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Original Research

Bioleaching Potential of Indigenous Bacterial Consortia from Gold-Bearing Sulfide Ore of Ta Nang Mine in Vietnam

Ngoc Tung Quach[1,3#], Can Pham-Ngoc[2#], Thi Lien Bui[1], Trong Hoa Tran[2], Thi Phuong Ngo[2], Van The Nguyen[1], Thi Hanh Nguyen Vu[1,3], Tuan Anh Tran[2], Hoang Ha Chu[1,3], Quyet Tien Phi[1,3]*

[1]Institute of Biotechnology, Vietnam Academy of Science and Technology, Hanoi 10000, Vietnam
[2]Institute of Geological Sciences, Vietnam Academy of Science and Technology, Hanoi 10000, Vietnam
[3]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi 10000, Vietnam

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Abstract

To date, bioleaching using bacterial consortia is widely regarded as an eco-friendly alternative to the traditional mining approaches due to its cost-effectiveness, feasibility, and sustainability. In the present study, for the first time, gold-bearing sulfide ore collected at Ta Nang mine, Vietnam was mineralogically characterized and subjected to bioleaching trial using indigenous bacterial consortia. The ore contains arsenopyrite, pyrite, galena, sphalerite, and chalcopyrite, of which the major metals were iron (4.78%), arsenic (1.73%), lead (0.43), and zinc (0.33%). After enrichment, a total of 19 iron- and sulfur-oxidizing bacteria were isolated and classified into six distinct genera including three previously described *Bacillus*, *Pseudomonas*, *Acidithiobacillus*, and three firstly reported heterotrophic *Glutamicibacter, Providencia,* and *Stenotrophomonas* from gold ore origin. Moreover, an autotrophic *Acidithiobacillus* sp. TNG6.3, sharing a 16S rRNA sequence of 95.1% identity with the closest sequence of the type strain *A. caldus* KU, represented a novel candidate species. The establishment of bioleaching utilizing enriched bacteria from gold ore consequently led to the removal of Ag (99.1%), Zn (37.9%), As (37.0%), and Fe (32.2%) from ore after 21 days of treatment, respectively. The present findings highlighted the potential of acidophilic bacteria originated from gold ores for extending applications in bioleaching of metals in Vietnam.

Keywords: acidophilic, arsenopyrite, bioleaching, consortia, gold

# The autors contributed equally to this work.
*e-mail: tienpq@ibt.ac.vn

Introduction

Over several decades, the yield for precious metal mining such as gold, silver, and copper has significantly increased due to the development of advanced technologies and global demands [1, 2]. However, the estimated reserves of some crucial metals Cu, Pb, Au, Ag will be depleted within 50 years or fewer [3]. New approaches will be necessary to recover metals from low-grade ore deposit and mine tailings as a part of circular economy.

Bioleaching is an economical and green technology for the recovery of precious metals from minerals. Notably, approximate 7% of the world's copper production was statistically produced from low-grade copper ore and mine tailing by using bioleaching technologies [4]. In fact, bioleaching technology has been shown to strictly rely on indigenous bacterial consortia colonizing sulfide ores, consisting of members of the genera *Acidithiobacillus*, *Pseudomonas, Leptospirillum, Sulfobacillus*, and *Bacillus* [1, 5, 6]. The most studied leaching bacteria are the genus *Acidithiobacillus*, which includes sulfur-oxidizing species (*A. caldus, A. thiooxidans, A. albertensis*) and sulfur- and ferrous-oxidizing bacteria (*A. ferrooxidans, A. ferrivorans, A. ferriphilus, A. ferridurans*) [7]. *Acidithiobacillus* spp., a group of acidophilic chemolithoautotrophic Gram-negative bacteria, are common inhabitants of extremely acidic metal-rich ecological niches, which were widely applied in the bioleaching of metal-sulfide ore minerals, ore concentrates, and waste sludge [8-10]. Despite the high bioleaching efficiency, the genus *Acidithiobacillus* is often found in low density in indigenous microbial consortia [1, 11], which has not been fully understood to date. By contrast, the most abundant genera commonly found in bioleaching models are *Acinetobacter, Bacillus, Pseudomonas*, and *Streptococcus* [11]. Presently, the genus *Acidithiobacillus* has not got much paid attention to taxonomic classification and consists of only 7 species, which leads to higher possibility of finding a novel species, especially in extreme habitats like acidic mine drainage or sulfide ore bioleaching systems.

It is widely recognized that the utilization of microbial consortia is effective in the bioleaching of sulfide ore and concentrate. Many previous studies reported the efficiency of using microbial consortia for removal of different heavy metals Cu, Ag, Au, and As under either anaerobic or aerobic conditions [12-14]. A huge amount of Cu (77.7 ± 3.6%) and Zn (70.6 ± 3.8%) extraction were obtained from low-grade copper ore by using mixtures of *A. ferrooxidans, L. ferriphilum,* and *L. ferrooxidans* [10]. More than 92% of Au was leached from carbonaceous-argillaceous shales by using the bacterial consortium comprising predominantly *Pseudomonas, Bacillus, Stenotrophomonas* [1]. Among these heterotrophs, the genus *Bacillus* has been emerging as a promising candidate for the extraction of heavy metals from non-sulfidic minerals such as Ni-laterite and silicate ores [15, 16]. Besides, mineralogical composition and operating condition contribute significantly to the success of this green technology.

The Ta Nang gold mine in central highland of Vietnam is hosted in the middle Jurassic siltstones, mudstones, and shales of the La Nga Formation, and has been exploited since the 1990s [17]. Soon afterward, all the rich gold veins has been continuously mined using a laborious processes requiring a myriad of technical steps, which gradually makes Ta Nang possible to be a secondary deposit. Therefore, the aim of this work was to shed light on the mineral composition of Ta Nang ore and bioleaching potential of indigenous bacterial consortia. To the best of our knowledge, this is the first report on acidophilic consortia and bioleaching of gold-bearing sulfide ore from the Vietnamese deposit.

Materials and Methods

Gold-Bearing Sulfide Samples

Mining residues and mine drainage samples were collected in January 2019 from different sites at the Ta Nang gold deposit, Duc Trong district, Lam Dong province, central highland of Vietnam (11°34'23''N; 108°32'33"W) for further study on mineralogical characteristics, bacterial identification and composition, and bioleaching efficiency using indigenous microbial consortia. Around 20 kg of the sample were crushed in a jaw crusher and then ground by a ball mill to the fraction with particle size of 1 mm [1]. Ground gold-bearing sulfide ores were then sieved through a mesh grid to obtain a size fraction of 74 μm. The concentrate was prepared by gravity separation using shaking tables. The samples were held at 4ºC in sterile containers and protected from contamination sources until they were used for experimental investigations.

Mineralogical and Morphological Characterization

The major important elements and components of the ore samples were determined by using atomic absorption spectrometry (Varian SpectrAA-220, USA) at the Institute of Chemistry, Vietnam Academy of Science and Technology. Mineralogical characteristics of ore minerals were studied by observing the polish section under a Leica Primotech polarized microscope at the Institute of Geological Sciences, Vietnam Academy of Science and Technology. High-resolution composition images were recorded using a back scattered electron (BSE) detector on a JEOL JSM 6510 LV scanning electron microscope (SEM). The chemical composition of minerals was determined by an OXFORD EDS (Energy Dispersive Spectroscopy) detector attaching to the above SEM at the Institute of Geology and Mineralogy, Siberian Branch, Russian Academy of Sciences.

Enrichment and Isolation of Acidophilic Bacteria

Ten grams or milliliters of samples were enriched in 250 ml flask containing 90 ml of sterile modified 0K mineral salts medium containing $(NH_4)SO_4$ 3 g/L, KCl 0.1 g/L, K_2HPO_4 0.5 g/L, $MgSO_4.7H_2O$ 0.5 g/L, $Ca(NO_3)_2$ 0.01 g/L, pH 3.0 adjusted by H_2SO_4 and autoclaved at 121°C for 15 min [6, 18]. The modified 9K medium was composed of the 0K medium supplemented with 4 g of $FeSO_4$ as the sole energy source, used for isolation of indigenous iron-oxidizing bacteria. The sulfur-oxidizing bacteria were isolated using the 0K medium supplemented with 10 g of S^o, namely S^o medium [6]. The flasks were shaken in a horizontal shaker at 150 rpm at 30ºC. After 7 days of incubation, 10 ml of the culture broth was transferred to a new flask containing 90 ml of a fresh medium and repeated twice. The bacterial cells in the culture were counted using Newbauer Chamber (Blau Brand, Germany), and harvested by centrifugation at 10,000 rpm for 10 minutes. The pellets were then re-suspended in 20 ml of sterilized distilled water, adjusted to pH 4.0. A 10-fold serial dilution was performed from 10^{-1} to 10^{-6} and 0.1 ml of diluted samples were then spread onto the modified 9K and S^o agar plates prepared as described previously [10, 19]. The agar plates were inoculated at 30°C for 14 days, and single colony was streaked out several times to obtain pure isolates and then stored in 15% glycerol at -80ºC. All the experiments were performed in triplicate.

Sequencing of 16S rRNA Gene and Phylogeny Analysis

The genomic DNA of each isolate was extracted by using a bacterial genomic DNA isolation kit (Norgen Biotek Corp). The 16S rRNA genes of bacterial isolates were amplified and sequenced using universal primers 27F (5'-TAACACATGCAAGTCGAACG-3') and 1429R (5'-GGTGTGACGGGCGGTGTGTA-3') [14]. The 16S rRNA gene sequences were analyzed for the homologies using a nucleotide Blast tool (http://www.ncbi.nlm.nih.gov/BLAST/). A neighbor-joining phylogenetic tree was constructed based on the 16S rRNA gene sequences obtained and corresponding sequences of reference strains retrieved from GenBank NCBI using MEGA7 with a branch support of 1000 bootstraps. The 16S rRNA gene sequences of isolates were deposited in GenBank (NCBI) under accession numbers MW821367-MW821381, MN209900, MW713141, and MW713142.

Characteristics of Acidophilic Bacteria TNG13.1 and TNG6.3

The morphology of cells was analyzed by using SEM (JEOL, JSM-5410, Japan) at a magnification of 7500x. The effects of different conditions, including pH, NaCl, temperature, energy sources, and heavy metals on bacterial growth were performed as described previously [20].

Bioleaching Experiment

To increase bioleaching activity, 190 ml of fresh 9K and S^o media were inoculated with 10% (w/v) sulfidic gold ore concentrate at 30ºC for 14 days [6]. After three sequential continuous subcultures, the bioleaching experiment was carried out when the final cell density of each culture was about 2.0×10^7 cells/ml. A 10% (w/v) microwave-sterilized concentrate was added to the 5-liter-bioreactor (Bioflo, New Brunswick Scientific Inc., NJ, USA) containing 3 liters of 0K medium without energy sources. The experiment was performed by adding a ratio of 1:1 (v/v) mixture of the iron- and sulfur-oxidizing bacteria to a bioreactor at a final inoculum of 5% (v/v), followed by incubation at 30°C under agitation rate of 100 rpm. The loss of water content in the bioreactor was replenished with sterile water. The experiment was terminated after 21 days of incubation. All heavy metals in the concentrate and leach solution were analyzed using atomic absorption spectrometry (Varian SpectrAA-220, USA) and inductively coupled plasma (Perkin Elmer Optima 3300RL, USA). The efficiency of extracted heavy metal was calculated from aqua regia extraction before and after the experiment. The morphological features of the ore residues were observed by a polarized microscope, and bacterial cells were observed by SEM.

Results and Discussion

Mineralogical Characteristics of the Ta Nang Gold Mine

In Ta Nang ore, arsenopyrite (FeAsS) and pyrite (FeS_2) were found to be the most abundant iron sulfide-bearing minerals, followed by galena (PbS), sphalerite (ZnFeS), and chalcopyrite ($CuFeS_2$) (Table 1), which are consistent with numerous studies previously describing arsenopyrite and pyrite being of the most frequently found sulfide minerals [21, 22]. Furthermore, SEM morphological studies visualizing the occurrences of Au in the ore indicated the dominant proportion of arsenopyrite and pyrite (Fig. 2). Under the polarized microscope, arsenopyrite was pale yellow, and mostly brecciated (Fig. 1b,c). Pyrite was brass-yellow, mostly formed assemblage with arsenopyrite (Fig. 1a,c) and occurred as tiny inclusion in sphalerite (Fig. 1b,d). Sphalerite and galena were dark gray and light gray color, respectively. Both formed an assemblage and replaced or filled in fractures of arsenopyrite as cementation. Besides, low-grade chalcopyrite was observed as tiny inclusions in sphalerite. These resulting observations led to a hypothesis that arsenopyrite and pyrite can be recovered by flotation and bioleaching bacteria.

Table 1. Percentage of the sulfide ores in the concentrate.

Sulfidic ore concentrate	Content (%, w/w)
Arsenopyrite (FeAsS)	20.6
Pyrite (FeS_2)	9.30
Galena (PbS)	1.90
Sphalerite (ZnFeS)	1.48
Chalcopyrite ($CuFeS_2$)	0.05

Using a polarized microscope and SEM, the gold mineralization in Ta Nang mine was classified into 2 types including visible and invisible gold grains. Visible gold occurs as free gold grains intermediate- to coarse-grained in arsenopyrite and quartz (Fig. 2a, d). Size of visible gold grains varied from dozens to hundreds of micrometers. On the other hand, invisible gold occurs as fine to ultra-fine grained (less than 1 to 10 micrometers). This type of gold grains is locked inside arsenopyrite and sphalerite. Under SEM observation, the invisible gold was shown to be too small to liberate and unable to be recovered by using the conventional approaches. Many studies showed Au being locked up in sulfide minerals such as arsenopyrite and pyrite [23, 24]. However, invisible gold has also been found mainly in pyrite and altaite determined in tellurium-bearing ore from Sunshin mine, Korea [13]. To liberate Au, bacterial bioleaching is considered an effective strategy since acidophiles accelerate the oxidation of arsenopyrite and enhance its floatability, thus improving the recovery of gold from ores [9, 25].

Chemical Composition of the Ta Nang Gold Mine

Chemical analysis of multiple elements was performed to determine valuable elements in the feeding ore and concentrate of the Ta Nang gold mine. As shown in Table 2, the most abundant elements in the feeding ore sample were Fe (4.78%), S (2.20%), and As (1.73%), which are corresponded to the greatest proportion of arsenopyrite in the sulfidic concentrate. The presence of Pb and Zn observed was in agreement with the low content of galena and sphalerite. In addition, the samples were found to contain precious metals such as Ag (126 mg/kg), Cu (95 mg/kg), and Au (31.8 mg/kg). As described previously, arsenopyrite comprises more chemically bound Au than pyrite, and Au within the arsenopyrite is a gold solution Au^+ [26]. Ag is predominantly associated and recycled with Au, raising the potential for simultaneous recovery of both Au and Ag [27]. Ta Nang ore held higher metal contents including Fe, As, Ag, and Au than other ores collected at the Ural Federal District, Orenburg region, Russia [1]. The difference could be due to the geographic location of the reservoir and the formation of rock [28]. After the gravitation enrichment, the heavy metal contents such as Cu, Ag, and Au in the concentrate enriched

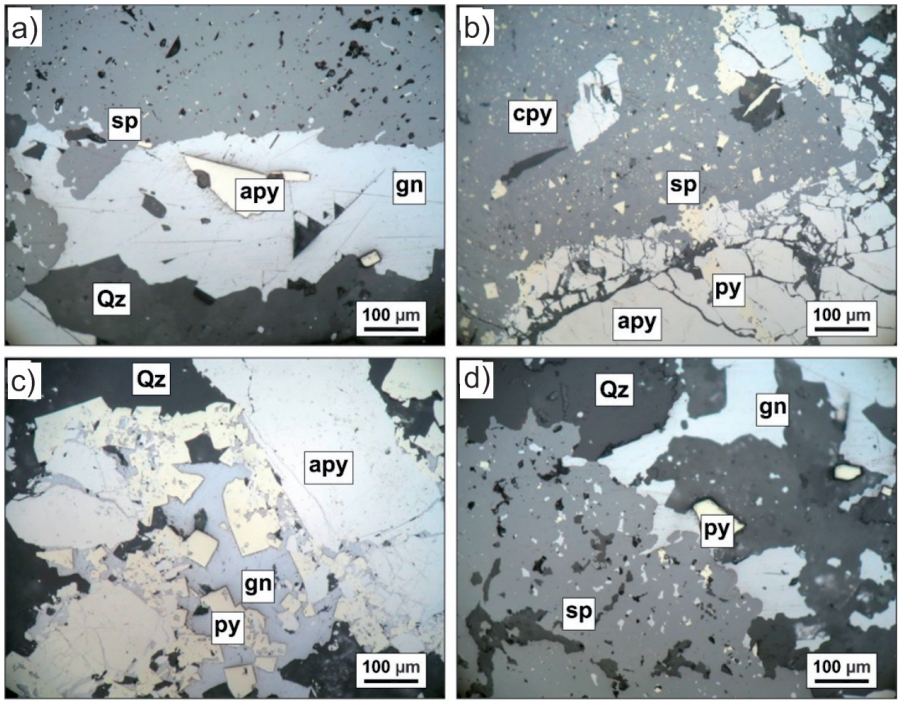

Fig. 1. Photomicrographs show the mode of occurrences of sulfide minerals in the concentrate. a) Inclusions of arsenopyrite in sphalerite-galena assemblage. b) breccias of arsenopyrite-pyrite assemblage were cemented by sphalerite, pyrite and chalcopyrite occur as inclusions in sphalerite. c) Galena fill in the fracture of arsenopyrite-pyrite assemblage. d) Pyrite occurs as euhedral grains and inclusions in the sphalerite. Abbreviations: apy, arsenopyrite; py, pyrite; cpy, chalcopyrite; sp, sphalerite; gn, galena; Qz, quartz.

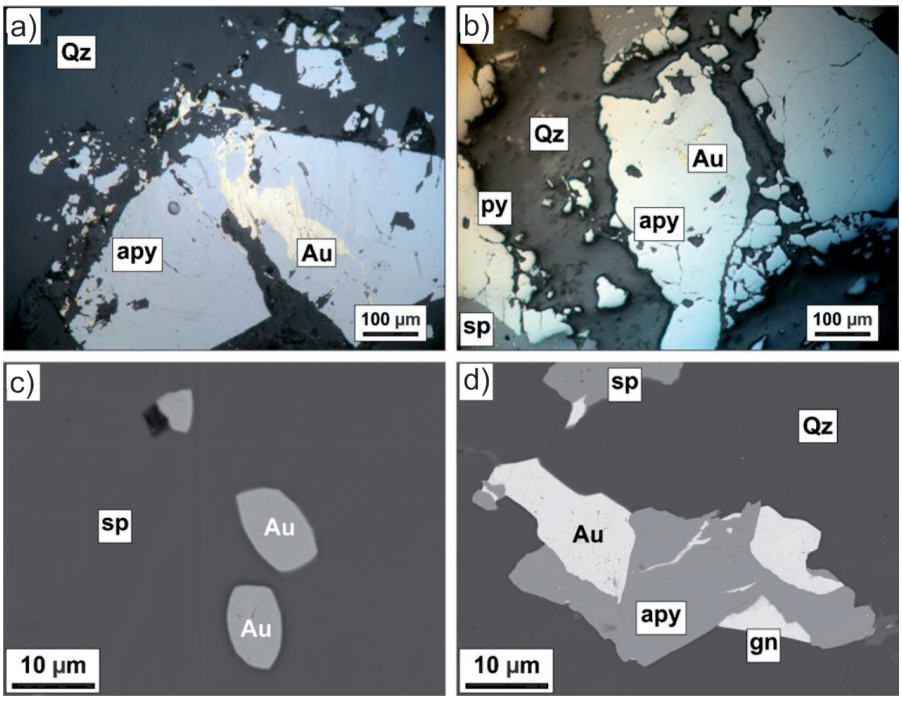

Fig. 2. Photomicrographs show mode of occurrences of gold in the ore of the Ta Nang gold mine. a) coarse-grained native gold in fracture of arsenopyrite grain. b) fine-grained native gold locked in arsenopyrite grain. c) fine-grained native gold locked in sphalerite. d) fine-grained native gold in fracture of arsenopyrite. Abbreviations: Au, gold; apy, arsenopyrite; py, pyrite; sp, sphalerite; gn, galena; Qz, quartz.

significantly (Table 2), which was favorably used for further studies.

Occurrence of Indigenous Acidophilic Bacteria

After three times of enrichment, the color of modified 9K liquid medium used to isolate iron-oxidizing bacteria was altered from pale green to reddish-brown, while the S^o medium targeted to isolate sulfur-oxidizing bacteria appeared turbid and cloudy (Fig. 3a, b). The obtained results indicated the growth of indigenous iron- and sulfur-oxidizing bacteria in either 9K or S^o medium. A total of 10 morphologically distinct bacterial colonies growing on the modified 9K agar plates after 14 days of cultivation, followed by color change from beige to deep brown after 7-day elongated incubation were isolated (Fig. 3c). Nine sulfur-oxidizing bacteria were isolated on the modified S^o agar plates.

Table 2. Major constituents of the ore mineral and concentrate.

Elements	Ore minerals	Concentrate
Fe (%, w/w)	4.78	15.2
S (%, w/w)	2.20	10.1
As (%, w/w)	1.73	8.93
Pb (%, w/w)	0.43	1.67
Zn (%, w/w)	0.33	0.95
Cu (mg/kg)	95	171
Ag (mg/kg)	126	339
Au (mg/kg)	31.8	80.4

Analysis of 16S rRNA sequence revealed that majority of the isolates exhibited over 99% pairwise identity to the relative sequences of the type species deposited on GenBank (NCBI). Among isolated strains, the dominant genera were *Bacillus* (47.4%), followed by *Pseudomonas* (26.3%), and *Acidithiobacillus* (10.5%) (Fig. 3d). Interestingly, in the present study, the bacterial genera rarely reported to being isolated from the acidic environment as *Glutamicibacter, Providencia,* and *Stenotrophomonas,* were found with equally low occupation of 5.3% (Fig. 3d). Phylogenetic analysis consequently categorized all 19 isolates into 12 species belonging to 6 genera from three different phyla: *Bacillus* from Firmicutes phylum; *Glutamicibacter* from Actinobacteria phylum; *Providencia, Stenotrophomonas, Pseudomonas,* and *Acidithiobacillus* from Proteobacteria phylum (Fig. 4). The present findings are in agreement with a recent study demonstrating that *Pseudomonas* and *Bacillus* to be the most abundant genera in the bacterial consortia enriched from Au-containing ores taken at Ural Federal district, Russia [1]. As recently reported, heterotrophic *Bacillus* genus has paid attention to research and application of bioleaching to recover heavy metals from non-sulfidic ores originally lacking an energy source for autotrophic bacteria. Another recent report showed that *B. subtilis* was able to effectively solubilize

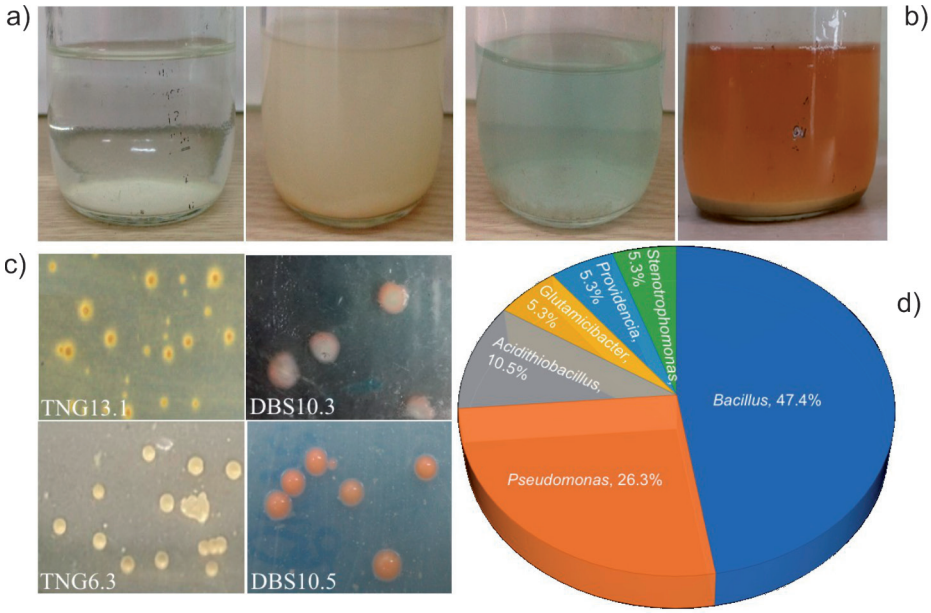

Fig. 3. Isolation and characterization of indigenous bacterial consortia in enrichment culture. Apparent color change in 9K a) and S^o b) culture media after inoculation with samples collected at Ta Nang mine. c) The colony characteristics of representative acidophilic bacteria including TNG13.1, DBS10.3, TNG6.3, and DBS10.5. d) Percentage of cultivable bacteria on genus level.

Ni metal from Ni-laterite ores through production of organic acids such as citric, gluconic and oxalic acids [15]. Among 9 *Bacillus* strains, six isolates including TNG6.2, DBS3.5, TNG3.1, TNG3.2, TNG3.4, TNG3.5 were classified as *B. subtilis* (Fig. 4). Especially, *Bacillus* spp. and *Pseudomonas* spp. are able to produce cyanide enabling gold dissolution from ores and urban solid wastes [29, 30]. To survive in an extreme environment, such acidophilic bacteria usually produce extracellular polymeric substances (EPSs) to enhance the attachment of bacteria to the solid material, and capture metal ions to protect cells [31].

Besides the above-mentioned *Bacillus* spp. and *Pseudomonas* spp., three species, three species *S. pavanii*, *P. rettgeri*, and *G. uratoxydans* were found from gold-bearing sulfide ores for the first time in this study. *Providencia* and *Stenotrophomonas* genera were previously used in bioleaching from low-grade copper ores [32], while the genus *Glutamicibacter* was only found in contaminated soils such as mercury and arsenic [33]. Since the genera *Providencia, Glutamicibacter,* and *Stenotrophomonas* are heterotrophs, the supplementation of carbon and nitrogen sources into batch culture is required to enhance the isolation step and bioleaching efficiencies. The growth of *Providencia* sp. JAT-1 improved in the presence of urea and sodium citrate, leading to the highest Cu extraction of 54.5% [32]. The use of acid-processed rice straw fostered the proportion of *Stenotrophomonas* in the bacterial community and Cu extraction efficiency [34]. Therefore, using metagenomic tool to fill the underlying gaps between microbial community and its functions can be an interesting subject for future studies.

Despite its low proportion observed, the genus *Acidithiobacillus* is the most extensively well-studied acidophiles for bio-mining and biodegradation of sulfide ore [10, 35]. Out of 19 isolates, the 16S rRNA sequence of isolate TNG13.1 displayed 99.9% homology with that of *A. ferrooxidans* ATCC 23270, leading to be a potential candidate for the bioleaching technique. As observed under SEM, the strain TNG13.1 was Gram-negative, non-spore-forming and rod-shaped, which is able to grow at a wide temperature range from 20 to 50ºC and pH range from 1.5 to 3.0 (Table 3). In addition to the growth under high ferrous concentration, the strain TNG13.1 also derived energy for growth by oxidation of sulfur and thiosulfate. Moreover, strain TNG13.1 proved to be resistant to an array of heavy metals such as arsenate, nickel, zinc, and copper, which can be due to the isolation source (Table 3). Combining morphological and genetic characteristics, the strain TNG13.1 was identified as *A. ferrooxidans* and deposited at VAST-Culture Collection of Microorganisms (VCCM) with the accession number VCCM 14177.

A. ferrooxidans has been widely isolated from various environmental sources such as ore, mine and wastewater, and coal [19, 36]. Under aerobic conditions, *A. ferrooxidans* oxidized the ferrous iron and/or reduced sulfur compounds present in ores to the ferric iron and sulfuric acid, respectively. As a result, the dissolution of sulfide minerals can be accelerated, which plays an important role in heavy metal extraction from ores and industrial wastes [5, 6]. In addition, application of bacterial consortia consisting of *A. ferrooxidans* for bioleaching are more effective than using pure bacterial strain [37].

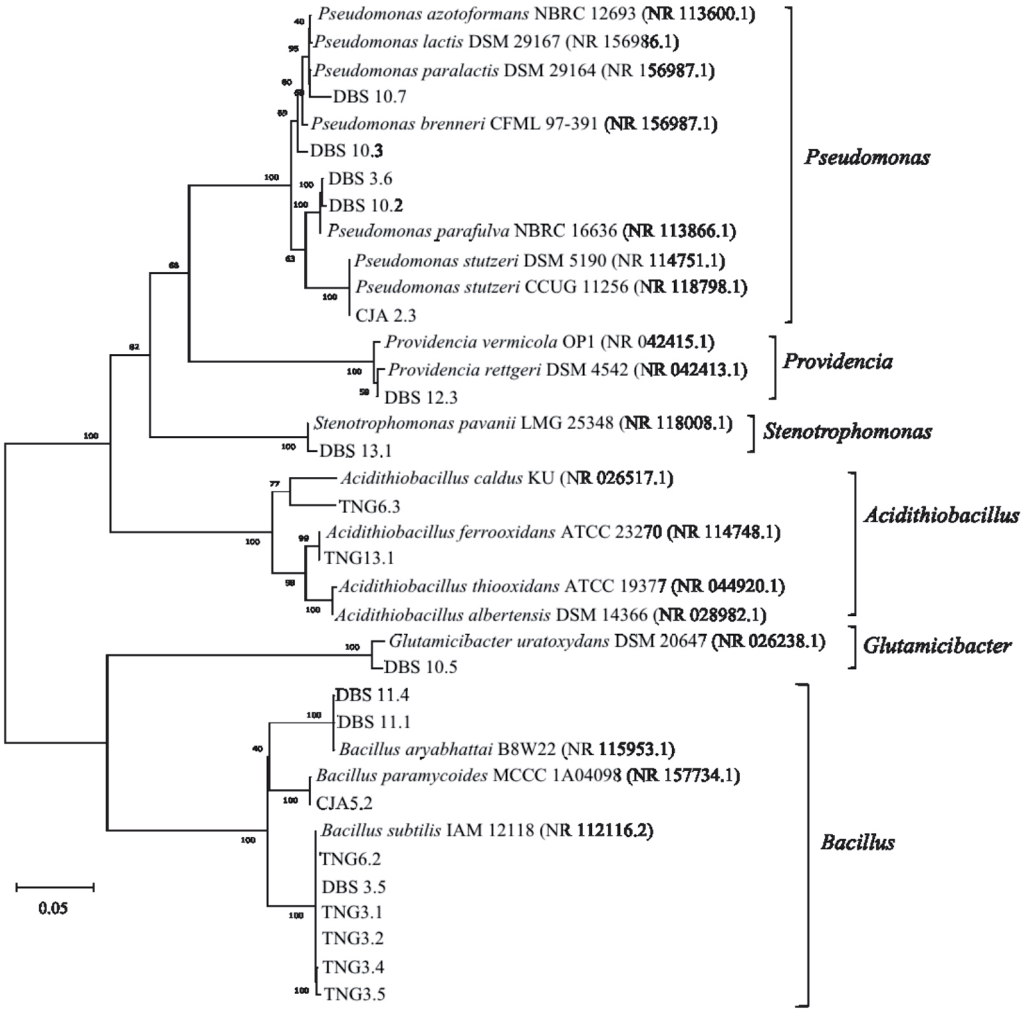

Fig. 4. Neighbor-joining phylogenetic tree of 19 isolates based on 16S rRNA sequence alignments with their closest reference strains (accession numbers appear in parentheses). Scale bar indicates 0.05 substitution per nucleotide; Bootstrap values greater than 50% are shown at the nodes and are based on 1000 replicates.

Notably, further analysis of 16S rRNA sequences interestingly revealed that the strain TNG6.3, originated from extremely acidic fluid leaking from mines, showed 95.1% sequence identity to the type strain *Acidithiobacillus caldus* KU (NR026517) (Fig. 4). To date, the genus *Acidithiobacillus* comprises seven species with validly taxonomic assignments as: *A. ferrooxidans, A. ferridurans, A. ferrivorans, A. ferriphilus, A. albertensis, A. caldus,* and *A. thiooxidans* [20, 38]. In addition, morphological characteristics indicated that the strain TNG6.3 was Gram-negative, rod-shaped and preferred temperature range from 30 to 50°*C* and pH range from 1.0 to 4.0 (Table 3). Strain TNG6.3 is much more resistant to As, Ni, Zn, Cu, and Pb than *A. caldus* and *A. ferrooxidans.* Moreover, this train did not grow on the medium supplemented with ferrous iron as the sole energy sources, but not with sulfur and thiosulfate. Thus, all above obtained results represented the strain TNG6.3 as a novel species in the genus *Acidithiobacillus.*

Application of Indigenous Bacterial Consortia for Bioleaching of Gold-Bearing Sulfide Ore Concentrate

In the present study, consortia of indigenous iron- and sulfur-oxidizing bacteria were assessed for heavy metal extraction from a sulfidic ore concentrate. During the bioleaching process, the pH remained consistently around 2.5. The *ex-situ* bioleaching yields of heavy metals reached the highest extraction of 99% for Ag after 21 days, followed by Zn, As, Fe, and Pb of 37.9%, 37%, 32.2%, and 13.8%, respectively (Fig. 5). Moreover, 34.1% of the second most abundant sulfur was leached from concentrate. Evaluation of the bioleaching efficiency by SEM showed the mass of ore structures damaged by bacterial oxidation, leading to tiny leaked grains with sizes ranging from 5 μm to 10 μm (Fig. 6).

Since Ta Nang gold ore contained only As in the form of arsenopyrite, the bioleaching efficiency of As may be due to the presence of genus *Acidithiobacillus* including *A. ferrooxidans* TNG13.1 and *Acidithiobacillus* sp.

Table 3. Physiological and phenotypic features of TNG13.1 and TNG6.3 strains.

Parameters	TNG13.1	TNG6.3
Morphological Characteristics		
Morphology	Rod	Rod
Gram staining	(-)	(-)
Physiological Properties		
NaCl range (%)	0 - 2	0 - 2
pH range	1.5 - 3.0	1.0 - 4.0
Temperature range (°C)	20 - 50	30 - 50
Electron donors		
Sulfur	+	+
Thiosulfate	+	+
Ferrous iron	+	-
Heavy metal resistance		
Arsenate (1000 mM)	+	+
Nickel (1000 mM)	+	+
Zinc (1000 mM)	+	+
Copper (1000 mM)	+	+
Lead (1000 mM)	-	+

TNG6.3. In addition, *A. ferrooxidans* TNG13.1 could oxidize Fe^{2+} to Fe^{3+}, leading to oxidation of the arsenopyrite to arsenic acid [39], while *Acidithiobacillus* sp. TNG6.3 was capable of oxidizing element sulfur and reducing inorganic sulfur compounds [40]. Comparing to single bacterial treatment, the advantage of using consortia of acidophiles in bioleaching can increase the resistance against the recalcitrant behavior of heavy metals and the attachments of bacterial cells to ore surface and stagnant solution [41]. Previous studies reported that *Bacillus* spp. and *Acidithiobacillus* spp. were able to produce a high number of EPSs to adhere to solid surfaces and overcome the hazard of extreme conditions [31, 42]. The retrieved results from trials therefore led to the hypothesis that bacteria interactions might take place under stress conditions. However, the bioleaching efficiency obtained in the present study was lower when comparing with previous studies [5, 6, 37]. The lower yields of bioleaching in present study model could be explained by the high content of heavy metals in treated ores, especially As and Fe, leading to the significantly inhibiting the growth of bacterial consortia. Apart from above-mentioned factors, Ag^{+} is also an important element involved in bioleaching efficiency. The absence of Ag^{+} in the leaching solution leads to the formation of passivating products, such as As_2S_2, As_2S_3, and S^{o}, on the surface of arsenopyrite particles [43]. Subsequently, a passivating film is generated and causes severe impedance to the subsequent leaching process. By contrast, the presence of Ag^{+} promotes the bioleaching of arsenopyrite through the formation of Ag_2S that causes the removal of the passivating film. Then, Ag_2S is oxidized by Fe^{3+} leading to the release of Ag^{+} back to the bioleaching solution [43]. Our result was comparable with this recent study, verifying the circular function of Ag in bioleaching ore.

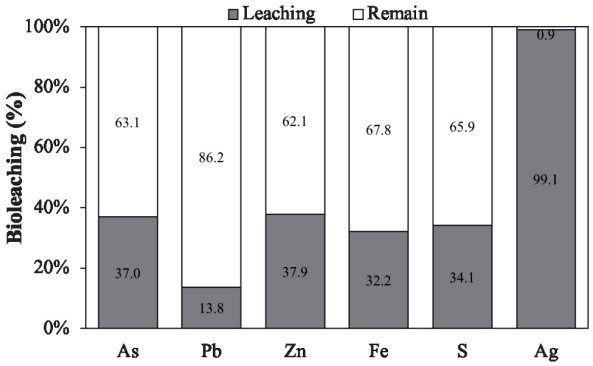

Fig. 5. Metal extraction from sulfide ores with indigenous bacterial consortia after 21 days of processing

On the other hand, the bioleaching achieved from present result also yielded low extraction of Zn and Pb from ore. Sajjad reported previously that the Zn bioleaching efficiency of up to 70.6% was gained under optimized conditions when using consortia of indigenous iron-oxidizing bacteria [10]. However,

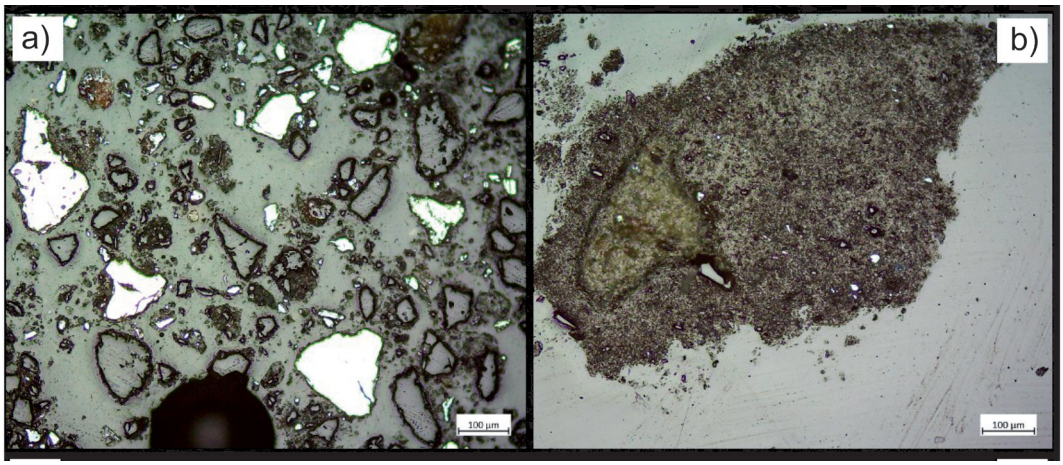

Fig. 6. Photomicrographs of the ore unexposed and exposed to indigenous bacterial consortia. a) Untreated ore. b) Treatment by indigenous bacterial consortia after 21 days of inoculation.

Zn and Fe contents in ore sample collected at Baiyin copper mine, China were 19-fold and 11.5-fold less than those in Ta Nang ore concentrate, respectively. So far, many studies reported that that the quality and chemical composition of the ore have contributed significantly contributed to the efficiencies of bioleaching processes [10, 13, 44]. The high content of heavy metals, including As, S, Fe, and Pb in Ta Nang concentrate, could be a reason for low extraction efficiency as compared to previous studies [10, 12, 13]. Since bioleaching efficiency depends on many factors such as pH, pulp density, and temperature, optimizing conditions for bioleaching precious metals of Ta Nang ore are required for further studies to improve extraction yield of metals.

Conclusions

The current study sheds light on bacterial consortia present in gold-bearing sulfide ore from the Ta Nang mine and its bioleaching potential. The refractory gold-bearing ores consist of several phases of sulfides such as arsenopyrite, pyrite, galena, and sphalerite, which holds main metals including Fe, As, Pb, Ag, Cu and especially Au. Enrichment of Ta Nang mine-surrounding samples and isolation of indigenous iron- and sulfur-oxidizing bacteria showed 19 acidophilic strains belonging to genera including previously described *Bacillus*, *Pseudomonas*, *Acidithiobacillus*, and three firstly reported heterotrophic *Glutamicibacter, Providencia,* and *Stenotrophomonas.* Among those, *Acidithiobacillus* sp. TNG6.3 represented a candidate of novel species. In addition, bioleaching using indigenous bacterial consortia was effective for the extraction of Ag (99.1%), Zn (37.9%), As (37.0%), and Fe (32.2%) from ore after 21 days of treatment. The new findings in this study therefore can open a potential opportunity for biobleaching of gold-bearing ores by indigenous acidophilic bacteria in Vietnam.

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Conflict of Interest

The authors declare no conflict of interest.

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