PROKARYOTIC DIVERSITY OF ACID MINE DRAINAGE PONDS IN ORE ENRICHMENT PLANT

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ABSTRACT
The biodiversity of acidophilic prokaryotes was determined in three AMD ponds (pH 2.7-6.5) in Turkey (Izmir-Halköy antimony ore enrichment plant) using 16S rRNA cloning and denaturing gradient gel electrophoresis methods. Water samples were taken two times in March 2014 and June 2015. The microbial diversity identified includes species such as Acidiphilium angustum, Acidocella sp., Ferrophilus acidophilus, Acidithiobacillus ferruginosus, Acidithiobacillus ferrooxidans, Acidiphilium rubrum, Thiomonas sp., Acidiphilium multivorans, Acidiphilum cryptum, Ferrovum mysofaciens, Acidocella alumimutans with the used techniques. In addition to, it has been determined that biodiversiti is variable in the operating mine pools. Acidithiobacillus ferruginosus, Acidiphilium angustum, and Acidiphilum rubrum are new records for Turkey.

Keywords: acidic mine drainage, acidophiles, prokaryotic diversity, Turkey

INTRODUCTION
Acid mine drainage (AMD) is the largest environmental problem caused by normally associated with mining activities (Garcia-Moyano et al., 2015). The mining wastewater is defined by properties such as low pH, high metal ions (e.g., iron, nickel, copper) and mineral concentrations. Acidophilic microorganisms living in this habitat are very interesting because of their adaptability to extreme pH values, their metabolic diversity, and their ability to be used in biomining applications. Especially, due to their availability in biomining and bioremediation applications, it is important to identify the acidophiles living in AMD. As determined in previous studies, the AMD microbial community changes over time (McGinness and Johnson, 1993; Edwards et al., 1999; Volant et al., 2014). The variety of microbial community is attached to seasonal changes and environmental conditions in AMD (Auld et al., 2017).

Classical microbial ecology methods remain limited in determining microbial diversity. Therefore, culture-independent methods such as 16s rRNA gene cloning, fluorescent in situ hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE) are often used to investigate the diversity of microbial community that adapts to this unique environments (Gonzalez-Toril et al., 2003; Nicomrat et al., 2006; Garcia-Moyano et al., 2015). Acidophilic chemolithotrophs such as Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Leptospirillum ferrooxidans have been identified in AMD which extremely low pH and high concentrations of iron, sulfates and other heavy metals (Edwards et al., 1999; Kuang et al., 2012). At the same time, the investigation of microbial community by molecular methods is difficult because of the inhibition of PCR by metals such as Fe and Cu (Nicomrat et al., 2006).

For the reason, DGGE and 16s rRNA gene cloning methods are used together to support each other in determining the microbial diversity of AMD.

The aim of this research was to determine the acidophilic prokaryotic community of acid mine drainage in ore enrichment plant Halköy, Izmir (Turkey). Our study area in Halköy is within the Menderes Massif in western Turkey. The antimony mine was discovered in 1870 and continued to operate until 1918 in Halköy area. After a long-standing period, production began again in 1974 (Akcay et al., 2006). Our results are the first knowledge about the prokaryotic community of the selected AMD area.

MATERIALS AND METHODS
Site description and sample collection
The water samples were collected from the operating antimony mine, ore enrichment plant Halköy site (38°5'28.09"N, 28°10'09.6"E) in Izmir, Turkey (Fig 1), in two different time (March 2014 and June 2015). The samples were taken three different points from mine area as drainage water (sample #1 and sample #4), iron oxide pool water (sample #2 and sample #5), and stationary water before iron oxide pool (sample #3 and sample #6) (Fig 2). Water samples were taken in sterile Duran bottles and were filtered from 0.2 um GTFP filter. In situ measurements for pH were made using a WTW Multi350i/SET (WTW, Germany). Metal concentrations of water samples were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Figure 1 Map of Halköy, Izmir (Turkey), where AMD ponds are located in antimony mine site
RESULTS

Characteristics of site and samples

The sampling site is antimony mine site being operated. The AMD samples were characterized by acidic pH values ranging from 2.7 to 6.5 and high concentrations of dissolved metals (Table 1). The sample points show the typical orange and red colors of dissolved ferric iron as shown in Figure 1. It was demonstrated that the samples had high iron, zinc, lead and manganese ratios. Treatment and neutralization studies of outlet water caused increase the pH value and decrease iron concentration between two sampling times (sample #5). This pH change also affected the prokaryotic diversity at the sample site (Table 1).

| Table 1 pH values and dissolved metal concentrations of water samples |
|-----------------|---|---|---|---|---|
| Samples code- pH | #1 | #2 | #3 | #4 | #5 | #6 |
| -----------------|----|----|----|----|----|
|                  | 3.0 | 2.9 | 2.7 | 2.8 | 6.5 | 3.6 |
| Elements         | Fe | 205.3 | 257.8 | * | 198.4 | 40.959 | 200.7 |
|                  | Zn | 1.693 | 7.547 | 28.98 | 2.006 | 5.328 | 15.952 |
|                  | Mn | 4.701 | * | * | 4.351 | 8.937 | * |
|                  | Cr | 0.185 | 0.302 | 0.784 | 0.145 | 0.267 | 0.935 |
|                  | Co | 0.066 | 1.794 | 6.643 | 0.045 | 1.539 | 5.628 |
|                  | Cu | 0.077 | 0.820 | 1.637 | 0.058 | 0.754 | 1.756 |
|                  | Ni | 0.241 | 1.624 | 6.481 | 0.321 | 0.954 | 4.522 |
|                  | Pb | 0.029 | 0.065 | 0.063 | 0.019 | 0.017 | 0.052 |

*Not determined because it has a high concentration.

Cloning

Clone library technique offers the opportunity to do very sensitive taxonomic studies. By this method, uncultured and uncategorized microorganisms are also possible to define (Sanz and Kochling, 2007). The clones from sample #1 and #3 YT_K1, YT_2, YT_K12, YT_K14, YT_K16 matched with an uncultured bacterium (%99), an uncultured archaeon (%97), Acidithiobacillus ferrivorans (%99), Acidithiobacillus ferr上午is (%99), and Acidithiobacillus rubrum, respectively. According to ARDRA of the plasmid insert, it has been determined that there are 4 and 14 different profiles from sample #2 and #5 (same points, different periods) coded samples taken in March 2014 and June 2015, respectively (Fig. 3). The sequence of clones (from water samples #2 YT_K3, YT_K4, YT_K7, and YT_K8 showed 99% similarities with Acidiphilum sp., Acidithiobacillus ferr上午is, Acidocella sp., Acidithiobacillus anguistrum, respectively. Clones from water sample #3 YT_K12 and YT_K13 matched with Acidithiobacillus ferrivorans (similarity 99%), YT_K14 and YT_K16 matched with Acidithiobacillus ferr上午is, Acidithiobacillus rubrum (similarity 99%), respectively. The archaeal profile was only determined on AMD samples #1 and #3. According to the results of sequence analysis the clones YT_K11, and YT_K20 matched with Ferroplasma acidithiobacillus (similarity 99%), and clone YT_K9 showed 99% similarities with Thermoplasmatales archaea. Other clones from water samples #4 and #6 showed 99% similarities with Acidiphilum sp. and Thiomonas sp. The sequencing results of the clones were given in Table 2.

Figure 3 ARDRA profiles of water samples (for bacteria: a, b, c, d, e, f, g: #1, #2, #3, #4, #5, #6; for archaea: h, i: #1, #3 respectively).
| Clone no | GenBank accession no | Organism | Water sample no | 16S rRNA gene, clone BioPlate3_A12 | GenBank similarity (%) | Number of sequencing base |
|----------|----------------------|----------|----------------|-----------------------------------|------------------------|--------------------------|
| YT_K1    | MH057124             | Uncultured bacterium partial 16S rRNA gene, clone BioPlate3_A12 | 1 | 99% | 754 |
| YT_K2    | MH057125             | Uncultured archaean for 16S rRNA, partial sequence, clone: H02859463 AB600346.1 | 1 | 97% | 650 |
| YT_K3    | MH057126             | Acidiphilium sp. DB5-4 16S ribosomal RNA gene, partial sequence EU003879.1 | 2 | 99% | 1004 |
| YT_K4    | MH057127             | Acidithiobacillus ferrooxidans strain DSM 100412 tyrosyl-rRNA synthetase gene, complete cds: K7052949.1 | 2 | 99% | 850 |
| YT_K5    | MH057128             | Acidocella sp. M21 16S ribosomal RNA gene, partial sequence AY765998.1 | 2 | 99% | 854 |
| YT_K6    | MH057129             | Acidiphilum angustum strain Colony6 16S ribosomal RNA gene, partial sequence K924944.1 | 2 | 99% | 873 |
| YT_K7    | MH057130             | Uncultured Thermoplasmatalesia archean clone B_DKE 16S ribosomal RNA gene, partial sequence KY582129.1 | 3 | 99% | 546 |
| YT_K8    | MH057131             | Uncultured archaean clone AMD-archD26 16S ribosomal RNA gene, partial sequence KC537536.1 | 3 | 98% | 941 |
| YT_K9    | MH057132             | Ferroplasma acidiphilum strain Y, complete genome CP015363.1 | 3 | 99% | 809 |
| YT_K10   | MH057133             | Acidithiobacillus ferrooxidans strain NO-37 16S ribosomal RNA gene, partial sequence NR_144620.1 | 3 | 102% | 1004 |
| YT_K11   | MH057134             | Acidithiobacillus ferrooxidans strain NO-37 16S ribosomal RNA gene, partial sequence KF031176.1 | 3 | 99% | 910 |
| YT_K12   | MH057135             | Acidithiobacillus ferrooxidans strain DSM 100412 tyrosyl-rRNA synthetase gene, complete cds: K7052949.1 | 3 | 99% | 1016 |
| YT_K13   | MH057136             | Acidiphilum rubrum strain Colony11 16S ribosomal RNA gene, partial sequence K924944.1 | 3 | 99% | 865 |
| YT_K14   | MH057137             | Uncultured Thiomonas sp. clone dw10 16S ribosomal RNA gene, partial sequence KF287769.1 | 4 | 99% | 862 |
| YT_K15   | MH057138             | Uncultured bacterium clone RTK11-ant10-eto8-W 16S ribosomal RNA gene, partial sequence JF737920.1 | 4 | 100% | 862 |
| YT_K16   | MH057139             | Ferroplasmid acidiphilum strain D-m 16S ribosomal RNA gene, partial sequence KX694511.1 | 3 | 99% | 747 |
| YT_K17   | MH057140             | Uncultured bacterium clone NC02n-bac_d12 16S ribosomal RNA gene, partial sequence KC619550.1 | 4 | 98% | 671 |
| YT_K18   | MH057141             | Uncultured bacterium partial 16S rRNA gene, clone BioPlate2_H11 HE587131.1 | 4 | 95% | 894 |
| YT_K19   | MH057142             | Acidiphilum multivorans strain AIU301 16S ribosomal RNA gene, complete sequence NR_073427.1 | 4 | 99% | 877 |
| YT_K20   | MH057143             | Uncultured bacterium clone LRE22B44 16S ribosomal RNA gene, partial sequence HQ420129.1 | 4 | 99% | 963 |
| YT_K21   | MH057144             | Acidiphilum crytopyt JF-5, complete genome CP000697.1 | 4 | 99% | 824 |
| YT_K22   | MH057145             | Uncultured bacterium clone A13 16S ribosomal RNA gene, partial sequence KF301176.1 | 5 | 98% | 805 |
| YT_K23   | MH057146             | Uncultured alpha proteobacterium clone AKY8835 16S ribosomal RNA gene, partial sequence KF92070.1 | 5 | 99% | 809 |
| YT_K24   | MH057147             | Uncultured bacterium clone BE326_BF2_out4 16S ribosomal RNA gene, partial sequence JX29644.7 | 5 | 96% | 848 |
| YT_K25   | MH057148             | Uncultured bacterium clone BD28WS24 16S ribosomal RNA gene, partial sequence KF841202.1 | 5 | 98% | 668 |
| YT_K26   | MH057149             | Uncultured bacterium clone T7-82 16S ribosomal RNA gene, partial sequence GQ487952.1 | 5 | 99% | 857 |
| YT_K27   | MH057150             | Uncultured bacterium clone 200T36 16S ribosomal RNA gene, partial sequence DQ110071.1 | 5 | 98% | 816 |
| YT_K28   | MH057151             | Uncultured bacterium partial 16S rRNA gene, clone Iron-rich microbial mat clone Hoffnungsstollen_5-1A_E11 LN870830.1 | 5 | 96% | 726 |
| YT_K29   | MH057152             | Uncultured bacterium clone SX2-12 16S ribosomal RNA gene, partial sequence DQ469219.1 | 5 | 99% | 858 |
| YT_K30   | MH057153             | Uncultured bacterium clone EPS09_OK_001A_57 16S ribosomal RNA gene, partial sequence JX521231.1 | 5 | 99% | 838 |
| YT_K31   | MH057154             | Uncultured bacterium clone F-19 16S ribosomal RNA gene, partial sequence HQ132424.1 | 5 | 97% | 702 |
| YT_K32   | MH057155             | Uncultured bacterium clone SH201209-31 16S ribosomal RNA gene, partial sequence KX508599.1 | 5 | 98% | 646 |
| YT_K33   | MH057156             | Uncultured bacterium clone SX2-10 16S ribosomal RNA gene, partial sequence DQ469201.1 | 5 | 99% | 752 |
| YT_K34   | MH057157             | Uncultured bacterium clone AMD1-Plate1-B06 16S ribosomal RNA gene, partial sequence JN127499.1 | 5 | 95% | 455 |
| YT_K35   | MH057158             | Uncultured bacterium clone BCWCWP1A42 16S ribosomal RNA gene, partial sequence FJ598380.1 | 5 | 99% | 925 |
| YT_K36   | MH057159             | Acidiphilum sp. BGR 75a 16S ribosomal RNA gene, partial sequence GU167999.1 | 6 | 99% | 843 |
| YT_K37   | MH057160             | Uncultured Thiomonas sp. clone S-K6-C18 16S ribosomal RNA gene, partial sequence EF612428.1 | 6 | 99% | 878 |
| YT_K38   | MH057161             | Uncultured bacterium partial 16S rRNA gene, clone BioPlate2_G10 HE587210.1 | 6 | 99% | 798 |
| YT_K39   | MH057162             | Uncultured bacterium partial 16S rRNA gene, clone BioPlate2_A10 HE587319.1 | 6 | 99% | 827 |
DGGE analyses were performed with each different sample to determine the level of microbial diversity in the mine area. (Fig 4). The samples collected from the same sample points determined to have different profiles. Especially, due to pH change, it was found that bacterial diversity quite different sample #5 and sample #2. Blast analyses of DGGE bands sequences are given in Table 3. Archaeal diversity was determined only in sample #1 and sample #3. The sequence of bands YT_D1, YT_D2, YT_D3, YT_D4, and YT_D5 showed similarity with uncultured archaean clones, as a show that in Table 3. Differences were observed in the DGGE profiles of taken water samples at different times from the same sampling points. It was determined that the sample #4 have more bacterial diversity from sample #1. According to sequence analysis results, there are bands matched with Ferrovum myxofaciens, Acidithiobacillus ferrooxidans, Acidithiobacillus ferrivorans, Acidithiobacillus ferriphilus and uncultured Acidithiobacillus sp. in sample #1. In the case of water sample #4, it was determined that the majority of the species are Acidocella (Table 3). Although samples #2 and #5 were taken from the same spot, it was thought that the change in pH at sample #5 caused the formation of different profiles. Bands at sample #5 were showed similarity with Acidocella aluminidurans, uncultured Acidocella sp. and uncultured Acidiphilium sp. In sample #3, bacterial diversity is less than in sample #6. The band of sample #6, it was determined to match with Thiomonas sp. (Table 3).

Accession numbers and construction phylogenetic tree of nucleotide sequences

16S rRNA gene sequences were deposited in GenBank under accession numbers MH057089-H057162. In order to determine the phylogenetic group, the phylogenetic tree was constructed with sequences obtained by 16 rRNA clone library and DGGE analyses (Fig 5, 6).
Table 3: Prokaryotes of environmental water samples closest matches of DGGE bands in GenBank

| Clone no | GenBank accession no | Organism | Water sample no | 16S rRNA Gene Sequence | Number of sequencing base |
|----------|----------------------|----------|----------------|------------------------|--------------------------|
| YT_D1    | MH057089             | Uncultured archaeon clone AMD-archI04 16S ribosomal RNA gene sequence | 3 | 99% | 520 |
| YT_D2    | MH057090             | Uncultured Thermoplasmatales archaeon clone B_DKE 16S ribosomal RNA gene, partial sequence KY825129.1 | 3 | 100% | 437 |
| YT_D3    | MH057091             | Uncultured euryarchaeote clone RT10A_3A_4 16S ribosomal RNA gene, partial sequence EF441876.1 | 1 | 99% | 457 |
| YT_D4    | MH057092             | Uncultured archaeon clone AMD-archF17 16S ribosomal RNA gene sequence | 1 | 99% | 449 |
| YT_D5    | MH057093             | Uncultured archaeon clone AMD-archE05 16S ribosomal RNA gene sequence | 1 | 99% | 458 |
| YT_D7    | MH057094             | Uncultured Acidiphilium sp. partial 16S rRNA gene, clone A4_89 AM940514.1 | 5 | 93% | 542 |
| YT_D8    | MH057095             | Uncultured Acidocella sp. clone M3O29 16S ribosomal RNA gene, partial sequence JX68779.1 | 5 | 94% | 519 |
| YT_D9    | MH057096             | Ferrovum mysofaciens strain P5G 16S ribosomal RNA gene, partial sequence NR_117782.1 | 1 | 97% | 477 |
| YT_D10   | MH057097             | Acidithiobacillus ferrooxidans strain S1 16S ribosomal RNA gene, partial sequence EF3913262.1 | 1 | 95% | 465 |
| YT_D11   | MH057098             | Acidithiobacillus ferrophilus strain M20 16S ribosomal RNA, partial sequence NR_147744.1 | 1 | 98% | 308 |
| YT_D12   | MH057099             | Acidithiobacillus ferrovarans strain NO-37 16S ribosomal RNA gene, partial sequence NR_114620.1 | 1 | 99% | 297 |
| YT_D13   | MH057100             | Acidithiobacillus sp. YB18 16S ribosomal RNA gene, partial sequence KM369991.1 | 1 | 99% | 443 |
| YT_D15   | MH057101             | Ferrovum mysofaciens strain EHSH 16S ribosomal RNA gene, partial sequence KC155322.1 | 1 | 99% | 522 |
| YT_D17   | MH057102             | Acidocella sp. CFR23 16S ribosomal RNA gene, partial sequence KC662252.1 | 2 | 98% | 529 |
| YT_D18   | MH057103             | Uncultured Acidocella sp. clone M3O29 16S ribosomal RNA gene, partial sequence JX68779.1 | 2 | 95% | 515 |
| YT_D19   | MH057104             | Acidiphilum sp. strain MPLK-302 16S ribosomal RNA gene, partial sequence KX689773.1 | 2 | 93% | 542 |
| YT_D20   | MH057105             | Uncultured Acidocella sp. clone M3O29 16S ribosomal RNA gene, partial sequence JX68779.1 | 2 | 93% | 511 |
| YT_D22   | MH057106             | Acidocella aquatica gene for 16S ribosomal RNA, partial sequence LC199502.1 | 3 | 93% | 562 |
| YT_D23   | MH057107             | Acidocella facilis strain PW2 16S ribosomal RNA gene, partial sequence NR_025852.1 | 3 | 95% | 520 |
| YT_D24   | MH057108             | Acidiphilum sp. strain MPLK-302 16S ribosomal RNA gene, partial sequence KX689773.1 | 3 | 91% | 532 |
| YT_D25   | MH057109             | Acidocella facilis strain PW2 16S ribosomal RNA gene, partial sequence NR_025852.1 | 3 | 86% | 523 |
| YT_D27   | MH057110             | Uncultured Acidiphilum sp. partial 16S rRNA gene, clone A4_89 AM940514.1 | 4 | 93% | 544 |
| YT_D28   | MH057111             | Acidiphilum alminidurans strain AL46 16S ribosomal RNA gene, partial sequence NR_112716.1 | 4 | 97% | 546 |
| YT_D29   | MH057112             | Acidiphilum angustum strain KLB 16S ribosomal RNA gene, partial sequence NR_025852.1 | 4 | 91% | 523 |
| YT_D30   | MH057113             | Uncultured Acidocella sp. clone G16O27 16S ribosomal RNA gene, partial sequence JX685682.1 | 4 | 95% | 533 |
| YT_D31   | MH057114             | Uncultured Acidocella sp. clone M2C26 16S ribosomal RNA gene, partial sequence JX686898.1 | 4 | 97% | 519 |
| YT_D32   | MH057115             | Uncultured Acidocella sp. clone M3O29 16S ribosomal RNA gene, partial sequence JX68779.1 | 4 | 93% | 525 |
| YT_D33   | MH057116             | Acidiphilum multivorans strain AU1301 16S ribosomal RNA, complete sequence NR_074327.1 | 4 | 94% | 513 |
| YT_D34   | MH057117             | Acidocella aminolytica strain 101 16S ribosomal RNA gene, partial sequence NR_025849.1 | 4 | 91% | 481 |
| YT_D35   | MH057118             | Uncultured alpha proteobacterium clone LKC_Acid_38p 16S ribosomal RNA gene, partial sequence EU038054.1 | 4 | 87% | 534 |
| YT_D36   | MH057119             | Acidocella facilis strain PW2 16S ribosomal RNA gene, partial sequence NR_025852.1 | 4 | 93% | 505 |
| YT_D38   | MH057120             | Acidocella alminidurans strain NBRC 104303 16S ribosomal RNA gene, partial sequence NR_114266.1 | 5 | 95% | 514 |
| YT_D40   | MH057121             | Acidocella sp. strain MPLK-72 16S ribosomal RNA gene, partial sequence KX689753.1 | 5 | 93% | 517 |
| YT_D41   | MH057122             | Uncultured alpha proteobacterium gene for 16S ribosomal RNA, partial sequence clone: AN037 AB809984.1 | 5 | 97% | 519 |
| YT_D43   | MH057123             | Thiemonar sp. Dg-E17 partial 16S rRNA gene, isolate Dg-E17 LN864672.1 | 6 | 96% | 521 |

437 organisms were identified. The similarity of matches ranged from 91% to 100%.
Determining of communities in AMD provides important clues in terms of the diversity and functions of these organisms with the development of molecular approaches. Furthermore, the development of sequencing technologies is rare in acidic environments allowing the identification of numerous taxa found (Kuang et al., 2012; Aliaga Goltzsm et al., 2014). The pH value and metal concentrations of the AMD ponds seem to be suitable for existence in the determined species. Especially, high iron concentration is determinant for the life of species such as Acidithiobacillus ferrivorans, Acidithiobacillus ferrilphilus. Mendez and coworkers have determined Acidithiobacillus ferrivorans with similar properties samples (pH 2.7, Fe: 38.100 mg kg⁻¹) (Mendez et al., 2008). One of the sequences identified in the study of microbial diversity of Xiang Mountain sulfide mine was matched with Ferrovoron myosofaciens, which was recently isolated from an abandoned copper mine (pH 3.0, Fe: 100.6 mg L⁻¹) (Hao et al., 2010). Acidiphilium sp. was determined by community composition analysis in acid mine drainage from Fankou Ph/Zn mine, China (pH 1.9, Fe 1240 mg L⁻¹) (Chen et al., 2013). Autotrophic and heterotrophic groups were identified from selected sample points in this study. It is noteworthy that archaea domain members cannot be determined from samples #4 and #6 in June 2015, while the archaea were determined in March 2014 (samples #1 and #3). It is thought that this may be due to the continuing effects of the mining activity. Changes in the environment created by anthropogenic effects are rapidly affecting microbial diversity. Ferroplasma spp., which was identified in this study, has been determined as dominant after the period of the acidification processes of mine wastes (Chen et al., 2013; Chen et al., 2014b).

As seen in Figure 7, the variety of prokaryotic diversity was observed by used molecular techniques in AMD ponds. As one of the reasons for this, some of the technical difficulties of the methods used can be shown. While sequencing over a short region of DNA by the DGGE method, the cloning longer base chain can be evaluated. With all of these drawbacks, both methods complement each other’s deficiencies so that can determine the prokaryotic diversity of AMD ponds to a significant extent. For example, Ferroplasma sp., Thiomonas sp., species could be identified only by 16S cloning, while Ferrovoron sp. could be identified only by DGGE method. Other less frequently detected bacterial taxa the heterotrophic grows Thiomonas spp. (Chen et al., 2016) that has been isolated was not detected by molecular techniques, probably reflecting its low abundance (Bruneel et al., 2005).

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Figure 6 Phylogenetic tree based on 16S rRNA gene sequences of DGGE bands.

Figure 7 The diversity profile of AMD ponds obtained by 16S rRNA clone libraries and DGGE methods.

Gonzalez-Toril and collaborators studied by DGGGE using 16S rRNA and, by 16S rRNA gene amplification for research molecular ecology an extreme acidic Tinto River (Spain). Comparative sequence analysis of DGGGE bands determined the identity of the respective microorganisms such like Leptospirillium spp., Acidithiobacillus ferrooxidans, Acidithiobacillus spp., Ferrimonas acidiphilum, Ferroplasma acidiphilum, and Thermoplasmata acidophilum (Gonzalez-Topor et al., 2003). Aytar and coworkers have identified prokaryotic diversity in two different AMD sites (Balya and Çan) in Turkey (Aytar et al., 2014). Some species identified in this study were as Acidithiobacillus sp., Leptospirillium spp., Ferroplasma sp., Saglam and colleagues determined bacterial diversity in the Acius effluent with cloning 16S rRNA sequences. The bacterial population was identified to occur Acidithiobacillus ferrooxidans, Ferrovoron myosofaciens, Leptospirillium ferrooxidans, Acidithiobacillus ferrooxidans, Acidocella facilis, Acidocella aluminodea, Acidiphilium curvatum, Acidiphilium multivorans, Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Acidiphilium curvatum (Saglam et al., 2016). Alphaproteobacteria (Acidiphilium, Acidocella) (Liu et al., 2011; Falagan et al., 2013). Betaproteobacteria (Thiomonas, Ferrovoron), (Mendez et al., 2008; Johnson et al., 2013). Acidithiobacillus (Acidithiobacillus) (Williams and Kelly, 2013) are seen in other studies. Betaproteobacteria, mostly belonging to the ‘Ferrovor’ genus, was clearly predominant in the community below middle pH conditions, whereas Alphaproteobacteria, Euryarchaeota, Gammaproteobacteria, and Nitrospira exposed a powerful adaptation to more acidic conditions (Kuang et al., 2012).

As a result of matches, it has been determined that some sequences are similar to those of Acidithiobacillus ferrooxidans (Nunez et al., 2017). Acidiphilium angustum, and Acidiphilium rubrum (Auld et al., 2013). These species are new records for Turkey. The iron-oxidizing acidithiobacillus Acidithiobacillus ferrilphilus was also isolated from different global locations such as metal-rich waters sample deep within the mine (Kay et al., 2014). The type strain M20 was isolated from a pond in a geothermal area of Montserrat (pH 1.5-3.0) (West Indies) which lived optimally pH 2.0 and 30 °C of temperature (Atkinson et al., 2000). It was determined later that this strain separated from other acidithiobacilli (Falagan and Johnson, 2016). The clones and DGGE bands sequences showed to match the most Acidiphilium genus. The mesophilic and obligately acidophilic bacteria Acidiphilium angustum grow in the pH range of 2.0-5.9. The clone YT_K8 matched with Acidiphilium angustum (99% similarity) was obtained from water sample #2 (pH 2.9). Aytar and coworkers have isolated Acidiphilium rubrum from an AMD site in Copper Cliff, Ontario (Auld et al., 2013). The isolate was isolated from AMD water at pH 2.5, similar to the water sample (pH 2.7) in which identified YT_K16.

CONCLUSION

Future studies will focus on the roles of these species on the biogeochemical cycles of the region where microbial diversity is determined. The AMD is also likely to contain new species. To better understand community dynamics in acid formation, more studies are needed to identify predominant species in AMD environments.
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