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BIOOXIDATION OF PYRITE, SULFIDE ORE AND COPPER CONCENTRATE BY NEW ISOLATED SULFUR AND/OR IRON OXIDIZING BACTERIA

S. KH. STEPANYAN, A.K. VARDANYAN, N.S. VARDANYAN

SPC "Armbiotechnology" of NAS RA
stepaniansk@gmail.com, nvard@sci.am

New original strains of iron oxidizing bacteria were isolated from natural biotopes and experimental systems of bioleaching. The highest activity in pyrite oxidation among isolated strains showed *Acidithiobacillus* sp. 13Zn, isolated from bioleaching pulp of zinc concentrate. *Aacidithiobacillus* sp. 13 Zn also demonstrated considerable activity in oxidation of Drmbon gold bearing ore and copper concentrate. Growth of the strain on ferrous iron is possible in the range of pH 1.4-2.6 with optimal pH 2.0, optimal temperature for growth is 37^0C. Phylogenetic analysis based on 16S rRNA gene sequences the isolates Tz and Dr were identified as *Acidithiobacillus ferrooxidans,* strain 13Zn can be represented as a new species of the genus *Acidithiobacillus*.

Iron oxidizing bacteria – bioleaching of pyrite – gold-bearing ore – copper concentrate

Բնական բիոտոպերից և տարրալուծման փորձարարական համակարգերից մեկուսացվել են երկաթ օքսիդացնող բակտերիաների նոր օրիգինալ շտամներ: Շտամներից պիրիտի օքսիդացման առավելագույն ակտիվություն ցուցաբերել է ցինկի խտանյութի կենսատարրալուծման պուլպից մեկուսացված *Acidithiobacillus* sp. 13Zn շտամը: Շտամը ցուցաբերել է նաև պղնձի խտանյութի և ոսկեբեր պղնձային հանքաքարի տարրալուծման բավարար ակտիվություն: Շտամի աճի օպտիմալ ջերմաստիճանը 37^0C է, pH-ը 2,0 է, աճը երկարժեք երկաթի վրա հնարավոր է pH 1.4-2.6 միջակայքում: 16 S ռՌՆԹ ի հաջորդականության ֆիլոգենետիկ վերլուծության հիման վրա մեկուսացված Tz և Dr շտամները նույնականացվել են որպես *Acidithiobacillus ferrooxidans*, իսկ *Acidithiobacillus* sp. 13Zn-ը կարող է ներկայացվել որպես *Acidithiobacillus*. ցեղի նոր տեսակ:

Երկաթ օքսիդացնող բակտերիաներ – պիրիտի տարրալուծում –
ոսկեբեր հանքաքար – պղնձի խտանյութ

Новые оригинальные штаммы железоокисляющих бактерий были изолированы из природных биотопов и экспериментальных систем биовыщелачивания. Наивысшую активность в окислении пирита проявлял *Acidithiobacillus* sp. 13Zn, изолированный из пульпы выщелачивания цинкового концентрата. *Acidithiobacillus* sp. 13Zn также показал значительную активность в окислении Дрмбонской золотоносной руды и медного концентрата. Рост штамма в присутствии двухвалентного железа возможен в пределах pH 1.4-2.6 с оптимальным значением pH 2.0, оптимальной температуре роста 37^0C. На основании филогенетического анализа нуклеотидной последовательности гена 16S рРНК выделенные штаммы Tz и Dr были идентифицированы как *Acidithiobacillus ferrooxidans,* штамм 13Zn может быть представлен как новый вид рода *Acidithiobacillus*.

Железоокисляющие бактерии – выщелачивание пирита –

Bioleaching is an alternative resource saving and environmentally friendly method for metal recovery from ores and concentrates [3]. It is based on the abilities of acidophilic bacteria and archaea to oxidize sulfide minerals.

Previously, bioleaching of metals was more often associated with mesophilic bacteria from genus *Thiobacillus* (now *Acidithiobacillus*): *Acidithiobacillus ferrooxidans* and *Acidithiobacillus. thiooxidans*. Later on, studies have shown that *Leptospirillum* and other thermophilic and thermotoleratnt bacteria are dominating in bioleaching niches [2, 4, 7-9]. The exceptional diversity of eco-geographical conditions of Armenia and the richness of metal ores provide great possibilities to isolate and study iron oxidizing bacteria perspective for metal biotechnology.

In this paper we have carried out investigations for studying communities of chemolithotrophic bacteria in different natural ecosystems and technological conditions, as well as revealing physiological peculiarities of isolated original and active strains. The potential of isolated original and active strains and their communities for the application in bioleaching of pyrite, gold-bearing ore and copper concentrate has been evaluated.

Materials and methods. Strains of iron oxidizing chemolithotrophic bacteria (Tz, Dr, CC, 15 and 13Zn) were isolated from natural and technogenic biotopes (acid drainage water, ore dumps) of Tandzut polymetallic, and Drmbon copper gold-bearing ores of Armenia as well as from experimental systems of bioleaching. To isolate iron oxidizing bacteria, Mackintosh medium [5] with ferrous iron as a source of energy was inoculated with acid mine drainage water, ore dump or bioleaching pulp samples and incubated at 30, 37 and 45°C for 7-10 days.

Pure cultures were obtained by transferring the yellow and yellow-brown colonies growing on Manning [6] and $FeTSB_0$ [4] solid media to the above mentioned liquid medium. Isolation, cultivation and characterization of iron oxidizing strains were performed in our previous work [10].

DNA extraction, PCR of 16S rRNA, phylogenetic analysis. DNA extraction was carried out according to the method described by [1].

PCR amplification was performed in 25 μl reaction mixture which contained 10 μg of the genome DNA, 5 μl buffer (GoTaq®Flexi DNA Polymerase M8291), 2 mM $MgCl_2$, 100-200 μM dNTPs, 10 pmol of each primers (27F-AgA gTT TgA TCM TGG CTC Ag and 1492R-TAC ggY TAC CTT gTT Acg ACT T), 1 unite taq-polymerase (PROMEGA®), nuclease-free water.

Extraction and purification of PCR-product from agarose gel were done using Zymoclean ™ Gel DNA Recovery Kit (ZYMO RESEARCH). The purified PCR products of approximately 1,400 bp were sent to Korea (MACROGEN) for sequencing. Sequencing was performed using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA).

The 16S rRNA gene nucleotide sequences of isolated strains were analyzed using BLAST (http://ncbi.nlm.nih.gov/BLAST). Construction of phylogenetic trees was done by MEGA 6.06 software using neighbor-joining method. Bootstrap analysis was carried out on 1000 replicate input data sets.

Bioleaching of pyrite: The bacterial strains were grown on Mackintosh medium containing Fe(II) as an energy source [5]. In the logarithmic phase of growth the cells were collected by centrifugation at 6000 g for 10 min, washed with acidified Mackintosh medium and resuspended in the same medium without iron. Pyrite (FeS_2) from Shamlugh ore, copper concentrate (Armenia) and Drmbon gold-bearing ore (Nagorno Karabakh) ground to 45-63 μm were placed into 250 ml Erlenmeyer flasks and sterilized. Then 50 ml of Mackintosh medium without Fe(II), pH 2.0 adjusted by H_2SO_4 and washed bacterial suspension (10^8 cells/ml) were added to the flasks. The bioleaching experiments were carried out at 37^0C in shaking conditions (180 revs/min). The pulp density was calculated as pyrite or sulfide ore ratio to the volume of the medium. Sampling was performed at 24 h intervals and pH, ferric Fe(III) and ferrous (Fe(II)) ions in the medium were analyzed. Concentration of copper and total iron were determined by atomic absorption spectrometer AAS 1N (Germany).

Results and Discussion. Original strains of iron oxidizing chemolithotrophic bacteria (CB) were isolated from natural biotopes of polymetallic (Tandzut) and copper gold-bearing (Drmbon) ores in Armenia and from experimental systems of bioleaching

and studied. At first screening of isolated strains in respect of pyrite oxidation was performed. The strains were tested for oxidation of pyrite (FeS_2) from Shamlugh ore (Armenia).

Comparative activities of isolated cultures of iron oxidizing bacteria in pyrite oxidation (Shamlugh, Armenia) are presented in fig. 1. As seen from the fig. 1, *Acidithiobacillus* sp. Tz and *Leptospirillum sp.* CC showed similar activities in pyrite oxidation. Efficiency of *Acidithiobacillus* sp. Dr and *Acidithiobacillus* sp. 15 was inferior to its above mentioned strains. The highest activity in pyrite oxidation showed *Acidithiobacillus* sp. 13Zn. Pyrite oxidation activity of *Acidithiobacillus* sp. 13Zn exceeded that of *Acidithiobacillus* sp. Tz and *Leptospirillum sp.* CC about 2.1-2.3 and 2.4-3.0 times respectively (fig.1).

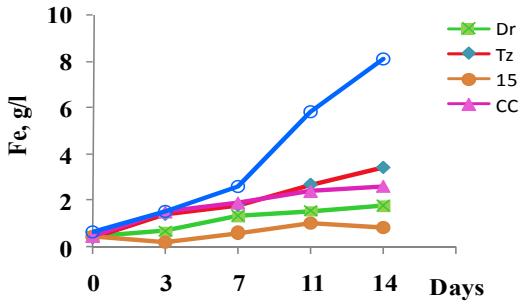

Fig.1. Bioleaching of pyrite by isolated iron oxidizing bacteria *Acidithiobacillus* sp. Tz, Dr, 15, 13Zn and *Leptospirillum* sp. CC (PD- 4%, t 30^{0}C, pH 2)

Thus, further investigations were carried out with *Acidithiobacillus* sp. 13Zn. The main physiological properties of the mentioned strain were revealed. Optimal temperature for growth was 37^{0}C. Growth of the strain on ferrous iron is possible in the range of pH 1.4-2.6 with optimal pH 2.0 (fig.2).

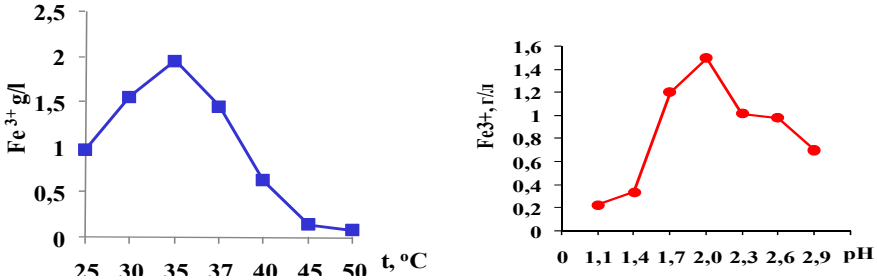

Fig 2. Influence of temperature (a) and pH (b) on oxidation of iron by *Acidithiobacillus* sp. 13Zn

16S rRNA gene sequences analysis. The 16S rRNA genes of isolated strains were amplified and the PCR amplification product was detected by 1.0% agarose gel electrophoresis. Preliminary analysis of 16S rRNA gene nucleoide sequences of strains was done using BLAST (http:www.ncbi.nlm.nih.gov/blast). Construction of phylogenetic trees was done by MEGA 6.06.

According to dendogram pesented in fig. 3, Isolate 13Zn was clustered into *At.ferrooxidans* and possessed 91% sequence similarity with the type strain *Acidithiobacillus ferrooxidans*. Thus, according to phylogenetic analysis based on 16S rRNA sequences, the strain 13Zn can be presented as a new species of the genus *Acidithiobacillus*.

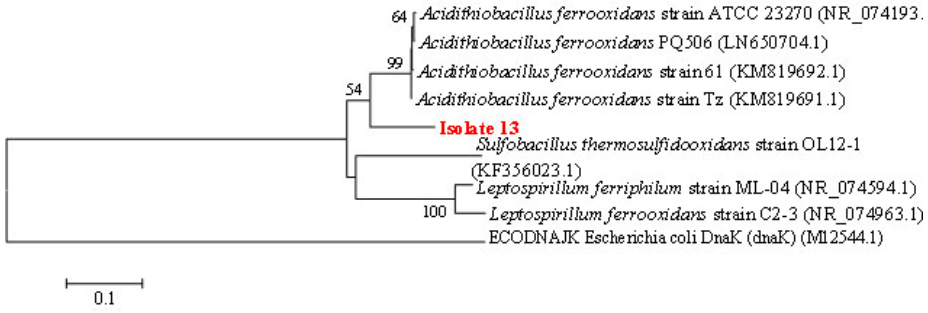

Fig. 3. Phylogenetic tree of isolated strain *Acidithiobacillus* sp. 13Zn. The evolutionary history was inferred using the Neighbor-Joining method. Evolutionary analyses were conducted in MEGA6.

Isolated strains Dr, and Tz were clustered into *At.ferrooxidans* and possessed 99% sequence similarity with the type strain *Acidithiobacillus ferrooxidans* ATCC 23270 and were identified as *At.ferrooxidans* [10].

Isolated bacteria were deposited in the Microbial Depository Center of the SPC of "Armbiotechnology" of NAS of Armenia and got the MDC numbers (Dr-MDC 7042, Tz-MDC 7043, CC-MDC 7047, 13Zn –MDC 7054).

Bioleaching of gold bearing ores and copper concentrates. *Acidithiobacillus* sp. 13Zn was also tested in respect of Drmbon gold- bearing ore (Nagorno Karabakh). As shown in fig. 4, *Acidithiobacillus* sp. 13Zn accelerates oxidation of the mentioned sulfide ore approximately 5.3 times in comparison with uninoculated control (chemical oxidation).

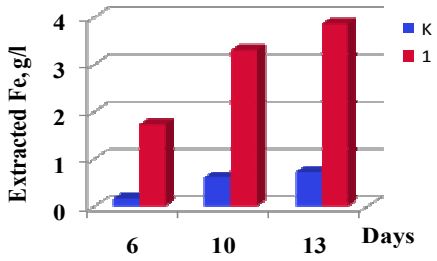

Fig. 4. Bioleaching of iron from Drmbon concentrate by *Acidithiobacillus* sp. 13Zn (PD 10%, t-37°C)

Biooxidation of copper concentrate by *Acidithiobacillus* sp. 13Zn is presented in fig.5. Data obtained indicated that extraction of copper and iron from copper concentrate by *Acidithiobacillus* sp. 13Zn increased about 7-8 and 3 times respectively (fig. 5).

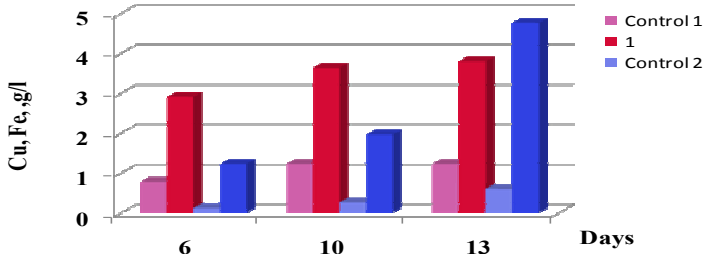

Fig. 5. Bioleaching of iron (1) and copper (2) from copper concentrate by *Acidithiobacillus* sp. 13Zn (PD 10%, t-37°C)

Thus, we can conclude that new isolated strain *Acidithiobacillus* sp. 13Zn may serve as an efficient candidate for development and performing high efficient bio-oxidation process of pyrite-containing ores especially gold bearing ores as well as copper concentrates.

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