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# Acidophilic Bacterial Communities of Soil and Enrichment Cultures from Two Abandoned Mine Sites of the Korean Peninsula

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**Abstract − Bacterial diversity based on the denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S rRNA gene sequences was determined for soil samples from two abandoned mine sites and the corresponding enrichment cultures using soil sample as key inoculum. Sequencing analysis of DGGE bands obtained from both the soil samples matched mostly with sequences of uncultured and newly described organisms, or organisms recently associated with the acid mine drainage environment. However, the enrichment of soil samples in ferrous sulfate and elemental sulfur media yielded sequences that were consistent with well-known iron- and sulfur-oxidizing acidophilic bacteria. Analysis of enrichment cultures of soil samples from Dalsung mine revealed abundant γ-*Proteobacteria*, whereas that of Gubong mine sample displayed acidophilic groups of γ-*Proteobacteria*, α-*Proteobacteria*, *Actinobacteria* and *Firmicutes*. Chemical elemental analysis of the mine samples indicated that the Dalsung site contained more iron and sulfate along with other toxic components as compared with those of the Gubong site. Biogeochemistry was believed to be the primary control on the acidophilic bacterial group in the enrichment samples.**

**Key words : acidic soil, acidophilic bacteria, bacterial community, enrichment, PCR-DGGE**

## INTRODUCTION

Environmental pollution from abandoned mines is a serious concern due to acid mine drainage (AMD) laden with various toxic metals (Liang and Thomson 2009). AMD also contains high concentrations of Fe (III) and sulfate, and is a particular problem in abandoned mines with exposed sulfidic seams. AMD is also harmful to the environment because of sulfuric acid-based acidity (pH < 4.0). Environmental impacts due to acidity include dissolution of aluminum and iron in rocks and structures (e.g., steel), potentially impacting a human health and resulting in damage to installations and constructions. Exposure of soils and sediments to heavy metals can lead to their accumulation in the tropic chain, ultimately exacting serious ecological and health consequences (Schippers *et al.* 2000).

Coal and metal mining was an active industry in Korea in the early 20$^{th}$ century, but most of the mines were closed in the 1990s due to poor productivity and depletion of ore reserves. Due to mismanagement, the mines were abandoned during the early years of the present century. In recent years, heavy metals from abandoned mines have become an environmental health problem to people living in the nearby areas (Jung 2001). The source of the AMD problem has a microbiological origin, but microorganisms in abandoned Korean mine environments have been overlooked. Some previous studies have reported that extreme acidic conditions promote

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proliferation of acidophilic chemolithotrophs such as *Acidithiobacillus* and *Leptospirillum* spp. and heterotrophs such as *Acidiphilium*, *Acidimicrobium*, and *Alicyclobacillus* spp. (Kim *et al.* 2009; Denef *et al.* 2010). Analysis of indigenous microorganisms in abandoned mine environments is a prerequisite to understanding their activities and optimizing their geomicrobiology in these engineered ecosystems.

Several culture-dependent and culture-independent studies have addressed the microbial diversity in AMD impacted environments (Nicomrat *et al.* 2006; Tan *et al.* 2008). However, there are fewer studies examining microbial communities in acidic soil environments of mining areas. These studies need to be focused carefully, as the contaminated soil would affect the vegetation and health of the area of concern. Moreover, these acidic soils often contain acidophilic bacteria which have bio-hydrometallurgical importance (Gonzalez-Toril *et al.* 2010; Mishra and Rhee 2010). The consortia present in such soils may play a greater role in the metal dissolution process than axenic cultures. Mostly bioleaching processes have been reported to predict better leaching behavior in the case of mixed acidophilic consortia than axenic cultures (Qiu *et al.* 2005). If we would able to isolate and identify the major acidophilic bacteria those use in industrial bioleaching process it would be more effective as mixed culture has robustness to survive in extreme conditions.

The purpose of this study was to examine bacterial communities in soil samples from two abandoned sulfide mines in Korea: the Dalsung mine and the Gubong mine. Using soil samples from these sites, acidophilic bacteria (iron and sulfur oxidizers) that help in biomineral leaching process were enriched and identified. A culture-independent approach using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified 16S rRNA gene segments was used to analyze the bacterial population in samples from these sites. DNA was extracted directly from the soil samples as well as enrichment cultures for the PCR-DGGE and efforts were also made to characterize the DGGE profiles by sequencing analysis.

## MATERIALS AND METHODS

### 1. Sampling sites

The Dalsung mine is located in Sangwon-ri, Gachang-myeon, Dalsung-gu, which is 235 km south-east of Seoul (Fig. 1). The site was one of the biggest copper (Cu) mines in South Korea with maximum production in the 1960s. The ore minerals of the site contain key minerals such as; chalcopyrite ($CuFeS_2$) and wolframite [$(Fe, Mn)WO_4$] associated with bismuthinite ($Bi_2S_3$) and pyrite ($FeS_2$). The mine, closed and abandoned since 1973, has been reported to release high concentrations of metals such as Cu, Zn, Pb, Cd and Fe, along with sulfate, nitrate, and phosphate to an adjacent creek that merges to the Sincheon stream that flows through Daegu

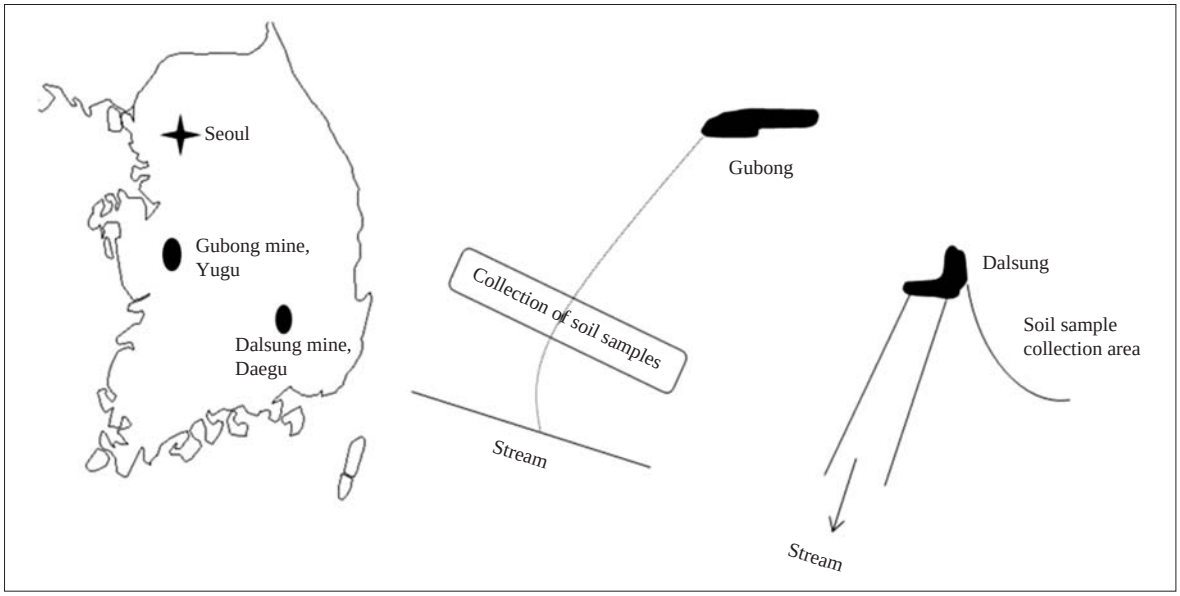


**Fig. 1.** Map of Gubong and Dalsung mines location and sampling stations. Soil samples were collected near to mining area.

City (Jung 2008).

The Gubong mine is located in the Yugu-Kwangcheon Metallogenic province, Chungnam-do, which is 125 km south of Seoul (Fig. 1). The major sulfide minerals are pyrite, sphalerite (ZnS), arsenopyrite (FeAsS), chalcopyrite, galena (PbS), and marcasite ($FeS_2$). The gangue minerals are quartz, K-feldspar, calcite, and chlorite. The Gubong mine was developed from 1908 to 1971. It is anticipated that in the next few years AMD will dispersing Cu, Zn, Pb, and Cd to the surroundings.

### 2. Sampling and chemical analysis

Sampling at both sites was conducted during the dry season when the ambient temperature was 15～20°C. Sampling sites were chosen for the presence of metal contaminated soils located at a distance of nearly 500 m away from the mining location. Surface soil samples (0～10 cm depth) from both mine-impacted areas were collected in polyvinyl bags and stored at 4°C for 24 h until analyzed. Both sets of samples were marked for identification; D=Dalsung soil, and G=Gubong soil. Samples for chemical analysis were air-dried for 48 h and ground with a pestle and mortar. A standard amount of sample was digested in aqua-regia and diluted to the required level before analysis. Partial elemental composition was analyzed with inductively coupled plasma emission spectroscopy JY38 Plus (Jobin Yvon, Instruments SA, France). The samples were analyzed in triplicate to find out the standard error and the standard deviation was fixed at (2.0). The obtained analysis value (mg $L^{-1}$) of the elements was finally normalized to wt%. Sulfate was analyzed gravimetrically (Jaffery *et al.* 1989). The pH was measured in the supernatant soil and deionized water in a 1 : 1 ratio after shaking for 2～3 h.

### 3. Enrichment cultivation

The collected soil samples were used as key inoculums to isolate the typical acidophilic iron and sulfur oxidizers during enrichment process. For enrichment of iron and sulfur oxidizing bacteria ferrous sulfate ($FeSO_4$) or elemental sulfur ($S^o$) was used as sole substrates in liquid medium, respectively. A slightly modified 9 K medium was used for the cultivation of iron oxidizing bacteria. The medium contained (per liter) 33.5 g $FeSO_4 \cdot 7H_2O$, 3.0 g $(NH_4)_2SO_4$, 0.5 g $K_2HPO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, and 0.1 g KCl, pH 2.0～2.10 adjusted with 1 N $H_2SO_4$. A similar chemical composition was used for the enrichment of sulfur oxidizing bacteria, except in place ferrous sulfate, elemental sulfur (1% w/v) was added and pH of the medium was adjusted to 2.8～3.0. The medium was inoculated with 5% (w/v) of soil from the mine sites. Cultures were incubated at 28°C on a shaker at 180 rpm. After 4 weeks, the solids were separated by filtration (Whatman# 42) and biomass was harvested by centrifugation (8000 g for 30 min) of culture filtrates.

### 4. DNA extraction and PCR amplification

DNA was extracted directly from soil samples as well as from the enrichment cultures. For the soils, a FastDNA Kit (MP Biomedicals, Ohio, USA) was used according to the manufacturer's protocol. DNA from enrichment cultures was extracted with a G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology, Daejeon, Korea). The DNA samples were subsequently used as templates for PCR amplification of the partial 16S rRNA gene. The V3 region (*E. coli* position 341～534) was amplified using nTaq-Tenuto DNA polymerase (Enzynomics, Korea) and primers, GC341F (5′-GCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGCCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) (Muyzer *et al.* 1993). The final PCR mixture (50 μL) contained 1.5 μL of each primer, 1 μL deoxynucleotide triphosphate mixture, 5 μL of 10X *Taq* buffer and 0.3 μL of *Taq* DNA polymerase. PCR was performed at 94°C for 5 min, 35 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec, followed by final extension at 72°C for 7 min. A control PCR without DNA was used to check for non-specific amplification.

### 5. DGGE analysis

PCR products (approximately 250 bp) of genomic DNA were separated by DGGE on 10% (w/v) polyacrylamide gels (acrylamide : bisacrylamide=37.5 : 1) with a 35～65% linear gradient of denaturant (100%=7 M urea with 40% (v/v) formamide). Gels were run for 16 h at 60 V in 1X TAE (Tris-acetate-EDTA) buffer at 60°C. Denaturing gels were run using DCode Universal Mutation Detection System (Bio-Rad, California, USA). The gels were visualized in an ultraviolet transilluminator after staining for 3 h in Gel-red (100 μL $L^{-1}$) in 1X TAE buffer. Gel images were obtained with the ChemDoc (Bio-Rad) gel documentation system. Indivi-

dual bands were selected for sequence analysis, excised, and held overnight in distilled water. Excised DNA samples were re-amplified with the same primers without having a GC clamp. PCR products were purified and sequenced by SolGent (Daejeon, Korea). Further details on DGGE analysis are described in a previous report (Lee *et al.* 2011).

Previously identified sequences with high similarity to the obtained results were searched using the BLAST database available from National Center for Biotechnology Information (NCBI). The highest similarity value along with the affiliated phylum and accession numbers are selectively reported in a tabular form in this study. Phylogenetic analysis was performed using MEGA 4.0 software (Tamura *et al.* 2007). Neighbor-joining method was employed to infer the tree topology. The reliability of the trees was tested by bootstrapping 1000 replicates generated with a random seed.

## RESULTS AND DISCUSSION

### 1. Chemical characterization of soil samples

The chemical characterization of the Dalsung and Gubong mine soil samples is given in Table 1. The Dalsung soil contained more iron (9.37 wt%) than the Gubong soil (5.72 wt%) and the sulfate content was also higher in the Dalsung sample. Furthermore, the Dalsung soil had higher levels of Cu, Cd, and Sb as compared the Gubong sample, whereas the latter contained more Al, Mg, Mn, and Ca. The pH values of the Dalsung and Gubong samples were 2.8 and 3.5, respectively. Due to low pH of Dalusng soil sample, the concentration of metal dissolution was higher. The level of acidity of contaminated soil sample might be proportional to metal ion concentration. As expected, these samples are mostly affected by AMD, which usually contains high concentration of iron and sulfate by oxidation and microbial mediated reactions.

**Table 1.** Physico-chemical analytical data of the soil samples collected from the two abandoned mines

| Element | Dalsung (wt%) | Gubong (wt%) |
|---|---|---|
| Al | 3.92 | 7.96 |
| Fe | 9.37 | 5.72 |
| Cu | 0.14 | 0.022 |
| Mg | 0.19 | 0.48 |
| Mn | 0.038 | 0.044 |
| Cd | 0.032 | 0.014 |
| Bi | < 0.004 | ND[a] |
| Sb | 0.034 | ND |
| Ca | 0.41 | 0.86 |
| Zn | 0.020 | 0.035 |
| Pb | 0.009 | 0.008 |
| As | < 0.01 | < 0.01 |
| $SO_4$ | 2.1 | 1.71 |
| pH | 2.8 | 3.5 |

[a]ND: not detected.

### 2. DGGE analysis of the soil samples

The V3 region of 16S rRNA gene was amplified from the DNA extracted from the soil samples and the enrichment cultures. The gel electrophoresis of 16S rDNA revealed more intense bands for the Dalsung sample as opposed to the Gubong sample (Fig. 2). The two soil samples showed a distinct band pattern having a number of faint bands. A total of fifteen and eight bands were excised from the gels of the Dalsung and Gubong soil samples, respectively. The nucleotide

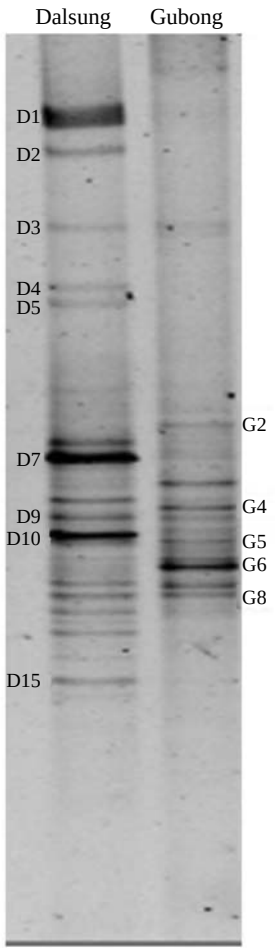


**Fig. 2.** DGGE profiles of 16S rRNA genes amplified from soil DNA from the Dalsung and Gubong sampling sites. Bands excised for sequencing are identified with numbers.

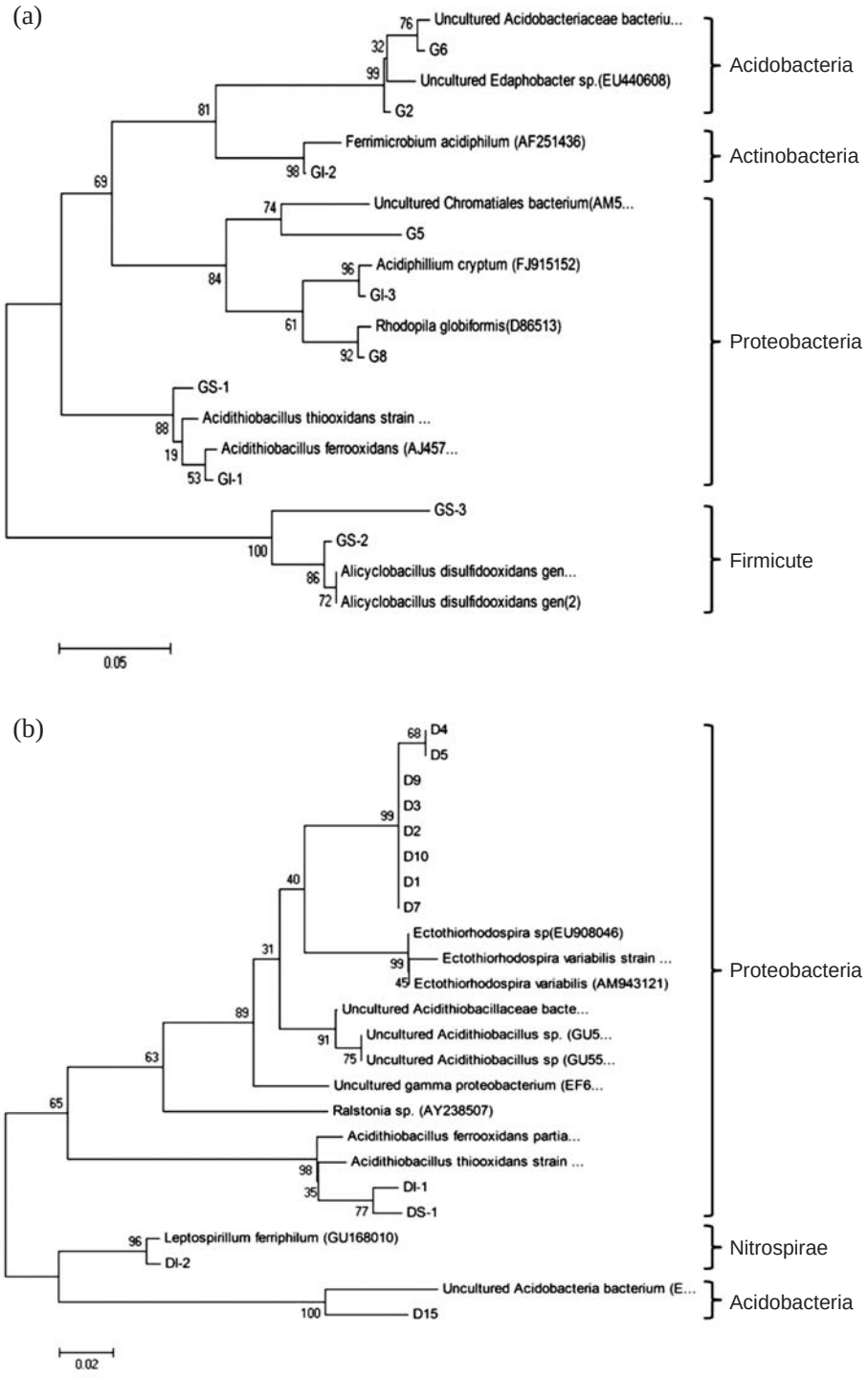


**Fig. 3.** Neighbour-joining phylogenetic tree of the bacteria in Gubong (a) and Dalsung (b) soils based on 16S rDNA sequences.

sequences of the bands were determined and aligned with those of reference strains in GenBank. However, the sequencing analysis yielded nine and five bands for Dalsung and Gubong samples, respectively, thereby indicating the rest of the bands showed either ambiguity or noise during the sequencing process. The sequence similarities to the known closest relatives of the strains are listed in Table 2 and the phylogenetic positions of the strains are shown in Fig. 3.

The sequencing results of the nine bands obtained from the Dalsung soil were related to *γ-*, *β-Proteobacteria*, and

**Table 2.** Sequence matches of 16S rRNA genes amplified from the soil samples and grouped according to DGGE pattern

(a) Dalsung soil

| Band name | Closest relative | Accession number | Similarity (%) | Cluster |
|---|---|---|---|---|
| D1 | *Uncultured Acidithiobacillaceae* bacterium | EF562120 | 98.2 | *γ-Proteobacteria* |
| D2 | *Ectothiorhodospira variabilis* strain | AM943122 | 96.5 | -do- |
| D3 | Uncultured *Acidithiobacillus* sp. | GU556432 | 98.2 | -do- |
| D4 | Uncultured gamma proteobacterium | EF663756 | 99.1 | -do- |
| D5 | *Ectothiorhodospira variabilis* | AM943121 | 96.5 | -do- |
| D7 | *Ectothiorhodospira* sp. | EU908046 | 96.5 | -do- |
| D9 | *Ralstonia* sp. | AY238507 | 81.1 | *β-Proteobacteria* |
| D10 | Uncultured *Acidithiobacillus* sp. | GU556432 | 98.2 | *γ-Proteobacteria* |
| D15 | Uncultured *Acidobacteria* bacterium | EF457374 | 93.3 | *Acidobacteria* |

(b) Gubong soil

| Band name | Closest relative | Accession number | Similarity (%) | Cluster |
|---|---|---|---|---|
| G2 | Uncultured *Edaphobacter* sp. | EU440608 | 92.9 | *Acidobacteria* |
| G4 | Uncultured *Actinobacterium* | AJ232701 | 87.8 | *Actinobacteria* |
| G5 | Uncultured *Chromatiales* bacterium | AM501856 | 85.1 | *γ-Proteobacteria* |
| G6 | Uncultured *Acidobacteriaceae* bacterium | AM940758 | 98.4 | *Acidobacteria* |
| G8 | *Rhodopila globiformis* | D86513 | 98.5 | *α-Proteobacteria* |

*Acidobacteria* phyla (Table 2a and Fig. 3). Sequences in two DGGE bands matched with 96.5% similarity to purple sulfur bacteria, *Ectothiorhodospira varibalis*. The match with the genus *Ectothiorhodospira* is unique because, to our knowledge, this genus has not been previously observed in soil communities in mine sites. It is purple, photosynthetic sulfur bacteria that have previously been isolated from soda lakes (Kulp *et al.* 2008). More investigations are needed related to its existence in mining area.

One of the bands, D9, had the most abundant sequence similarity with *β-Proteobacteria* groups. The sequencing matching showed low similarity with *Ralstonia* sp., which is a key soil bacterium and presumed to be a new genus found in the present soil sample. Additional analysis of the band D15 revealed the phylogenetic similarity with *Acidobacteria* group.

Likewise, the sequencing results of five bands of the Gubong soil sample exhibited the presence of *Acidobacteria*, *Proteobacteria*, and *Actinobacteria* as the major groups (Table 2b and Fig. 3). Among the studied bands, only G8 band matched with an identified species, particularly *Rhodopila globiformis* with 98.5% similarity, whereas the others showed phylogenetic similarity with uncultured bacteria.

Most of the findings in soil samples of Dalsung and Gubong stations were related to the presence of uncultured or newly isolates studied in different mining sites. These groups are reported to tolerate toxic heavy metals both in organic and inorganic media and able to live under natural and AMD conditions (Sahl *et al.* 2008). To identify the key and required acidophilic iron and sulfur bacteria, enrichment process was preferred.

### 3. DGGE analysis of the enrichment culture

The isolated DNA bands from enrichment culture were analyzed by DGGE. The bands obtained during DGGE experimental process were designated DI or DS for iron and sulfur oxidizers, respectively, in the Dalsung enrichment culture samples, and GI or GS for the corresponding respective Gubong enrichment culture samples (Fig. 4). The enrichment of the Dalsung samples in ferrous sulfate and elemental sulfur media resulted in growth of iron and sulfur oxidizers (Fig. 4). Bacteria enriched from the Dalsung soil matched with major phylotypes of γ-*Proteobacteria* in the sample. Analysis of bands DI-1 and DS-1 showed that the iron-oxidizing enrichment culture had a 98.1% similarity with *A. ferrooxidans*, whereas the sulfur-oxidizing culture matched (97.4%) with the sulfur-oxidizing chemolithoautotroph *A. thiooxidans* (Table 3a). *A. ferrooxidans* also appeared among sulfur oxidizer lineages, as this autotrophic bacterium can oxidize both iron and sulfur (Rawlings and Johnson, 2007). Additional analysis of the DI-2 band revealed 98.1% sequence similarity with *Leptospirillum ferrooxidans* (*Leptospirillum* group I).

The identified bacteria from enriched culture are typical bio-mining bacteria that could play an important role in mineral solubilization (Mishra *et al.* 2005). Abundance of *Aci-*

*dithiobacillus* spp. has also been widely reported in different AMD sites (Rawlings 2002). *Leptospirillum* spp. are often associated with the iron-oxidizing *Acidithiobacilli* (Rawlings and Johnson 2007). It has been reported that these bacteria are widely used in different processes such as heap, dump or tank bioleaching where they can tolerate very high concentrations of heavy metals. Particularly, *A. ferrooxidans* can have high level of metal resistance to Cu, Co, Ni, Zn, Cd and U and therefore the same has industrial importance in biohydrometallurgical process (Rawlings 2002; Mishra *et al.* 2008). The soil sample of Dalsung mine was found to be mixed up with various contaminated metals (shown in Table 1), and the enrichment media for iron oxidizers could suitably help in growing of these strains while using the same soil sample as key inoculums. Therefore, it can be predicted that these mesophilic acidophiles have tolerance power to survive or adapt with the toxic metal ions present in the soil vicinity. Moreover, the ambient conditions of the mining sites must be favorable for the sustainability of these strains.

On the other hand, the enrichment of the Gubong sample in iron and sulfur media was successful and yielded several bands of DNA from DGGE analysis (Fig. 4). The iron-oxidizing enrichment culture showed phylogenetic similarity with *A. ferrooxidans* (GI-1), and the sulfur-oxidizing enrichment culture could be matched with *A. thiooxidans* (GS-1) (Table 3b), as also was the case of the Dalsung samples. There was no lineage of the *Nitrospirae* group in this sample. The sequence analysis of band GI-2 showed a close match with family *Acidimicrobiaceae*, a gram-positive group, specifically with a heterotrophic iron-oxidizing *Ferrimicrobium acidiphilum* with 98.5% similarity. Similar heterotrophic iron-oxidizers have been found in microbial community studies at the Iron Mountain Mine which are generally associated with the autotrophs (Druschel *et al.* 2004).

In addition to this, lineages of *α-Proteobacteria* was obtained in the iron oxidizing enrichment culture of Gubong soil sample and showed the closest match with iron-reducing

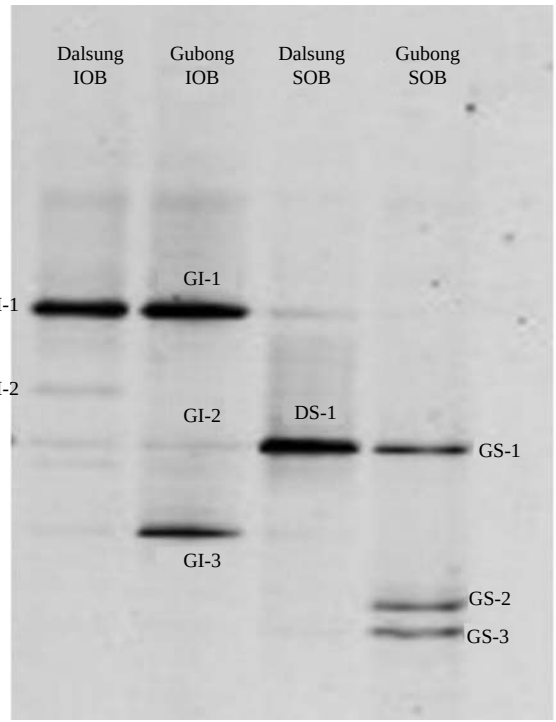


**Fig. 4.** DGGE profiles of 16S rRNA genes amplified from soil DNA from the Dalsung and Gubong sampling sites (Denaturant - 35%～65%, 250-bp fragments). Bands excised for sequencing are identified with letter/number codes. Iron oxidizing bacteria (I) and sulfur oxidizing bacteria (S) from the enrichment cultures are labeled.

**Table 3.** Identification of the closest matches of 16S rRNA genes amplified from the enriched Dalsung (a) and Gubong (b) cultures, based on DGGE

(a) Dalsung enrichment culture

| Band name | Closest relative | Accession number | Similarity (%) | Cluster |
|---|---|---|---|---|
| DI-1 | *Acidithiobacillus ferrooxidans* | FN811931 | 98.1 | *γ-Proteobacteria* |
| DI-2 | *Leptospirillum ferrooxidans* | GU168010 | 98.1 | Nitrospirae |
| DS-1 | *Acidithiobacillus thiooxidans* | GQ254654 | 97.4 | *γ-Proteobacteria* |

(b) Gubong enrichment culture

| Band name | Closest relative | Accession number | Similarity (%) | Cluster |
|---|---|---|---|---|
| GI-1 | *Acidithiobacillus ferrooxidans* | AF362022 | 98.1 | *γ-Proteobacteria* |
| GI-2 | *Ferrimicrobium acidiphilum* | AF251436 | 98.5 | Actinobacteria |
| GI-3 | *Acidiphilium cryptum* | FJ915152 | 98.5 | *α-Proteobacteria* |
| GS-1 | *Acidithiobacillus thiooxidans* | EU084705 | 97.4 | *γ-Proteobacteria* |
| GS-2 | *Alicyclobacillus disulfidooxidans* | AB089843 | 98.1 | *Firmicutes* |
| GS-3 | *Alicyclobacillus disulfidooxidans* | AB233323 | 87.0 | *Firmicutes* |

heterotrophs *Acidiphilium* spp. Detailed analysis of the sequences (GI-3 band) revealed close matches with *A. cryptum* of 98.5% similarity (Table 3). Such strain has been isolated from acid mine drainage sites or mine dumps and mine tailings (Tan *et al.* 2008). Finding of *Acidiphilium* with the lineage of iron oxidizing bacteria revealed that such heterotrophs have strong association with these co-cultures in the same medium. Previously the similar association has often found (Gurung and Chakraborty 2009).

The DNA bands of GS-2 and GS-3 represented presence of the *Firmicutes* group with a close relationship with *Alicyclobacillus* spp., which are heterotrophic gram-positive sulfur-oxidizers. The closest phylogenetic match was observed with *Alicyclobacillus disulfidooxidans*. It is known as a mixotroph that oxidize sulfur autotrophically and can simultaneously use organic carbon (Dufresne *et al.* 1996). Our results related to phylogenetic similarity with *A. disulfidooxidans* corroborating the data obtained in a study of heterotrophic acidophiles in an AMD treatment plant (Joe *et al.* 2007).

The detected bacteria in enrichment culture carrying Gubong soil sample are of autotrophs as well as heterotrophs, although the enrichment media was initially maintained in autotrophic conditions. It is believed that autotrophic acidophiles are known to excrete organic compounds that may be utilized simultaneously by acidophilic heterotrophs, which is a mutual association in the mine environment. In most of the mining waste dump, tailings, such association has been observed (Schippers *et al.* 2010). In the environmental samples these bacteria can thrive at high metal concentrations of different heavy metal ions. Most of the mining sites are complexed with hazardous toxic metal ions (e.g., As, Cu, Cd, Zn, Ni, Co, Hg, U) and these bacteria, with their adaptation property, can able to grow up at such metal-stress conditions. In the present context, both of the soil samples contain different toxic metal ions. The metal concentration in Gubong soil is comparatively less than Dalsung, although the pH lies in highly acidic zone. Such acidic pH zone might have stimulated the presence of acidophilic bacteria in such vicinity and therefore the enrichment process was conducted to find these cell cultures.

These acidophilic bacteria have been used in industrial process of bioleaching. Therefore, the enrichment process can be a suitable option to isolate such strains from the environmental sample, like mining sites, for further use in laboratory or industrial process. Moreover, the mixed culture of acidophiles has more robustness while using in different metal bioleaching process than the pure culture one. So, such enrichment process must be encouraged to find out the key isolates so that the bacteria strain can be used purposefully (Schippers *et al.* 2010).

## CONCLUSIONS

PCR-DGGE-based molecular approach has been an appropriate tool to find out the bacterial community in environmental samples. The initial DGGE analyses of soil samples only revealed the presence of uncultured and some bacteria species that have been recently described in environmentally contaminated samples. The enrichment process could easily propagate the presence of expected acidophilic iron and sulfur oxidizers by inoculating soil samples in bacterial specific media. The enrichment process could help in isolating specific iron oxidizers such as *A. ferrooxidans, L. ferrooxidans, Ferrimicrobium acidiphilium*, and sulfur oxidizers like *A. thiooxidans, Alicyclobacillus sulfidooxidans*. Additionally, lineage of heterotrophic iron reducer, *Acidiphilium cryptum*, was also found with these autotrophs. These bacteria are very well known in biomining consortia that are generally applied in mineral or ore bioleaching process to recover metal values. Additionally, these bacteria can able to tolerate high metal concentration and isolation of such mixed culture of industrially important would help in our further research on bacterial leaching process.

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