high concentration of heavy metal ions (Fe up to 20 g/L, Cu up to 0.7 g/L, Zn up to 0.56 g/L, As and Cr)	The cultural and molecular biological (DGGE, FISH, phylogenetic analysis) methods of investigation revealed members of the genera *Acidithiobacillus, Leptospirillum, Acidiphilum, Ferrimicrobium*, and of the two archaeal genera: *Ferroplasma* and *Thermoplasma.* The three dominant species were: *At. ferrooxidans, L. ferrooxidans* and *Acidiphilium* spp. *(Ac. organovorum, Ac. cryptum*, and *Ac. multivorum*). Other microorganisms such as *At. thiooxidans, L. ferriphilum, 'Ferrimicrobium acidiphilum', Desulfosporosinus* spp. or archaea *F. acidiphilum* and *Thermoplasma acidiophilum* were present in minor quantities.	[39–41]
	Anoxic sediments of the Tinto River	*At. ferrooxidans, L. ferrooxidans*, and heterotrophic bacteria of the genera *Acidiphilium, Acidocella* and *Acidosphaera* were identified in most of the samples. Some samples were shown to contain representatives of the β-*Proteobacteria*. The clones close to the γ-*Proteobacteria* were detected at most of the observation stations. High level of diversity was noted within the phylum *Actinobacteria*, where several clones were clustered in the genus *Ferrimicrobium* but other clones could represent a novel actinobacterial genus. Within the phylum *Firmicutes*, several clones belonged to the genera *Desulfosporosinus* and *Clostridium*. All archaeal sequences were identified as members of the class *Thermiplasmata*.	[42]
Acidic mine waters and biofilms	Drainage waters of outcrops and undeveloped underground copper ore, North Wales, England	In some sampling sites, iron-oxidizing microbes were predominant; in other sites, iron oxidizers and heterotrophic acidophiles were present in similar amounts. The β-*Proteobacteria* constituted 80–90% of the total number. The next largest group of bacteria was γ-*Proteobacteria*, with predominance of *At. ferrooxidans* (25–90%). "*Ferrimicrobium*"-like bacteria were detected.	[43]
	Mine waters and biofilms from three different sites of the deposit of undeveloped pyrite ore, North Wales, England	*At. ferrooxidans, Acidocella* ssp., *Acidiphilium* and *Sphingomonas* sp. were isolated from the biofilms on solid media. However, culture-independent studies (construction and analysis of clone libraries, FISH) revealed a distinctly different composition. The dominant microbes in most of the studied films were nonculturable β-*Proteobacteria*; α-, β- and γ-*Proteobacteria, Actinobacteria, Acidobacteria*, and *Nitrospira*. *Bacilli* were detected as well, though in lesser numbers. *At. ferrooxidans* and *Actinobacteria* dominated in the mine water samples. Archaea were detected in the ore samples.	[44]
	Acidic mine waters of two different copper deposits in Zhong Tiaoshan, Shanxi Province (China)	Phylogenetic analysis showed that the bacteria from five samples fell into four major groups: *Proteobacteria* (α-, β-, γ-, and δ-proteobacteria), *Nitrospira* (*Leptospirillum*), *Firmicutes*, and *Bacteroidetes*. Proteobacteria such as *At. ferrooxidans* and *At. albertensis* were dominant in some samples and *Nitrospira* such as *L. ferrooxidans* and *L. ferriphilum* were dominant in other samples. Firmicutes were found in two samples. *Bacteroidetes* were found in one sample. Archaea were found in two samples. Representatives of *Thermoplasma* and *Ferroplasma*, as well as *Sulfolobales* and microorganisms of the genus *Methanothermus* in minor quantities, were also detected in the latter samples	[45]

Table 4. (Contd.)

Ecosystem	Isolation site	Species composition	Literature cited
	Acidic mine drainage (pH 1.8, high concentration of ferrous iron, 301 mg/L) during the oxidation of abandoned sulfur ore (Japan)	Broad diversity of bacterial species was shown even under conditions of very high acidity. *'Ferribacter polymyxa'* was found. The obligate heterotrophic bacterium *Burkhol(i)deria cepacita* was predominant. *Chitinophaga* of the phylum *Bacteroidetes* was identified. Representatives of *Actinobacteria* and *Proteobacteria* were revealed. With due time, facultative chemolithotrophs were replaced by heterotrophs.	[46]
	Two samples of acidic drainage waters from Yunfu sulfide ore in the Guangdong province (China)	Phylogenetic analysis showed that bacteria fell into four tentative groups: *Nitrospira*, α-*Proteobacteria*, β-*Proteobacteria*, and 4 families of γ-*Proteobacteria*. Representatives of the genera *Acidithiobacillus* and *Gallionella* predominated in two samples.	[47]
	Acidic mine waters of different origin	Phylogenetic and physiological diversity of acidophilic bacteria in acidic mine waters was represented by: *At. ferrooxidans, At. ferrivorans, Leptospirillum* spp., *Acidiphilium* spp., *Acidocella* spp., *Ferrimicrobium acidiphilum, Acidimicrobium ferrooxidans, 'Ferrovum myxofaciens', Thiomonas* sp., *Halothiobacillus* sp., *Acidobacterium*-like spp., and *Ferroplasma* spp. Acidobacteriaceae were found in the sedimentary materials of acidic mine waters.	[48]
	Acidic drainage waters at the abandoned pyrite deposit Ouro Preto and in the experimental system of nickel sulfide bioleaching (Brazil)	*At. ferrooxidans* was not detected in the samples of acidic drainage waters, possibly due to the relatively high pH value when iron salts precipitate, probably together with the adsorbed iron-oxidizing microorganisms. However, the presence of *At. thiooxidans* was confirmed. Some *Burkholderia* species were found. *At. ferrooxidans* was also present in bioleaching tanks.	[49]

Table 4. (Contd.)

Ecosystem	Isolation site	Species composition	Literature cited
	Hot streams along the Rio Agrio belonging to the Copahue volcano system in Neuquén. This extreme environment is characterized by a broad range of temperatures, pH range of 1 to 8, and high concentration of heavy metals (Argentine)	Chemolithoautotrophic bacteria, archaea, heterotrophic bacteria, yeasts, and mycelial fungi were detected. *L. ferrooxidans, At. ferrooxidans, At. thiooxidans*, and *Acidianus* species were isolated in pure culture.	[50]
Ores and coal fields	Sulfide ores, Malanjkhand copper ore (pH value in different samples varied from 3.3 to 8.0) (India)	Heterotrophic gram-negative bacteria growing on the medium with sodium thiosulfate with glucose or yeast extract, acid-tolerant heterotrophs capable of growing on reduced sulfur compounds, and autotrophic sulfur and iron oxidizers were detected. *At. ferrooxidans, At. thiooxidans, Thiobacillus thioparus, T. versutus*, and *T. intermedius* were identified based on their morphological, biochemical and physiological characteristics. In different sites, heterotrophic and autotrophic iron-oxidizing acidophilic bacteria were present in different proportions.	[51]
	Jaduguda uranium ores (India)	Five soil samples were shown to contain representatives of the *Proteobacteria, Acidobacteria, Bacteroidetes, Firmicutes, Cyanobacteria* as the major phyla. About 46% of the sequenced clones were identified as *Proteobacteria*. About 20% of clone sequences showed low affinity to the known taxa and are likely to be novel organisms adapted to this habitat.	[52]
	Jaenschwalde, the largest European brown coal field, 150 km to the south from Berlin (Germany). In this region there are acidic lakes as a result of marcasite (FeS_2) oxidation	Thirty-two samples were analyzed. *At. ferrooxidans* and *At. thiooxidans* were identified in 23 and 10 samples, respectively. The *L. ferrooxidans* signal was received from 9 samples. The PCR genes of archaeal 16S rRNA were amplified in 4 samples. The sequencing of two samples revealed their identity to the unidentified and unculturable archaea found in the NCBI database. These data show that iron- and sulfur-oxidizing microorganisms are abundant in these sites.	[53]

Table 5. Species composition of the communities of acidophilic chemolithotrophic microorganisms in technogenic ecosystems

Ecosystem	Isolation site	Species composition	Literature cited
Biogeotechnological processes. Tank leaching	Laboratory reactor (1–4 l), continuous cultivation bioreactors used for commercial bioleaching of zinc ore concentrate	*At. ferrooxidans, At. thiooxidans, 'L. ferrooxidans'*, and *Acidiphilium cryptum* strains were identified in the natural sites and in batch cultivation bioreactors. Only *'L. ferrooxidans'*, a moderately thermophilic strain of *At. thiooxidans*, and a moderately thermophilic iron-oxidizing bacterium were isolated from the process of continuous cultivation.	[54]
	Continuous bioleaching of cobalt- and iron-containing concentrate in four reactors	*L. ferriphilum* and *At. caldus* were predominant. The former one predominated in the beginning of the process, while the latter increased its population density in the course of the process. Other microorganisms were detected in lesser quantities. These were *Sulfobacillus* and *Ferroplasma*.	[55]
	Copper leaching from pyrite-rich ores	The changes in bacterial community were analyzed in the course of pyrite bioleaching under continuous or periodic aeration at 30°C. Phylogenetic analysis of the 16S rRNA gene fragments showed that most of the sequences belonged to representatives of the genera *Leptospirillum, Sulfobacillus*, and *Acidithiobacillus.* The rest of the clones were clustered together with the genera *Afipia* and *Sporacetigenium*.	[56]
	Bioleaching of chalcopyrite-containing concentrate from the Aguablanca Ni–Cu ore under the conditions of continuous cultivation in multistage reactors at 70°C (Spain) and mixed Cu concentrate from the Bor ore (Serbia)	T-RFLP analysis showed the presence of only one archaeon. The communities from the Aguablanca and Bor systems consisted of two different microorganisms. The predominant archaeon (up to 98% of the total number of detected archaea) was *Acidianus brierleyi* and the other one was *Metallosphaera sedula.* A minor quantity of *Sulfolobus* spp. was detected.	[57]
	Bioleaching of cobalt–iron pyrite by two microbial communities	The first community of microorganisms was represented by an indigenous population from commercial bioreactors used for the processing of cobalt–iron pyrite Kasese Cobalt. The second one was comprised of four organisms dominating in the indigenous population. In the beginning of the process, three major bacterial strains were detected in nearly equal quantities. However, at the end of the process the population was almost completely dominated by *L. ferriphilum* with a minor quantity of *S. benefaciens.* On the contrary, *At. caldus* was not detected after 19 hours. The results showed that the second community did not leach the same amount of cobalt from the mineral as the already adapted indigenous community.	[58]
	Bioleaching at 60°C	According to RFLP, none of the strains found in the course of bioleaching corresponded to the inoculum strains. Four representatives of the two major clonal groups of bacteria had a 96–100% homology with the *L. ferriphilum* isolate but showed no common restriction samples with the inoculum *L. ferriphilum.* Non-inoculum populations of microorganisms developed in the liquid phase. The source of these organisms was ore. Microorganisms with the maximum growth efficiency under these conditions were selected during the process.	[59]

Table 5. (Contd.)

Ecosystem	Isolation site	Species composition	Literature cited
Biogeotechnological processes. Heap leaching	Bioleaching of low-grade copper sulfide ore Escondida (Chile) in an industrial heap	Phylogenetic analysis of the sequences from genetic libraries showed that the community consisted mostly of microorganisms belonging to *At. ferrooxidans* (two strains), *At. thiooxidans, L. ferrooxidans, L. ferriphilum*, and the *Ferroplasma* archaea.	[60]
	Slag tailings from the primary copper ore Sáo Domingos containing various heavy metals: copper, zinc, lead, antimony, silver, mercury, and cadmium (Portugal)	The population of indigenous acidophiles was restricted to gram-negative sulfur-oxidizing and gram-positive sulfur/iron-oxidizing bacteria. The study of the microbial population before and after bioleaching confirmed the presence of *At. thiooxidans.* Though other bacteria were identified as firmicutes, strain identity varied when using different methods of analysis.	[61]
	Heap leaching of chalcocite MICCL Monywa	Enrichment cultures were obtained from the samples of solid and liquid phases of the heap and 6 strains were isolated (BH1–BH6). Phylogenetic analysis of the 16S rRNA genes showed that the strains BH3 and BH4 were close to the acidophilic bacterium *At. caldus*, while the strains HB5 and HB6 were similar to *L. ferriphilum.* A novel archaeal species *'Ferroplasma cyprexacervatum'* was isolated. The species was phylogenetically closest to *F. acidiphilum* with a sequence similarity of 95%.	[62]
	Mineral heaps and ore waters of different age in actively developed and abandoned copper ores: (1) 11-year old leaching heap of Bingham Canyon mine, Salt Lake City (United States); (2) two abandoned mineral heaps piled 50 year ago in Sáo Domingos mine in the south of Portugal; and (3) well-weathered barren rock piled 120 years ago in the Mynydd Parys ore, North Wales (England)	Extremely acidophilic mineral-oxidizing *Firmicutes* were predominant in the first two sampling sites. The population of the Sáo Domingos heap was very simple, while in the bioleached heap it was compositionally diverse. The barren rock from Mynydd Parys was much more weathered and therefore less extreme by the conditions for microorganisms. The populations were dominated by moderately acidophilic heterotrophic γ-*Proteobacteria*, though the total microbial population was dominated by unculturable *Actinobacteria* and unclassified *Bacteria.* Biodiversity was associated not with age but rather with the pH of the heap and the concentration of easily extracted metals. Since the content of unoxidized sulfide minerals decreased with the time of heap piling, microbial population became less dependent on mineral oxidation and more heterotrophic.	[63]
	Generalization of the results of analysis of microbiological communities in 30 sulfide ore heaps	Molecular biological studies showed that bacteria predominated over archaea and eukaryotes and that the genus *Acithiobacillus* was most abundant in the processes of Fe^{2+} oxidation. Anaerobes such as sulfate reducers or Fe^{3+}- and Mn^{4+}-reducing *Geobacteraceae* were presented in lesser quantities in all sites.	[64]

Table 5. (Contd.)

Ecosystem	Isolation site	Species composition	Literature cited
	Sulfide ore waste heaps (Germany)	Most of the cultivated iron oxidizers belonged to 4 genera: *Acidithiobacillus, Acidimicrobium, 'Ferrimicrobium'* and *Leptospirillum*. All of the analyzed *Acidithiobacillus* strains were identified as *At. ferrooxidans*. The strains of leptospirilla were of the species *L. ferriphilum* or *L. ferrooxidans*. Gram-positive strains represented by the genera *Acidimicrobium* or *'Ferrimicrobium'* were more diverse phylogenetically than strains of the genera *Acidithiobacillus* and *Leptospirillum* and were grouped into three separate clusters. Whereas several strains could be identified as related (by 16S rDNA) to *'Ferrimicrobium acidiphilum'*, the other two 16S rDNA clusters were weakly connected and could be attributed to the novel species and even genera. In addition, a few strains close to *Acidiphilium acidophilum* were detected and formed a single 16S rDNA cluster.	[65]
	Leaching solution from the heap of low-grade copper ore Escondida (Chili)	Six bacteria and one archaeon were isolated on a solid medium and identified on the basis of phylogenetic analysis of the 16S rRNA genes as the strains of *At. ferrooxidans, At. thiooxidans, L. ferriphilum, Acidiphilium cryptum*, and *F. acidiphilum*. The 16S rRNA gene sequences from the isolated strains showed high similarity to those detected previously by culture-independent analysis of the samples obtained from the pilot plant of this process. Out of the three known species of leptospirilla, only *L. ferriphilum* was detected.	[66]
Biotechnological processes. Column leaching	The ore of black crystalline schists rich in the sulfide minerals containing nickel, copper, zinc, and cobalt	Only 1% of the cells grew from the column material on a solid medium. Iron-oxidizing acidophiles (*At. ferrooxidans*) were isolated from the cultivated cells; their number exceeded the numbers of other acidophiles tens of times. The changes in the population were monitored by molecular techniques (T-RFLP and SSCP). *At. ferrooxidans* were predominant in these experiments as well. As a result of temperature increase in the column from 20 to 35°C by heating up the recycling fluid or air, the content of *At. ferrooxidans*-like cells decreased and the number of unidentified bacteria increased. Total cell number varied insignificantly under temperature increase.	[67]
	Bacterial populations from solutions and minerals in the column and from heap process of bioleaching of low-grade ore Escondida (Chili, Spain)	Microorganisms in solutions and minerals from the experimental column and in the two samples taken from the industrial heap of Escondida copper ore in Chili were compared. The bacterial population was analyzed by Real-Time PCR and CARD-FISH techniques. Cell density was slightly higher for the mineral than for the solution when total cell numbers were counted. The predominant microorganisms in both types of the samples were *At. thiooxidans, At. ferrooxidans* D2, and *L. ferriphilum*. In the samples from the industrial heap, *At. ferrooxidans* and *At. thiooxidans* were predominant at the beginning and at the end of the leaching cycle, respectively.	[68]

Table 6. Mineralogical compositions of the concentrates

Type of concentrate	Ore deposit	Mineralogical composition
Pyrite–arsenopyrite	Nezhdaninskoe, eastern Yakutiya	FeS_2, FeAsS, ZnS, $CuFeS_2$, PbS
Arsenopyrite–pyrite	Albazinskoe, Khabarovsk krai	FeAsS, FeS_2
Arsenopyrite-pyrite	Kokpatasskoe, Central Kyzylkum district	FeS_2, $CuFeS_2$, ZnS, PbS, FeAsS, Sb_2S_3
Arsenopyrite–pyrite	Natalkinskoe (Matrosovsky mine), Magadan region	FeAsS, FeS_2, ZnS, FeS, $CuFeS_2$
Pyrite–arsenopyrite	Maiskoe, Chaunsky district of the North–East	FeS_2, FeAsS, Sb_2S_3
Pyrrhotite-containing pyrite–arsenopyrite	Olympiadinskoe, Krasnoyarsk krai	FeS_2, FeAsS, FeS, Sb_2S_3, PbS, ZnS, $CuFeS_2$
Pyrite–arsenopyrite	Kyuchusskoe, the north of the Verkhoyansk district of the Republic of Sakha, Yakutiya	FeS_2, FeAsS, Sb_2S_3
Copper–nickel	Shanchukskoe, the Kamchatka Peninsula	FeS, $(Fe, Ni)_9S_8$,$CuFeS_2$, FeS, $(Ni, Fe)S_2$

Table 7. Species composition and number of cells/mL in the communities of microorganisms during bacterial–chemical oxidation of different types of concentrates of gold–arsenic ores under mesophilic conditions

Ore deposit	Cell number, cells/mL				
	At. ferrooxidans, strain	*At. thiooxidans*, strain	*L. ferrooxidans*, strain	*Sulfobacillus* sp., strain	*F. acidiphilum*, strain
Nezhdaninskoe	TFN-d, 2.5×10^7	TTN-d, 2.5×10^4	LFN-d, 1.0×10^5	10^6	Y-7, 10^5
Albazinskoe	TFAl, 2.5×10^9	TTAl, 6.0×10^5	n.d.	10^4–10^5	Y-5, 10^6–10^7
Kokpatasskoe	TFKpt, 1.8×10^9	TTKpt, 5.5×10^7	n.d.	10^4–10^5	Y-6, 6.0×10^5
Natalkinskoe	TFMt, 2.5×10^6	TTMt, 2.5×10^3	LFMt, 1.0×10^5	10^5	n.d.
Maiskoe	TFM, 2.5×10^7	TTM, 6.0×10^9	LFM, 0.4×10^5	1.3×10^6	n.d.
Olympiadinskoe	TFO-2, 2.5×10^8	TTO, 2.5×10^9	LFO, 10^5–10^6	10^7–10^8	Y-2, Y-4, 10^5

Note: n.d., not determined.

chemical elements in the concentrates, pulp density, aeration conditions, the presence of organic substances, and technological peculiarities. We have also analyzed the changes in the species and strain compositions of microbial communities at different stages of oxidation of sulfide mineral concentrates.

Stable trophic relations are formed in microbial communities during the oxidation of sulfide minerals. Some microorganisms predominate in the beginning of this process and, as a result, ferrous iron and S^{2-} are released into the liquid phase. Other microorganisms actively oxidize ferrous iron or reduced sulfur compounds. At the end of the process, S^0 and its reduced compounds are oxidized to SO_4^{2-}, Fe^{2+} is oxidized to Fe^{3+}, and insoluble iron and arsenic compounds are formed.

Predominance of different *Acidithiobacillus* species in the community was observed in the course of oxidation of pyrrhotite-containing pyrite–arsenopyrite concentrate under mesophilic conditions in a pilot plant at the gold-processing factory of the Polyus Gold Mining Company. *At. ferrooxidans* predominated in the first reactor during the oxidation of sulfide minerals and ferrous iron (Fig. 1) [79]. On accumulation of elemental sulfur and reduced sulfur compounds in the liquid phase of the second reactor, the predominance in the microbial community passed on to *At. thiooxidans*.

The species ratio of the microbial community changed when the iron content in the incoming concentrate for bacterial–chemical oxidation decreased from 32.5 to 12 g/L (Fig. 2). The quantity of leptospirilla decreased most drastically. Archaea were most resistant to decrease in the iron content. Increase of

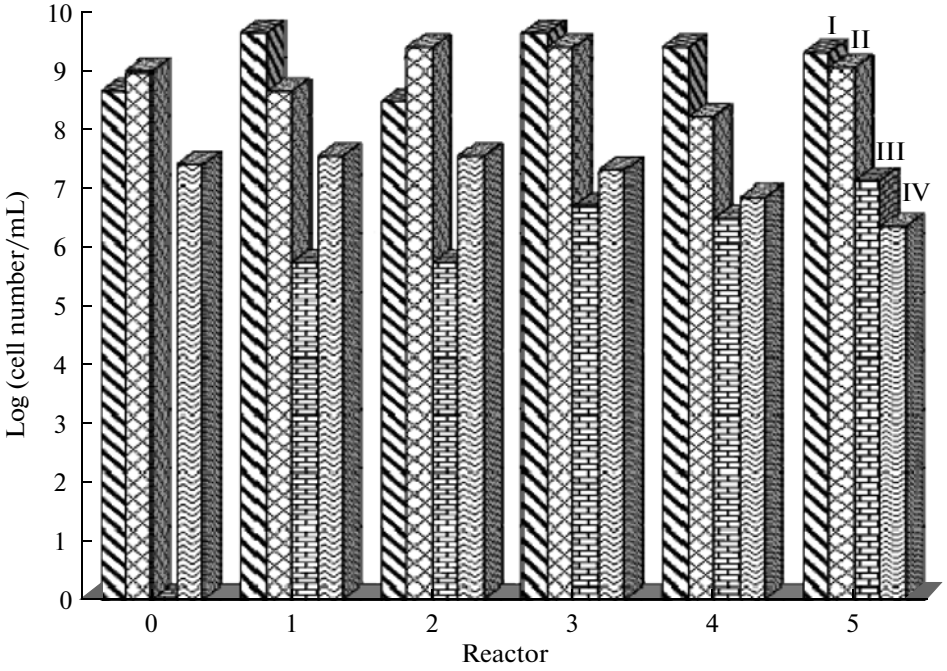

Fig. 1. Dynamics of the numbers of ACM cells in the pulp from reactor sequences of the Olympiadinskaya gold-mining factory: *At. ferrooxidans* (I), *At. thiooxidans* (II), *L. ferrooxidans* (III), and *Sulfobacillus* sp. (IV).

the iron content in the concentrate resulted in increased total numbers of microorganisms, including the relative abundance of leptospirilla.

In different years of the monitoring of the composition of ACM communities in reactor pulp during oxidation of the floatation concentrates of gold–arsenic ore (2005–2007), the dominant strains and species of sulfobacilli were replaced by other strains and species: '*S. olympiadicus'* S-5 ⟶ '*S. olympiadicus*' Ol-6 ⟶ *S. thermosulfidooxidans* Ol-7.

Substitution of predominant microorganisms was demonstrated in the course of oxidation of the high-antimony ore concentrate at 46°C [76]. The strain Sb-K with the high rates of growth and oxidation of the initial substrate was dominant in the first reactor, the strain Sb-F with the high substrate specificity to Fe^{2+} predominated in the second reactor, and the strain Sb-S actively oxidizing reduced sulfur compounds predominated in the third reactor. All three strains could oxidize elemental sulfur, ferrous iron, and sulfide minerals, including antimonite, in the presence of 0.02% yeast extract. The strains differed in the structure of chromosomal DNA analyzed by the method of pulsed-field gel electrophoresis. The isolates were similar to sulfobacilli in their morphological properties and mixotrophic metabolism. The study of the phenotypic properties of the strains Sb-K, Sb-F and Sb-S showed their differentiating characteristics: capacity for lithotrophic, organotrophic, and mixotrophic growth, as well as the optimal and ultimate pH and temperature values. Based on DNA–DNA hybridization data, the 16S rRNA gene sequence analysis and phenotypic characteristics, the Sb-K, Sb-F and Sb-S isolates were identified as novel strains of the species *S. thermotolerans, S. sibiricus*, and *S. thermosulfidooxidans*.

This work showed that microbial communities in the technological process of biooxidation of sulfide minerals at 46°C contained different species of sulfobacilli, with substitution of the dominant strains of different *Sulfobacillus* species in the course of the oxidation of energy substrates: from oxidation of sulfide minerals with iron and sulfur leaching, via oxidation of the easiest substrate for these microorganisms (ferrous iron), to oxidation of elemental sulfur and its reduced compounds with formation of sulfate.

Monitoring of the composition of microbial communities from different biogeotechnological processes showed not only the substitution of the dominant microbial strains of the same species in the communities or the substitution of species of the same genus in bacterial–chemical processes of the oxidation of sulfide minerals in the concentrates obtained from the ore of the same deposit, but also substitution of representatives of the dominant microbial genera.

For example, in the community of microorganisms oxidizing the floatation concentrate of gold-containing pyrite–arsenopyrite ore from the Olympiadinskoe deposit at 39–40°C, sulfobacilli were playing the key role according to the results of isolation of pure cultures of the dominant microorganisms and their phylogenetic analysis. Analysis of the composition of the microbial community in one of the pulp samples from

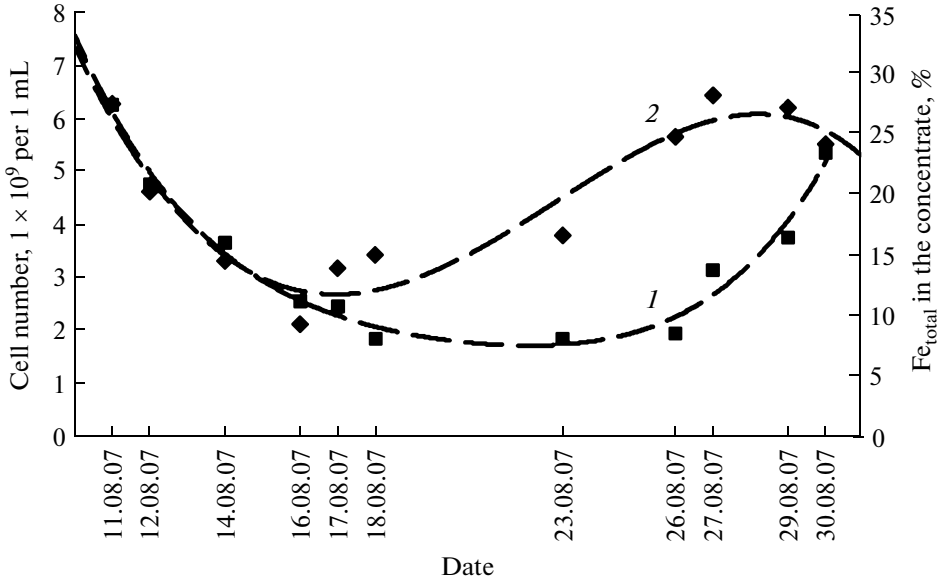

Fig. 2. Effect of decreasing iron content in the oxidized concentrate in the pulp of reactors of the Olympiadinskoe gold-mining factory on the cell number: cell number/mL (*1*); iron content, % (*2*).

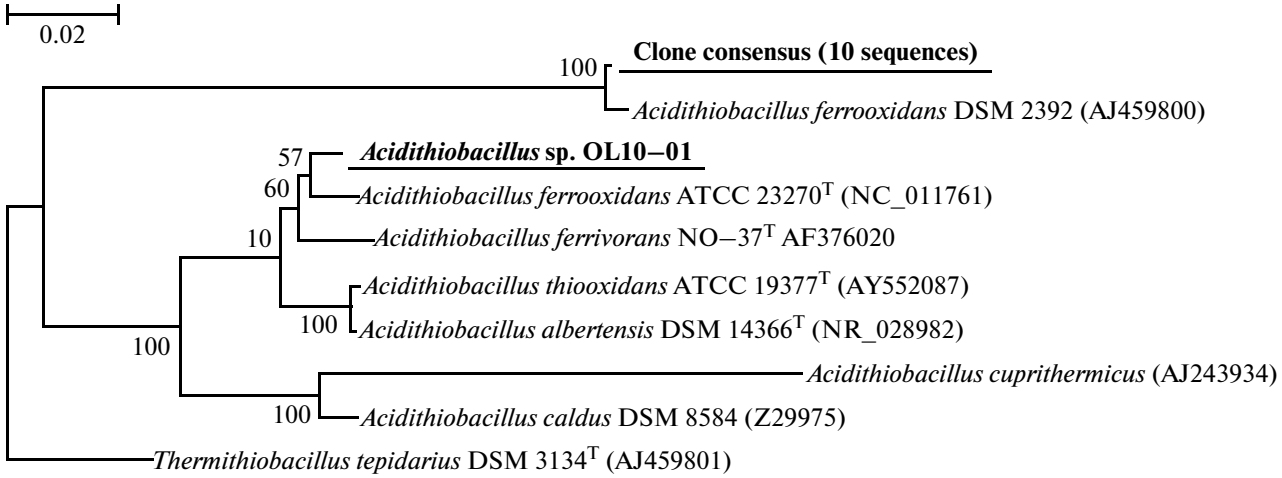

Fig. 3. Phylogenetic tree of the genus *Acidithiobacillus* constructed based on the nucleotide sequences of the 16S rRNA genes showing the position of the strain *Acidithiobacillus* sp. OL10-01 and of the consensus phylotype obtained. The numerals show the reliability of bootstrap analysis. The sequences obtained by the authors are in bold. The scale of evolutionary distances is on top left. The algorithm is neighbor-joining. The 16S rDNA sequence of *Thermithiobacillus tepidarius* DSM 3134^T (GenBank AJ459801) was used as outgroup.

the Polyus Co. reactors by construction of the 16S rRNA gene clone libraries showed the dominance of a strain from the genus *Leptospirillum* [80]. Fifty-one clones were obtained and analyzed. Based on the results of BLAST analysis, the clones were divided into two groups: 12 clones belonged to the genus *Acidithiobacillus* and 39 clones belonged to the genus *Leptospirillum*. All nucleotide sequences of the genus *Acidithiobacillus* formed a single phylotype, most closely related to *At. ferrooxidans* DSM 2392 (Fig. 3). All sequences of the *Leptospirillum* clones formed a single cluster together with the nucleotide sequence of *Leptospirillum ferriphilum* sp. BY EF025341, which was phylogenetically different from the clusters, including the sequences of type strains of the species *Leptospirillum ferriphilum* ATCC 49881^T and *Leptospirillum ferrooxidans* DSM 2705^T (Fig. 4). It was shown that the archaeal component of this community was practically a monoculture (Fig. 5). Analysis of the clones revealed no nucleotide sequence related to the *Sulfo-*

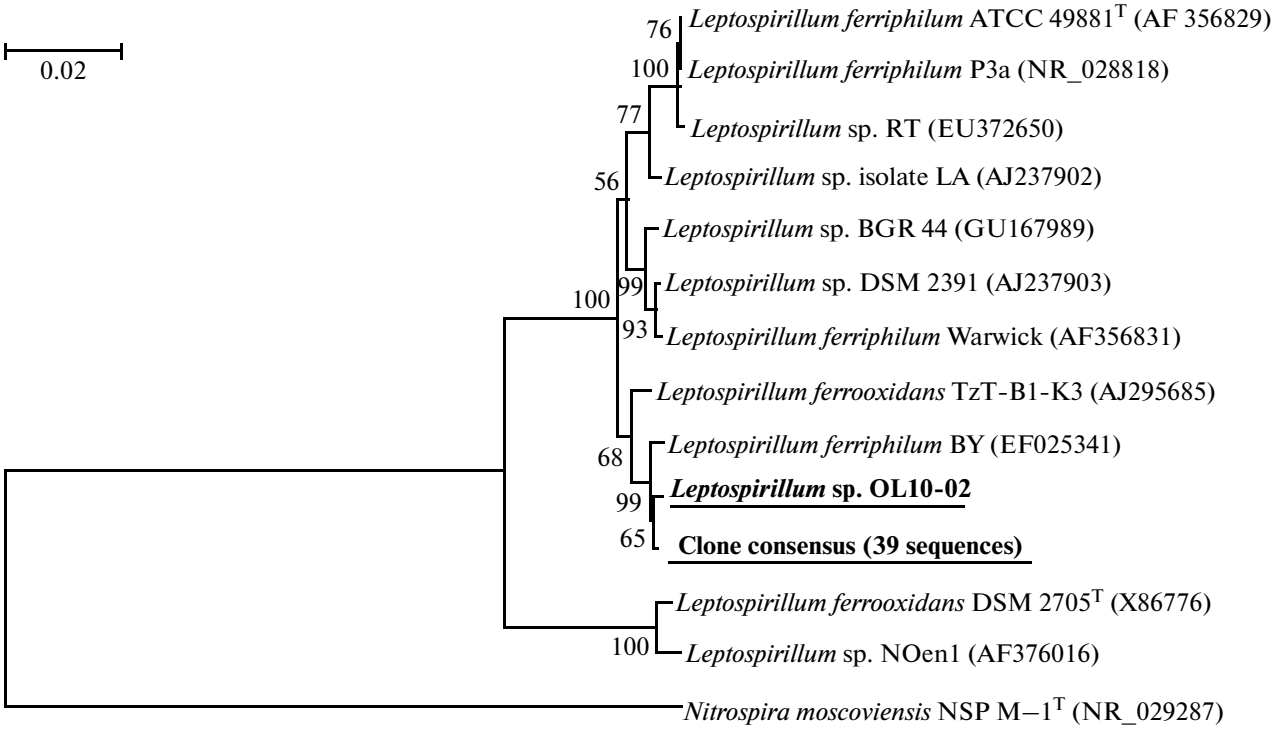

Fig. 4. Phylogenetic tree of the genus *Leptospirillum* constructed based on the nucleotide sequences of the 16S rRNA genes showing the position of the strain *Leptospirillum* sp. OL10-02 and of the consensus phylotype obtained. The numerals show the reliability of bootstrap analysis. The sequences obtained by the authors are in bold. The scale of evolutionary distances is on top left. The algorithm is neighbor-joining without considering inserts/deletions. The 16S rDNA sequence of *Nitrospira moscoviensis* NSP M-1^T (GenBank NR_029287) was used as outgroup.

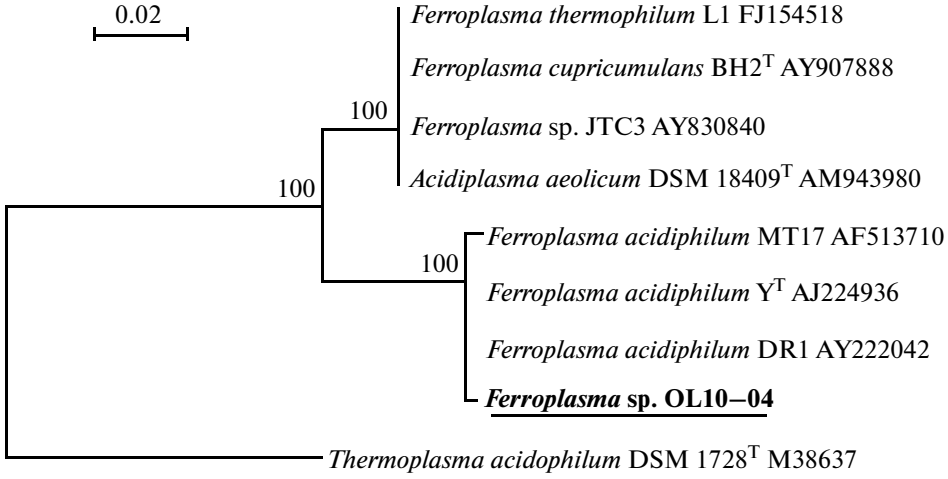

Fig. 5. Phylogenetic tree of the genus *Ferroplasma* constructed based on the nucleotide sequences of the 16S rRNA genes showing the position of the strain *Ferroplasma* sp. OL10-04. Numerals show the reliability of bootstrap analysis. The sequences obtained by the authors are in bold. The scale of evolutionary distances is on top left. The algorithm is neighbor-joining without considering inserts/deletions. The 16S rDNA sequence of *Thermoplasma acidophilum* DSM 1728^T (GenBank M38637) was used as outgroup.

bacillus species. This fact may be explained by the low numbers of *Sulfobacillus* cells in the microbial community under study.

Analysis of the clone libraries of the 16S rRNA gene obtained from the enrichment culture grown at 39–40°C on the concentrate of antimonite-containing gold–arsenic pyrite–arsenopyrite ore of the Kyuchusskoe deposit was carried out [81]. For the eubacterial component, 65 independent clones were analyzed and divided into 2 groups according to the

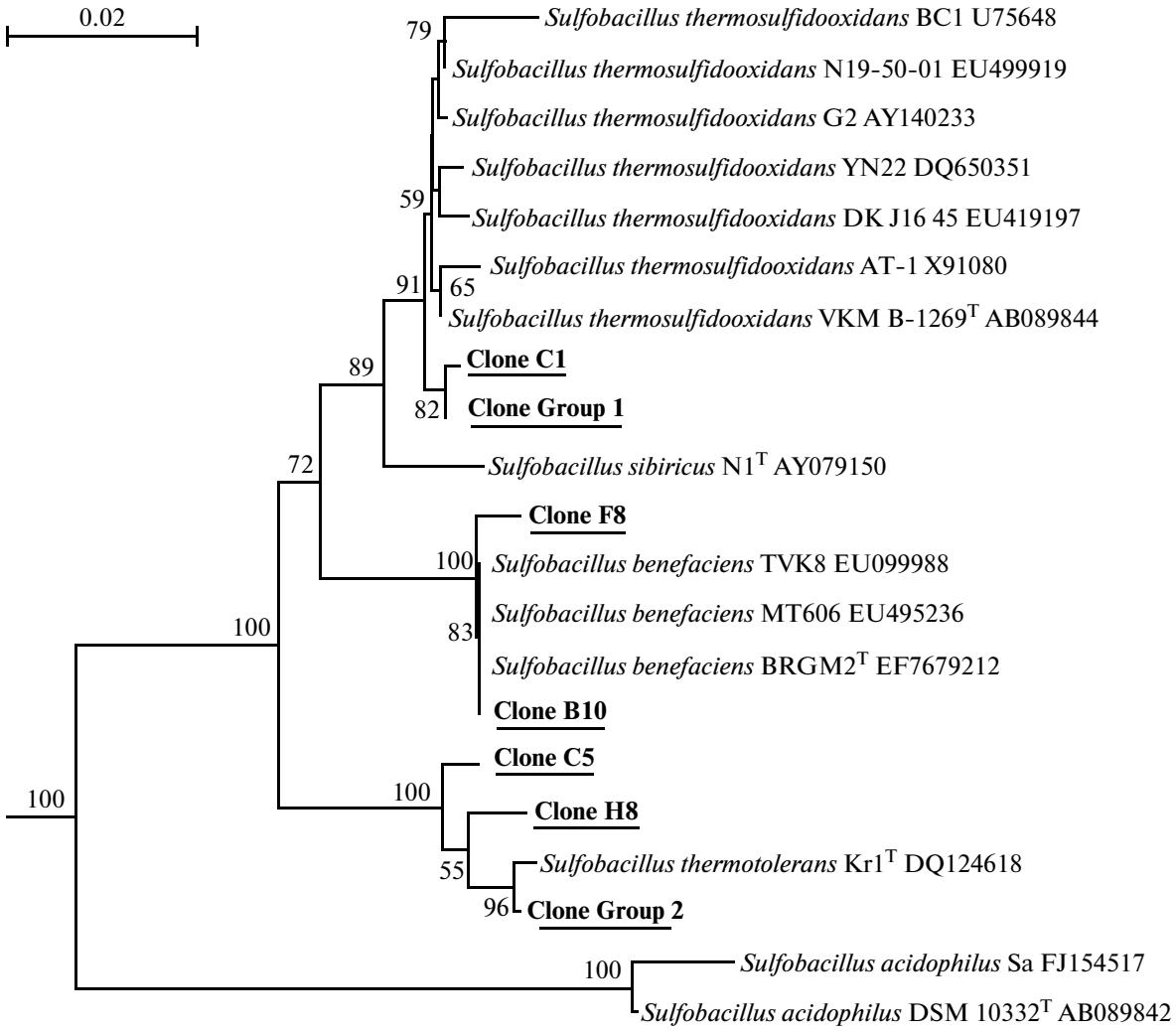

Fig. 6. Phylogenetic tree of the genus *Sulfobacillus* constructed based on the nucleotide sequences of the 16S rRNA genes. The numerals show the reliability of bootstrap analysis. The sequences obtained by the authors are in bold. The scale of evolutionary distances is on top left. The algorithm is neighbor-joining. The 16S rDNA sequence of *Thermaerobacter litoralis* KW1 (GenBank AY936496) was used as outgroup.

results of the primary BLAST analysis. Thirty-two of the analyzed clones were attributed to different species of the genus *Sulfobacillus* based on the sequences of ribosomal DNA, while 33 clones were bacteria of the genus *Leptospirillum*. The results of preliminary analysis of the nucleotide sequences made it possible to divide 32 clones of sulfobacilli into three clusters: the cluster of *S. thermosulfidooxidans*, the cluster of *S. benefaciens*, and the cluster of *S. thermotolerans*. As a result of analysis of the phylogenetic tree constructed by the 16S rDNA nucleotide sequences of *Sulfobacillus*, all of the above clones were distributed as follows: *S. thermosulfidooxidans*, 18 clones; *S. benefaciens*, 2 clones; and *S. thermotolerans*, 12 clones (Fig. 6). According to the data of preliminary BLAST analysis, the clones belonging to the genus *Leptospirillum* were a homogeneous group of strains. They formed a single cluster on the phylogenetic tree, related to the cluster formed by two isolates of the genus *Leptospirillum* and potentially representing a novel species within the genus *Leptospirillum*. Thirty-seven independent clones were analyzed for the archaeal component and it was supposed to contain no more than three components, according to the results of preliminary sequencing of total amplificate. During the construction of the phylogenetic tree, the sequences of all clones, except for the clone A1, formed a single cluster with the nucleotide sequences of the species *F. acidiphilum*.

Thus, the method of construction and analysis of the clone libraries of the 16S rRNA gene showed that the communities involved in bacterial–chemical processes on the concentrates of refractory gold–arsenic sulfide ores of different mineralogical composition varied both in the ratio of species and in generic affiliation of the dominant species [80, 81].

At present, ACM communities are studied by both cultural and molecular biological methods. A substantial advantage of PCR-based methods is the possibility of determining the composition of microbial communities without isolation of microorganisms in pure cultures. Obtaining the DNA preparations is the crucial stage of the application of these techniques. Importantly, the quality of these preparations depends on the quantitative representation of particular microorganisms in a community, peculiarities of their cell wall structure, conditions of efficient cell lysis, and specificity of the primers applied. The efficiency of cell lysis and isolation of nucleic acids from different representatives of ACM (gram-positive bacteria, gram-negative bacteria, and archaea) varies depending on the cell wall structure. Therefore, the data on the quantitative composition of a microbial community under study may significantly depend on the methods of DNA extraction from this community. In addition, the fact that phylogenetically close ACM strains may significantly differ in their physiological properties, as has been shown by the studies of strain polymorphism in ACM, prevents adequate ascertainment of the functional role of the so-called phylotypes revealed in the course of molecular ecological studies of bioleaching processes.

The cultural methods of research make it possible to isolate the known and studied microorganisms in a pure culture on selective media under the optimal conditions, even if their number in the community is very low. However, these methods cannot reveal uncultivable microorganisms.

Thus, it is obvious that a comprehensive concept of species diversity of ACM communities and the role of each microorganism in a community may be obtained only by combining the culture-dependent and culture-independent methods of research. The study of the physiological characteristics of microorganisms leads to conclusions about the interspecies interactions in ACM communities, which are important for understanding the functioning of such communities in natural and technogenic habitats.

What are the origins of this genetic diversity of microbial communities, the determining environmental factors, and the mechanisms of its generation?

ENVIRONMENTAL FACTORS DETERMINING GENETIC DIVERSITY OF THE COMMUNITIES OF ACIDOPHILIC CHEMOLITHOTROPHIC MICROORGANISMS

Temperature

Two strains, *At. ferrooxidans* and *At. thiooxidans*, predominated in the temperature range of 28–35°C during oxidation of the floatation concentrate of pyrrhotite-containing pyrite–arsenopyrite gold–arsenic ore of the Olympiadinskoe deposit; leptospirilla and sulfobacilli were present in minor quantities. Sulfobacilli predominated (60–80%) in the process carried out with the same floatation concentrate at 39–40°C, while leptospirilla and archaea were present in the ratios of (%): 10–20 : 10–20 [15].

The study of semicontinuous cultivation in a two-stage temperature regime (35–37°C in reactors 1 and 2 and 38–42°C in reactors 3 and 4) showed that thiobacilli were dominant in all reactors, whereas the numbers of sulfobacilli were much lower in both temperature regimes [82]. At a temperature of 38–42°C, the abundance of thiobacilli slightly decreased, and the numbers of sulfobacilli, on the contrary, increased from $1.34–1.42 \times 10^8$ to $1.64–2.08 \times 10^8$, indicating enhanced activity of moderate thermophiles at elevated temperatures. The quantities of leptospirilla and archaea remained negligible throughout the process.

In the course of oxidation of the gravitational concentrate of the same gold–arsenic ore inoculated with the cultures of the mesophilic *At. ferrooxidans* and moderately thermophilic *S. thermosulfidooxidans* in the two temperature regimes (30 and 42°C), a decrease in the abundance of mesophilic bacteria and increase in the numbers of moderately thermophilic bacteria could be clearly traced under elevation of temperature [83].

Table 8 shows the change in the abundance of different ACM strains under temperature increase during the oxidation of ore concentrates from two deposits: decrease in the numbers of *Acidithiobacillus* and increase in the numbers of *Sulfobacillus*. The number of cells of the *L. ferrooxidans* strain from the community on the ore concentrate from the Maiskoe deposit increased by 2 orders of magnitude at an elevation of temperature from 30 to 37°C, while the number of cells of the *L. ferrooxidans* strain from the community on the ore from the Olympiadinskoe deposit, which is more complex in the mineralogical composition (see Table 6), decreased by 3 orders of magnitude at a temperature increase to 39°C. It was evidence that the two communities contained different strains of *L. ferrooxidans* varying in optimal growth temperature.

Energy Substrate

As discussed above (Tables 4–7), the changes in energy substrate, including its mineralogical composition, resulted in variation of not only the ratio of different species in microbial communities, but also their strain composition. The rates of ACM growth and oxidation of energy substrates also depended on the physical, chemical, and electrophysical characteristics of specific sulfide minerals [84–86]. Five pyrite samples of different origin used in this work varied in density, microhardness, sulfur and iron content, the presence and composition of trace contaminants, the type of conductivity, the value of thermal EMF (electromotive force) coefficient, and resistance. The microorganisms *At. ferrooxidans, S. thermosulfidooxidans* and *F. acidiphilum* differed in their capacity for adaptation to the same pyrites. The adaptation to pyrites with p-

Table 8. The effect of temperature on the cell number of microorganisms during oxidation of ore concentrates from the Mayskoye and Olympiadinskoe deposits

Ore deposit	Temperature, °C	Cell number, cells/mL				
		At. ferrooxidans, strain	*At. thiooxidans*, strain	*L. ferrooxidans*, strain	*Sulfobacillus* sp., strain	*F. acidiphilum*, strain
Mayskoe	30	TFM, 2.5×10^7	TTM, 5.0×10^9	LFM, 0.4×10^5	S-5, 1.3×10^6	n.d.
"	36–37	TFM, 2.3×10^5	TTM, 1.6×10^7	LFM, 1.6×10^7	S-5, 1.8×10^8	n.d.
Olympiadinskoe	30	TFO-2, 2.5×10^8	TTO-2, 2.5×10^9	LFO-2, 1.0×10^5–1.0×10^6	S-5, 1.0×10^6	Y-2, Y-4, 1.0×10^5
"	39	TFO-4, 2.0×10^2	TTO-4, 6.0×10^3	LFO-4, 1.0×10^3	S-5, 1.0×10^7	Y-9 and Y-10, 1.0×10^8

type and mixed conductivity was not observed for *F. acidiphilum*. *At. ferrooxidans* had the maximum adaptation capacity. The microorganisms differed in their rates of growth and pyrite oxidation. The strains *S. thermotolerans* Kr1 and *F. acidiphilum* showed the maximum and minimum rates of oxidation of pyrite with the p-type conductivity, respectively. In the case of *At. ferrooxidans* TFV-1 and TFBk, the latter strain isolated from the gold–arsenic concentrate with more complex composition of sulfide minerals had the highest rate of growth on different pyrites.

These results demonstrate that the forming of the species and strain composition of ACM communities depends not only on the mineralogical composition of the energy substrates (sulfide ore, ore concentrate) but also on the physical, chemical, and electrophysical properties of sulfide minerals comprising the ore or the concentrate.

The Presence of Organic Substrates

Most of ACM, except for *At. ferrooxidans, At. thiooxidans* and *L. ferrooxidans*, belong to mixotrophs needing the presence of a minor quantity (0.02%) of organic substances in the medium (under laboratory conditions, usually yeast extract). The monitoring of the quantitative and species composition of the microbial community in the pulp of reactors at the gold-mining factory of Polyus Co., where sulfobacilli with mixotrophic metabolism were predominant, showed that decreased content of organic substances in the water (below 2 mg of O_2/L by BOD) used for preparing the liquid phase of the pulp resulted in lower quantity of sulfobacilli and archaea. The supply of water with a higher content of organic substances (over 4 mg of O_2/L by BOD) stabilized the species and quantitative composition of the community in 24 h.

Pulp density

Pulp density (solid/liquid phase ratio) is the most important index in the bacterial–chemical process of oxidation of sulfide minerals, which determines its economy and efficiency. An increase in pulp density of ore concentrates differently influenced the species composition and abundance of microorganisms in the community (Table 9) [69]. For example, on day 5 of oxidation of floatation concentrate of the ore from the Maiskoe deposit, the increase in pulp density affected only *At. ferrooxidans*: the cell number per 1 mL decreased from 1.0×10^7 to 1.0×10^4. At still higher pulp density, the number of *At. thiooxidans* cells considerably decreased on day 17: from 1.0×10^8 to 1.0×10^5. The number of *At. ferrooxidans* cells increased to 1.0×10^7, indicating the adaptation of this microorganism to the higher pulp density. The increase in pulp density from S : L = 1 : 5 to S : L = 1 : 2.5 had no significant effect on sulfobacilli. The quantity of all microorganisms decreased on day 23; however, *At. ferrooxidans* was present in equal amounts at both densities, while *At. thiooxidans* and *Sulfobacillus* spp. were present in much lower amounts as a result of cell lysis.

Table 9. The ratio of microbial species in the community under variation of pulp density during the oxidation of floatation concentrates of the ore from the Maiskoe deposit

Pulp density (S : L)*	Microorganism	Process duration, days		
		5	17	23
1 : 2.5	*Sulfobacillus* sp.	3.0×10^8	4.6×10^7	1.0×10^4
	At. thiooxidans	1.0×10^4	1.0×10^5	1.0×10^4
	At. ferrooxidans	1.0×10^4	1.0×10^7	1.0×10^5
1 : 5.0	*Sulfobacillus* sp.	2.5×10^8	9.9×10^7	5.4×10^6
	At. thiooxidans	1.0×10^4	1.0×10^8	1.0×10^8
	At. ferrooxidans	1.0×10^7	1.0×10^8	1.0×10^5

* S, solid phase; L, liquid phase.

Aeration Degree

The degree of pulp aeration is a very important parameter in the biohydrometallurgical technologies for sulfide mineral oxidation. Investigation of the effect of dissolved oxygen and reactive oxygen species on the changes in physiological and biochemical properties of thermoacidophilic chemolithotrophic bacteria of the genus *Sulfobacillus* isolated from different habitats made it possible to establish their response mechanisms [75, 87].

During the cultivation under conditions of oxygen deficiency of the strains isolated from well-aerated reactors, they exhibited repression of the synthesis of the enzymes of central carbon metabolism and of the antioxidant protection system: catalase, peroxidase, superoxide dismutase, glutathione peroxidase, and glutathione reductase, as well decreased content of the antioxidants in the cells, lower rates of substrate utilization, utilization of ferric iron as an additional sink for electrons, and generation of dormant forms [88]. In sulfobacilli from the habitats with lower oxygen levels (dumps/piles of sulfide ores, biofilms with developed matrix), repression of the synthesis of antioxidant protection enzymes in response to decreased levels of dissolved oxygen was much less pronounced, while, contrarily, the synthesis of a considerable part of the carbon metabolism enzymes (kinases, dehydrogenases, fructose bisphosphate aldolase, some carboxylases) was activated.

Thus, the regulation of enzyme synthesis during the changes in the oxygen regime was determined by the oxygen status of the habitats of thermoacidophilic sulfobacilli. The main programs of adaptation to the stress of oxygen limitation were regulation (induction or repression) of the synthesis of the carbon metabolism enzymes and of the antioxidant protection enzymes. The degree of aeration influenced the strain composition of ACM communities.

Technological Conditions

Investigation of the processes of bacterial–chemical oxidation of concentrates of the same gold–arsenic ore under different temperature regimes and technological conditions showed that the strains of *At. ferrooxidans* and *At. thiooxidans* were dominant in the ACM community at 28–35°C; sulfobacilli, leptospirilla, and archaea were present in minor quantities. The strain *'S. olympiadicus'* S-5 predominated at 39–40°C, leptospirilla and archaea were present in lesser quantities, acidithiobacilli and the fungus *Aspergillus niger* were detected. Bacterial culture designated as strain HT-4 was isolated from the pulp inoculated with the microbial community from reactors of the Polyus Co. gold-processing factory during the two-stage process of biooxidation of gold–arsenic ore concentrate chemically leached by ferric iron ions in a continuous mode at 39–40°C. Based on comparative phylogenetic analysis of the nucleotide sequences of the 16S rRNA genes, the strain HT-4 was assigned to the spe-

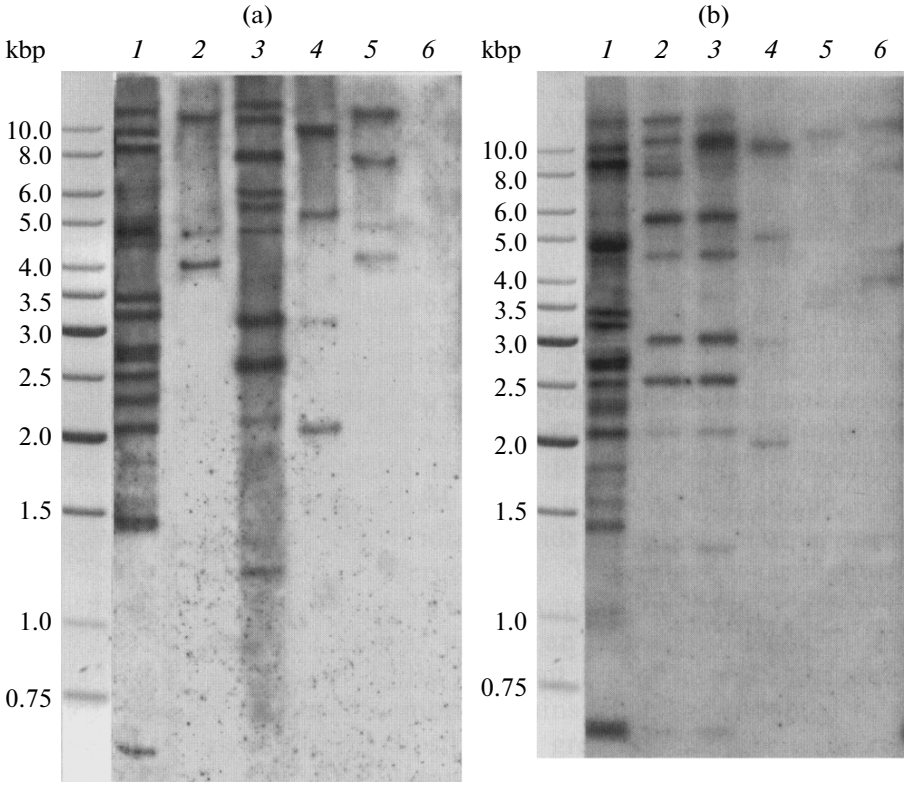

Fig. 7. Autoradiography of Southern blot hybridization of *Eco*RI fragments of the chromosomal DNA of *At. ferrooxidans* strains grown on the medium with ferrous iron (a) or adapted to elemental sulfur (b), with radioactively labeled DNA of full-size element IS*Afe*I. Strains: ATCC 19859^T (*1*); TFO (*2*); TFBk (*3*); TFL-2 (*4*); TFV-1 (*5*); TFN-d (*6*).

cies *S. thermosulfidooxidans* [72]. During tank biooxidation of the same ore concentrate at 50°C, two *S. thermosulfidooxidans* strains designated as HT-1 and HT-3 dominated in the community. The phylogenetic position of these strains was different from that of the strains used as inoculum [89]. The study of their properties showed substantial phenotypic and genotypic differences both from each other and from the strains prevailing in the processes of biooxidation of the concentrate of a similar composition under different conditions.

These results demonstrated the strain and species diversity of sulfobacilli in the associations of microorganisms involved in biooxidation of the same concentrate under different operating practices.

The main environmental factors determining the species composition of the communities of acidophilic chemolithotrophic microorganisms are temperature and the characteristics of the energy substrate. The strain composition is influenced by the characteristics of the energy substrate, temperature, concentrations of metal ions, and degree of aeration. Additional factors play an essential role in the technological processes: pulp density, the presence of organic substances and, indirectly, via the change in substrate characteristics, the peculiarities of the technological conditions.

GENOME CHANGES DURING THE ADAPTATION TO NEW ENERGY SUBSTRATES OR HIGHER CONCENTRATIONS OF HEAVY METAL IONS

During the adaptation of ACM strains to new energy substrates or to higher concentrations of heavy metal ions, both reversible and irreversible changes in the structure of chromosomal DNA were revealed by DNA cleavage with restriction endonuclease *Xba*I and analysis of the resultant products by pulse-field gel electrophoresis [70, 90–92]. The strains of different ACM species under switching of their metabolism from ferrous iron oxidation to the oxidation of pyrite, elemental sulfur, or sulfide ore concentrate, exhibited changes in the profiles of the chromosomal DNA fragments, which disappeared on return of the culture to the medium with ferrous iron. There were cases of irreversible changes in the structure of chromosomal DNA of the *At. ferrooxidans* strains. These changes in the structure of chromosomal DNA persisted during several years of cultivation on the standard medium with ferrous iron as an energy substrate. The strain *At. ferrooxidans* TFI-Fe, resistant to 50 g/L of Fe^{3+}, was obtained as a result of prolonged adaptation of the strain TFI to high ferric iron concentrations. The strain *At. ferrooxidans* TFO-2 was isolated from the lower horizons of less oxidized sulfide ore of the Olym-

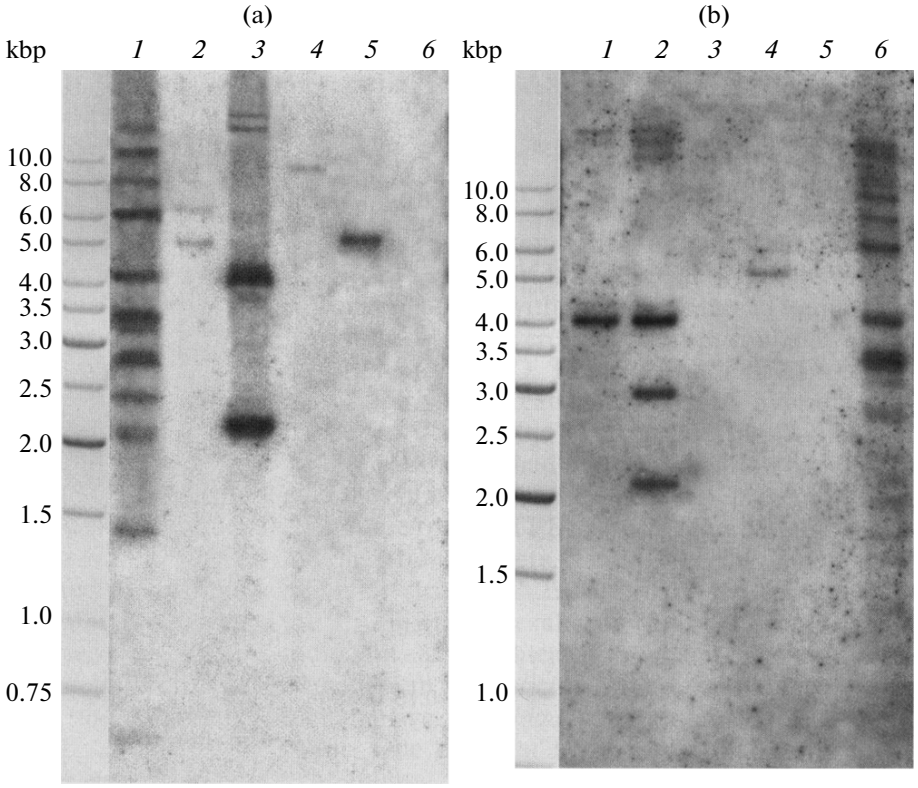

Fig. 8. Autoradiography of Southern blot hybridization of *Eco*RI fragments of the chromosomal DNA of *At. ferrooxidans* strains grown on the medium with ferrous iron (a) or adapted to elemental sulfur (b), with radioactively labeled DNA of full-size element IST2. Strains: (a) ATCC 19859 (*1*), TFO (*2*), TFBk (*3*), TFL-2 (*4*), TFV-1 (*5*), TFN-d (*6*); (b) TFO (*1*), TFBk (*2*), TFL-2 (*3*), TFV-1 (*4*), TFN-d (*5*), ATCC 23270^T (*6*).

piadinskoe deposit with the higher antimony content, whereas the strain TFO was isolated from more oxidized surface ore with the low antimony content. The strain 458M was obtained as a result of multiyear transfers of the strain *At. ferrooxidans* VKM B-458 on the concentrate of gold-containing refractory sulfide ore. It seems that, under both laboratory and natural conditions, new strains originate upon the changes in the characteristics of the oxidation substrates or the concentrations of heavy metal ions.

The adaptation of *At. ferrooxidans* strains to new energy substrates was coupled with the change in the number and localization of IS elements, as well as the number and sizes of plasmids (Figs. 7–9) [93–96]. The most substantial changes in the number and localization of element IS*Afe*I were observed in the strain *At. ferrooxidans* TFO isolated from an ore concentrate with high sulfur content during the switching of its metabolism from ferrous iron oxidation to elemental sulfur oxidation (Fig. 7). An additional band of hybridization of the *Eco*R1 restrict of the chromosomal DNA was observed in the strain *At. ferrooxidans* TFBk with radioactively labeled DNA of the IST2 element upon the switching of its metabolism from ferrous iron oxidation to elemental sulfur oxidation (Fig. 8). The change in the electrophysical characteristics of oxidized pyrites resulted in variation of the number and sizes of plasmids in the strain *At. ferrooxidans* TFV-1 (Fig. 9). It would be logical to suggest the involvement of variable genetic elements in the regulation of expression of the structural genes during the adaptation to new energy substrates.

CONCLUSIONS

Forming of species diversity of the communities of acidophilic chemolithotrophic microorganisms in natural ecosystems depends on temperature, characteristics of the energy substrate, pH value, aeration degree, and on the presence of organic substances. In technogenic ecosystems, it is also affected by pulp density, the iron/sulfur ratio in sulfide ore concentrates, and concentrations of heavy metal ions and toxic elements. These environmental factors also regulate strain diversity in the communities of acidophilic chemolithotrophic microorganisms both under natural conditions and in biotechnologies for mineral raw materials.

Genetic heterogeneity of the technogenic communities of acidophilic chemolithotrophic microorganisms allows them to adapt to the changes in the characteristics of energy substrate used for oxidation, and

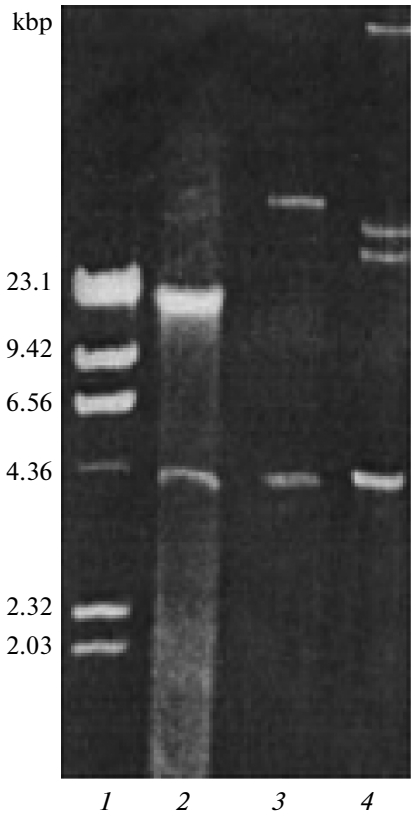

Fig. 9. Plasmid profiles of the strain *At. ferrooxidans* TFV-1 grown on the medium with Fe^{2+} (*2*) and adapted to pyrite 1 (*3*) or pyrite 2 (*4*); *1*, the λ phage DNA cleaved by restriction endonuclease *Hin*dIII.

to the changes in the ratio of both minerals and chemical elements (iron, sulfur, copper, zinc, nickel, arsenic, antimony, calcium, carbon, etc.) in sulfide ore concentrates. When a compositionally new floatation concentrate is supplied for oxidation in biohydrometallurgic technologies (it occurs rather frequently if its composition is not averaged during the production process), the clone which is best adapted to the oxidation of this substrate or is most resistant to the effects of high concentrations of heavy metal ions gains an advantage over other clones and begins to predominate in the process. Thus, the dominant strain of sulfide concentrate oxidation was *S. thermotolerans* Kr1, which essentially differed from other sulfobacilli in the number and size of plasmids and had high resistance to zinc ions (765 mM). The developed technique for isolation and purification of the largest plasmid, pST-S1, will make it possible to define its properties and functional significance.

The knowledge of the dominant strains of different species of microorganisms in the communities and of their physiological peculiarities affords optimization of the biogeotechnological processes as regards pH values, temperature, aeration degree, requirements to the composition of mineral salts and organic substances, pulp density, etc., which is a necessary condition for its stabilization, optimization, and enhancement of efficiency.

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