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Bacterial diversity of the abandoned mamut copper mine in Sabah, Malaysia and its correlation with copper contamination

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ABSTRACT

Aims: The former Mamut Copper Mine, acid mine drainage site represents an anthropogenic altered landscape characterized by its acidic topsoil which is contaminated primarily with copper. Even though the mining operation was ceased at 1999, the bacterial diversity in this area has never been investigated. This study was conducted to ascertain the bacterial diversity of this abandoned copper mine and correlate it to the copper concentration in the soil.
Methodology and results: Soil samples were collected from 7 sites near the mine pit and the vicinity. Soil samples were assessed for soil copper elemental concentration using inductively coupled plasma optical emission spectrometry and bacteria were isolated via serial dilution followed by culture on nutrient agar plates. Phylogenetic analysis was done based on the full-length sequences of 16S rRNA gene. Twenty-four phylotypes were obtained from the 7 locations which originated from the phyla Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria. The results of the study indicated that site 2 (6.030223°; 116.658030°), located in between the mine pit and the mine factory with a copper concentration of 88.96 ppm, possessed the most diverse bacterial community with a Shannon diversity index (H) of 1.68, evenness (E_H) of 0.94 and richness (S) of 6.
Conclusion, significance and impact of study: Current study revealed that there was a positive correlation between the copper concentration and the H index and the richness, but this was not reflected in the evenness. This is the first report of bacterial diversity from the former Mamut Copper Mine site. The data provided a valuable insight for the future monitoring of the bacterial community in this ecologically important niche.

Keywords: Copper mine, soil, bacteria, diversity, phylogenetic, 16S rRNA

INTRODUCTION

The former Mamut Copper Mine was the largest open pit copper mine in Malaysia, operated at Ranau, Sabah since 1975 and was the largest producer of gold and silver as by product of copper (Azizli *et al*., 1995). Followed by the production of minerals, the former copper mine had also generated several hundred million tonnes of mining waste and tailings. Since the operation ceased with the closure of the mine in 1999, the anthropogenic disturbance caused by mining activities had provided a great challenge to deal with the contamination at this site and its vicinity. With the help of consistent year-long precipitation in the tropical region, the overburden generated by the mining activities were leached into the surrounding water bodies. Acid mine drainage was evident in this area and it was reported in 2014 that the sediment collected from the adjacent Mamut River possessed high copper concentration that had exceeded the safety limit (Jopony and Tongkul, 2009; Ali *et al*., 2014). The concentration of copper was increased as much as 20-38 times as compared to the concentration tested in 2004 (Ali *et al*., 2004; Ali *et al*., 2014). For safety reasons, most of the activities in this area were suspended, leading to the abandonment of the mine site. Up to date, this former mine site and its surrounding areas were under the jurisdiction of the Mineral and Geoscience Department of Sabah (JMG).

Despite the hazardous effects caused by the mining waste in the surrounding areas of mine site, certain microbes such as bacteria have been reported to survive and thrive in this type of extreme environment (Mendez *et al*., 2008; Thavamani *et al*., 2017; Fernandes *et al*., 2018). The bacterial community profile for each mine site may differ based on the history of development, the types of mining products generated and the primary environmental factors such as pH, temperature, concentration of metals and so on. (Mendez-Garcia *et al*., 2015). Based on the theory proposed by previous studies, the effects of extreme environment are constantly resulting in new

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adaptations and shaping the microbial community through the process of colonization and species sorting (Baker and Banfield, 2003). However, certain bacteria are also adapted to the contamination at the mine site through different mechanisms such as biosorption and bioaccumulation of heavy metals (Timkova *et al*., 2018). Generally, bacteria that had been found to survive in mining areas associated with acid mine drainage belong to the phyla Proteobacteria, Nitrospirae, Actinobacteria, Firmicutes and Acidobacteria (Mendez-Garcia *et al*., 2015). These bacteria exhibit acidophilic characteristics and some of them are able to carry out natural processes such as nitrogen fixation to reduce the oxidative stress that is a direct result of exposure to heavy metals (Parro and Moreno-Paz, 2004; Valdes *et al*., 2008).

Previously, Mohamed *et al.* (2006) reported that the heavy metal pollutions caused by the mining activities had impacted the plant diversity at areas within the proximity of the former Mamut Copper Mine. However, other diversity study had never been reported since the establishment up till the abandonment of the mine site. Low *et al.* (2018a; 2018b) reported that a *Bacillus thuringiensis* strain MCMY1 isolated from the former Mamut Copper Mine had demonstrated ability to resist induced copper stress condition. In the interest of the soil bacterial community in this abandoned mine site, the current study aimed to determine the soil bacterial diversity of the former Mamut Copper Mine using culture-dependent method and correlate this with the concentration of copper in the soil samples. A phylogenetic study was done based on the 16S rRNA sequence analysis of all bacterial strains isolated and comparisons sequences retrieved from NCBI GenBank database.

MATERIALS AND METHODS

Sampling method

Soil samples were collected on January 16, 2019 during the sampling trip at the former Mamut Copper Mine site with the permission and assistance of Mineral and Geoscience Department of Sabah (JMG). The 7 selected sampling sites was based on the aim to study the soil bacterial community in at the former mine site and its vicinity (Figure 1). Soil samples were collected aseptically using a sterile auger and stored in sterile 50 mL Falcon tubes. The samples were immediately transferred back to the laboratory of Biotechnology Research Institute (BRI), Universiti Malaysia Sabah (UMS) and stored at 4 °C and -20 °C. The samples were processed for subsequent analysis within 24 h.

Figure 1: Locations of the sampling sites at the former mine site and its surrounding areas, plotted by using Google Earth Pro software.

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Soil pH and copper elemental concentration

All triplicates of soil samples were diluted with distilled water in 1:5 ratio and subjected to pH measurements by using Eutech Instruments Cyberscan pH 1500 bench meter pre-calibrated with buffers pH 10, 4 and 7. The soil samples were analyzed for copper elemental composition using inductively coupled plasma optical emission spectrometry (ICP-OES) on an Optima 5300 DV (perkin-Elmer). Aqua digestion method using Aqua Regia was employed on all the soil samples. All samples in triplicates were heated, filtered and analyzed using ICP-OES. The average copper elemental concentrations were calculated.

Serial dilution, isolation and count of bacteria

An amount of 1 g of soil samples was weighted and suspended in 9 mL of sterile distilled water in 15 mL Falcon tubes. The mixtures were thoroughly suspended by using Vortex mixer and serial dilutions were done up to 10^{-4}. Next, 100 µL of the supernatant of each dilution was aliquot and spread onto the nutrient agar (NA). The plates were then incubated at 37 °C for 5 - 10 days until the number of colony-forming unit (CFU) remained unchanged. Plates that contained 30 - 300 colonies were then selected to calculate the culturable bacterial counts. Individuals with different macroscopic morphological characteristics were sub-cultured, isolated and purified using the same medium.

Total DNA extraction, PCR amplification, and 16S rRNA gene sequencing

Total genomic DNA of each pure culture was extracted by using the QIAgen DNA Isolation Dneasy Blood and Tissue Kits, based on the manufacturer's protocol. PCR amplifications for each DNA template were conducted by using the universal prokaryotic 16S rRNA primer set: F27 (5'-GAGTTTGATCCTGGCTCAG) and R1492 (5'-GGTTACCTTGTTACGACTT) (McDonald *et al*., 1995; Yang *et al*., 2010). The PCR mixture consisted of 5 µL of 10x PCR buffer (final reaction buffer: 1x), 3 µL of 50 mM $MgCl_2$ (final concentration: 6 mM), 0.5 µL of 10 mM dNTPs (final concentration: 0.2 mM), 1 µL of each primer F27 and R1492 in 10 pmol/ µL (final concentration: 10 pmol each), 0.1 µL of *Taq* DNA polymerase in 5 U/µL (final quantity: 0.5 U), 1 µL of 250ng template DNA (final amount: 100ng) and the remaining are nuclease free water in a final volume of 25 µL. The PCR reaction was conducted in the Peltier Thermal Cycler machine (PT-200) with the following conditions: initial denaturation at 95 °C for 2 min followed by 15 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. Gel electrophoresis with 1% agarose gel at 100 V for 30 min was carried out to assess the results of the amplification. Ethidium bromide was used to stain the gel and visualized in the Bio-rad Gel Doc XR+ Gel Documentation System. The PCR products were purified by using the QIAquick PCR Purification Kit and sent to Apical Scientific Sdn. Bhd. for sequencing using Sanger sequencer. The 16S rRNA forward and reverse sequences of each bacteria were aligned and consensus sequences were created by using BioEdit sequence alignment editor. All sequences were deposited in GenBank and assigned with accession numbers: MN309878-MN309901.

Bacterial strain identification and nucleotide sequence analysis

The 16S rRNA sequences generated were used in the nucleotide BLAST (Blast Local Alignment Search Tool) system to search for the closest matching from the NCBI GenBank database. The 16S rRNA sequences of the closely related organisms were selected and downloaded from the GenBank on August 26, 2019 based on the similarities in terms of size in base pair value and overlapped length with the sequences in current study. Each sequence selected for comparison was at least published in a respective scientific journal. We used the 24 nucleotide sequences generated from current study and 17 downloaded nucleotide sequences to do a comparative analysis in order to study the bacterial biodiversity of the samples analyzed. These sequences were aligned using multiple sequence alignment by CLUSTALW. Phylogenetic reconstruction was done and depicted using maximum likelihood phylogenetic tree with 500 bootstrap replications using MEGA7 software.

Bacterial diversity analysis

The operational taxonomic units (OTUs) were defined by unique individual with < 99% 16S rRNA gene sequence similarity. The species richness, S is represented by the number of unique phylotypes and the relative diversity between the bacterial communities was evaluated by the measurement of alpha diversity index, namely the Shannon diversity index (H) and evenness (E_H). The calculations were done by using the following equations (Qing *et al*., 2006):

$$H = -\sum_{i=1}^{S} p_i \ln p_i = -\sum_{i=1}^{S} (N_i/N) \ln (N_i/N)$$

and

$$E_H = H/H_{max} = H/ \ln S$$

Where p_i represents the ratio between the number in a specific group (N_i) and the total number (N), H represents the Shannon diversity index recorded for each respective site, H_{max} is the maximum diversity of a sample found when all species are equally abundant. $H_{max} = \ln S$, where S represents the total number of species. The correlation and regression tests were done to determine the relationship between the alpha diversity indices and the soil copper elemental concentration by plotting the line of best fit and calculation of the P value with alpha value of 0.05.

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RESULTS AND DISCUSSION

Bacterial Isolation and enumeration

Bacterial colonies were starting to be seen after 24 h and the enumeration was made from the third days onward at 37 °C incubation for all samples. The number of colonies in all plates did not change from the fifth to tenth days. Several variations of physical colony morphologies were observed and pure isolates were established by taking account of their attributes in terms of color, form, margin and elevation. The isolation from the 7 soil samples managed to yield a total of 24 bacterial isolates as shown in Table 1. The highest colony forming unit per gram was counted from the soil of site 5 (6.032890°; 116.663779°), located at the blue lake with the value of 3.4 x 10^4 CFU/g followed by the site 2 (6.030223°; 116.658030°) with 2.6 x 10^4 CFU/g. No bacteria were isolated from the soil sample taken at site 7 (6.021545°; 116.652066°). The number of the phylotypes counted from each site ranged from 0 to 7 (Table 1).

Identification and phylogenetic analysis of bacterial strains

NCBI BLAST tool was used for searching and comparing of each bacterial 16S rRNA sequence with the available GenBank database. The identity matching of 1500bp for all 16S rRNA query sequences were ranged between 96% - 100% (Table 2). The overall abundance of the bacteria isolated revealed that 42% belonged to the Firmicutes, 31% to the Actinobacteria, 22% to the Proteobacteria and 5% to the Bacteroidetes. The present strains in the phylum of Firmicutes was dominated by the genus *Bacillus*. The members from the Actinobacteria group belonged to family of *Actinomycetaceae* and *Microccaceae*. Two different phylotypes from genus *Pseudomonas* were present from the phylum of Proteobacteria and the phylum of Bacteroidetes consisted of family *Flavobacteriaceae* and *Sphingobacteriaceae*.

Based on the phylogenetic tree reconstructed (Figure 2), it showed the classification of all 16S rRNA sequences in the five bacterial phyla that we had identified through NCBI BLAST tool. The most major bacterial domain, Gram-positive Firmicutes showed 11 strains that were clustered within the genus *Bacillus*. Among them, eight strains (Y2, Y6, Y13, IS7, IS9, IS12, IS15, S4) were associated with *Bacillus cereus sensu lato* group (96-100%) which included the sub lineages of *Bacillus pacificus* and *Bacillus wiedmannii* (Zhang *et al*., 2016; Liu *et al*., 2017). The *Bacillus wiedmannii* strain FSL W8-0169 (acc. no. 152692) which had matched to four of our strains (Y6, IS7, IS12, S4) was a psychrotropic strain and it has been proved to be involved in cytotoxic activities (Miler *et al*., 2016). Another two strains (IS10 and IS11) were related to *Bacillus oryzisoli* with GenBank accession number NR_151979 (96-98%) and the strain S2 was closely related to *Bacillus niacini* (acc. no. NR_024695) with 99% similarity.

In the domain of Actinobacteria, strain S3 was revealed by the BLAST tool to be 99% similar to the 16S rRNA sequence of *Arthrobacter woluwensis* A8-1 (acc. no. AB244293), strain IS3 were found to be 99% notably related to *Sinomonas susongensis* A31, accession number NR_134811 previously isolated from the surface of weathered biotite (Bao *et al*., 2015). The rest of the five strains are classified in the branch of genus *Streptomyces,* with strain IS23 related to *Streptomyces purpureus* (99%), strains IS4 and IS8 were related to *Streptomyces lactacystinicus* in 98% and 99%, respectively. Strains IS5 and IS25 both hit 98% similarities to the *Streptomyces neopeptinius* KNF 2047 (acc. no. NR_116261), which is a strain isolated from soil, previously described in 2008 and found to be a useful strain in producing antifungal substances (Han *et al*., 2008).

There were four strains found to be classified in the domain of Bacteroidetes. Strains IS21 and IS24 were both matched to *Chryseobacterium cucumeris* GSE06 (acc. no. NR_156145) with 96%. Another strain, S2 which was found to be closely related (98%) to species *Chryseobacterium arthrosphaerae* CC-VM-7 with GenBank accession number of NR_116977. Strain S1 shares a very high 99% similarities with *Mucilaginibacter carri* strain PR0008K (acc. no. NR_148857). Strains Y3 and Y15 were both classified in the domain of Proteobacteria, namely genus *Pseudomonas.* Strain Y15 was found to be 99% notably related to *Pseudomonas aeruginosa* strain R1-135, which is a strain that can carry out nitrogen fixing and responsible for biomass and seed production in plants (Madhaiyan *et al*., 2015). Strain Y3 was 96% related to *Pseudomonas stutzeri* CCUG 11256 (Table 2).

Correlation between the diversity and copper contamination

Based on the results generated from ICP-OES (Table 1), the soil copper elemental concentration revealed that the soil samples collected from the site 1, 2 and 3 had exceeded the maximum permissible potential toxic elements (PTE) of copper in soil (80ppm) (U.S. Department of the Environment, 1993). The highest concentration was recorded at site 3 (6.030019°; 116.661061°), the central of the mine factory with 125.06 ppm, followed by 96.88 ppm at site 1 (6.030324°; 116.657833°), the North-east edge of the mine pit and 88.96 ppm at site 2 (6.030223°; 116.658030°), in between the mine pit and factory. The lowest copper elemental concentration was recorded in the soil sample collected from site 7 (6.021545°; 116.652066°) with only 5.6 ppm which located at the stream bank inflow to the mine pit.

The pH values recorded for these soil samples were all acidic in general, which ranged from 3.8-5.1 (Table 1). The lowest pH value (3.8) was recorded at site 6 and 7. The highest pH value, although still acidic (5.1) was recorded at site 3 (6.030019°; 116.661061°), the mine factory central. According to the manual of U.S.

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Table 1: List of information analyzed according to each sampling site from the former Mamut Copper Mine.

Site	Latitude	Longitude	Soil samples descriptions	Soil pH	Copper concentration (ppm)*	CFU/g**	Shannon index (H)	Evenness (E_H)	Richness (S)
1	6.030324°	116.657833°	Soil from the North-east edge of the mine pit	4.1	96.88	6.5×10^3	1.49	0.77	7
2	6.030223°	116.658030°	Soil in between mine pit and factory	4.1	88.96	2.6×10^4	1.68	0.94	6
3	6.030019°	116.661061°	Soil in the mine factory central	5.1	125.06	1.2×10^4	1.18	0.85	4
4	6.030845°	116.661892°	Soil outside the sludge pool	4.1	23.15	4.8×10^3	0.72	0.66	3
5	6.032890°	116.663779°	Soil at the bank of blue lake, outflow from the mine pit	4.2	10.4	3.4×10^4	0.34	0.49	2
6	6.020545°	116.650809°	Soil from the South-west higher ground to the mine pit	3.8	5.88	9.7×10^3	0.52	0.75	2
7	6.021545°	116.652066°	Soil at the stream bank inflow to the mine pit	3.8	5.6	0	0	0	0

*Copper concentration (ppm): soil elemental copper concentration in part per million
**CFU/g: colony forming units per gram.

Table 2: List of the bacterial strains isolated, and their closely related species sequences retrieved from NCBI GenBank to conduct phylogenetic analysis.

Phylum	Site	Strain	Acc. Number	Closest relatives from NCBI GenBank	Sources
Firmicutes	1	Y2	MN309879	*Bacillus cereus* RJ1 JN159662 (96%)	Abbaspour *et al*., 2012
Firmicutes	1	Y13	MN309880	*Bacillus cereus* X5 FJ63651 (96%)	Wu and Liu, 2008
Firmicutes	4	IS9	MN309881	*Bacillus cereus* B2 MH384780 (100%)	Ozdemir and Arslan, 2009
Firmicutes	5	IS15	MN309882	*Bacillus pacificus* MCCC 1A06182 NR_157733 (100%)	Liu *et al*., 2017
Firmicutes	3	IS10	MN309883	*Bacillus oryzisoli* 1DS3-10 NR_151979 (96%)	Zhang *et al*., 2016
Firmicutes	3	IS11	MN309884	*Bacillus oryzisoli* 1DS3-10 NR_151979 (98%)	Zhang *et al*., 2016
Firmicutes	1	IS7	MN309885	*Bacillus wiedmannii* FSL W8-0169 NR_152692 (99%)	Miller *et al*., 2016
Firmicutes	4	Y6	MN309886	*Bacillus wiedmannii* FSL W8-0169 NR_152692 (98%)	Miller *et al*., 2016
Firmicutes	3	S2	MN309887	*Bacillus niacini* IFO15566 NR_024695 (99%)	Goto *et al*., 2000

(Continue Table 2)

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Firmicutes	3	S4	MN309888	*Bacillus wiedmannii* FSL W8-0169 NR_152692 (97%)	Zhang *et al*., 2016
Firmicutes	6	IS12	MN309889	*Bacillus wiedmannii* FSL W8-0169 NR_152692 (100%)	Zhang *et al*., 2016
Actinobacteria	3	S3	MN309878	*Arthrobacter woluwensis* A8-1 AB244293 (99%)	Nishiwaki *et al*., 2007
Actinobacteria	2	IS23	MN309893	*Streptomyces purpureus* LMG 19368 NR_042292 (99%)	Lanoot *et al*., 2005
Actinobacteria	2	IS3	MN309897	*Sinomonas susongensis* A31 NR_134811 (99%)	Bao *et al*., 2015
Actinobacteria	1	IS8	MN309898	*Streptomyces lactacystinicus* OM-6519 NR_136866 (99%)	Take *et al*., 2015
Actinobacteria	1	IS25	MN309899	*Streptomyces neopeptinius* KNF 2047 NR_116261 (98%)	Han *et al*., 2008
Actinobacteria	2	IS4	MN309900	*Streptomyces lactacystinicus* OM-6519 NR_136866 (98%)	Take *et al*., 2015
Actinobacteria	2	IS5	MN309901	*Streptomyces neopeptinius* KNF 2047 NR_116261 (98%)	Han *et al*., 2008
Bacteroidetes	1	IS24	MN309890	*Chryseobacterium cucumeris* GSE06 NR_156145 (96%)	Jeong *et al*., 2017
Bacteroidetes	2	IS2	MN309891	*Chryseobacterium arthrosphaerae* CC-VM-7 NR_116977 (98%)	Kampfer *et al*., 2010
Bacteroidetes	2	IS21	MN309892	*Chryseobacterium cucumeris* GSE06 NR_156145 (96%)	Jeong *et al*., 2017
Bacteroidetes	3	S1	MN309894	*Mucilaginibacter carri* PR0008K NR_148857 (99%)	Kim *et al*., 2016
Proteobacteria	1	Y15	MN309895	*Pseudomonas aeruginosa* R1-135 JQ659549 (99%)	Madhaiyan *et al*., 2015
Proteobacteria	1	Y3	MN309896	*Pseudomonas stutzeri* CCUG 11256 NR_118798 (96%)	Bennasar *et al*., 1996

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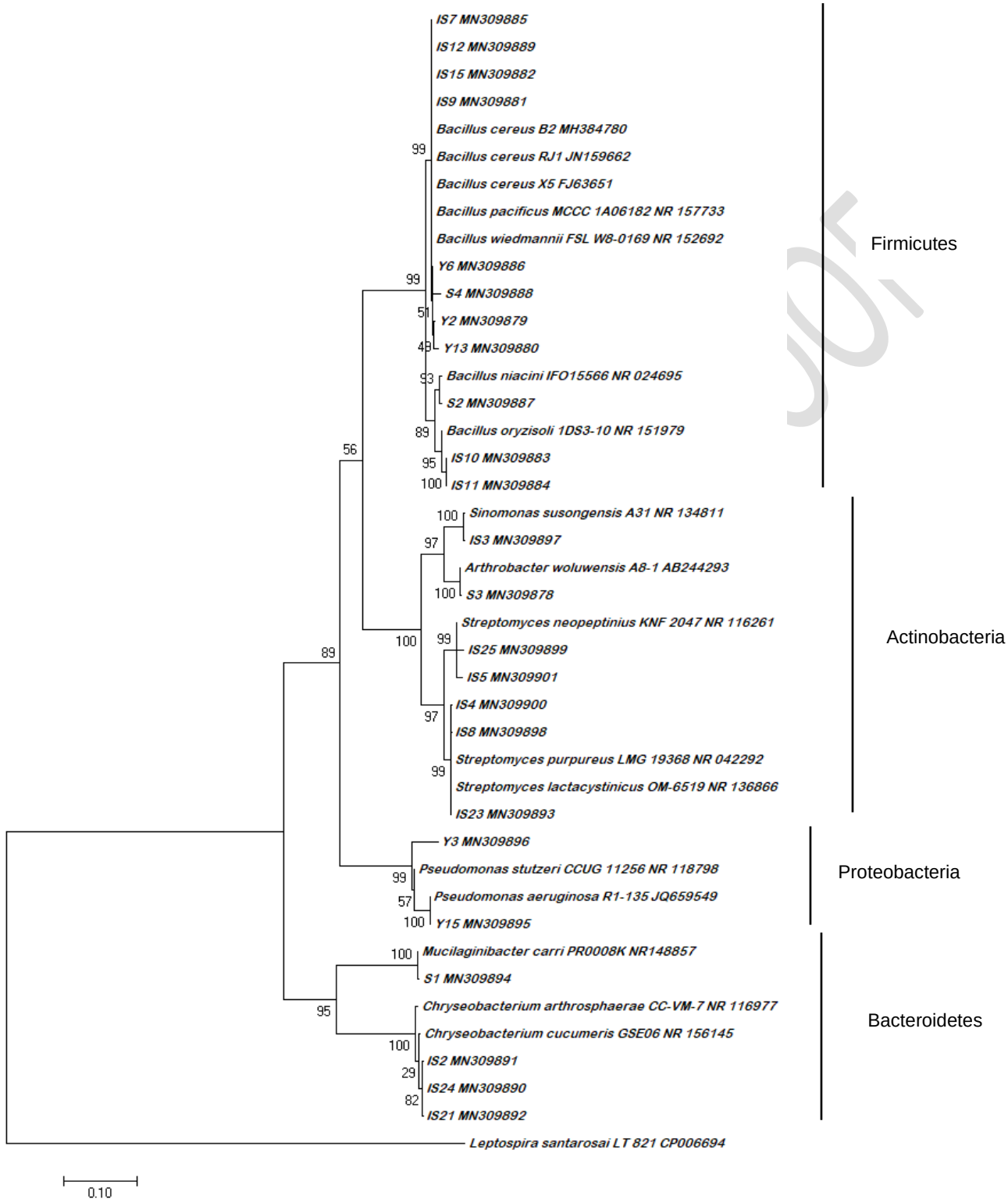

Figure 2: Maximum likelihood phylogenetic tree constructed with 500 bootstrap replications for all cultured bacterial 16S rRNA sequences and closely related species sequences retrieved from NCBI GenBank by using MEGA7 with CLUSTALW Multiple Sequence Alignment, *L. santarosai* CP006694 as outgroup.

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department of Agriculture, Natural Resources Conservation Service (Soil Survey Staff, 2009), the pH value for soil samples can be classified in the "extremely acidic" pH range (3.5 - 4.4), except for the soil sample collected from site 3 with the pH value of 5.1, that can be classified in the "strongly acidic" category (5.1 - 5.5).

The alpha diversity indices showed that the sample collected at site 2 (6.030223°; 116.658030°) in between mine pit and factory possessed the most diverse bacterial community among all the samples collected (H=1.68, E_H=0.94, S=6) followed by the sample collected from site 1 (6.030324°; 116.657833°) at the North-east edge of the mine pit (H=1.49, E_H=0.77, S=7) (Table 1). The soil sample collected from the site 7 did not contain any bacterial colony. We observed a positive relationship between the soil copper elemental concentration and the overall diversity indices (Figure 3). With the alpha value setting at 0.05, the regression test between the Shannon diversity Index (H) and the richness (S) against the soil copper elemental concentration (ppm) each shows the P-values of 0.013 and 0.040, respectively. This indicated that the directly proportional relationship between them is significant. On the other hand, the regression test between the evenness (E_H) and the soil copper elemental concentration showed a value of 0.11 which is greater than the alpha value, thus it is not significant. Generally, it was perceived that soil contaminated by heavy metal such as copper should have lower bacterial biomass and activity, however according to the investigation done at Sossege Mine by Pereira *et al*. (2014), bacteria with strong resistance can proliferate and diversify after long term exposure to the contaminated environments and high concentration of heavy metal such as copper can be correlated to the higher bacterial diversity.

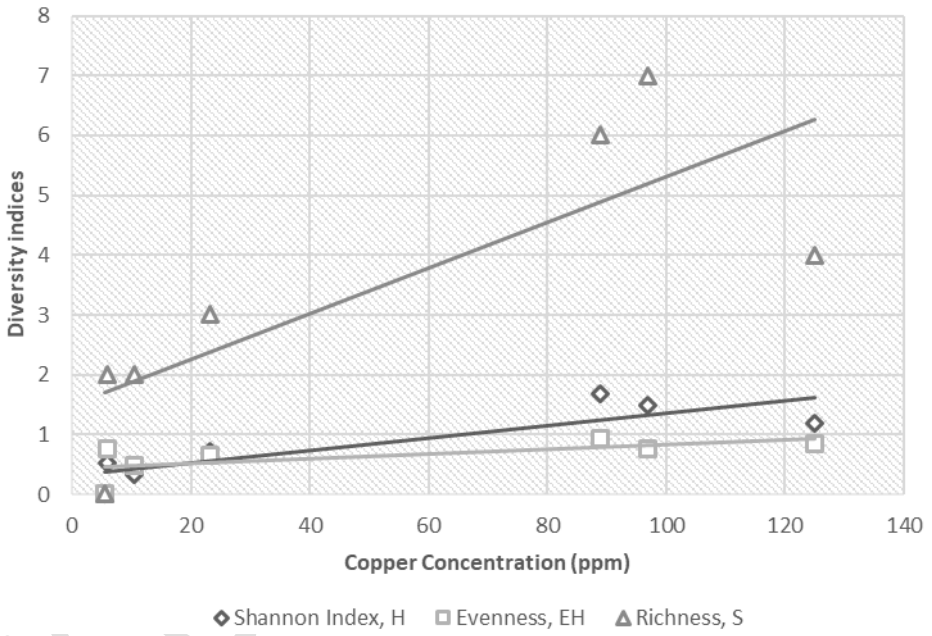

Figure 3: Correlation and regression test for the average elemental soil copper concentration (ppm) and the corresponding Shannon diversity index, evenness and richness for each sampling sites.

Another study done by Bernard *et al*. (2009) discussed that high copper contamination level in soil can affect the soil bacterial community structures, which it can inhibit the opportunistic populations, for instance GammaProteobacteria, but in return the copper contamination can promote the slower growing bacterial population, such as the members from phylum Actinobacteria. In current study, we had rather seen the dominance of the bacilli, in which they were isolated from almost all soil samples collected from the former Mamut Copper Mine either with copper contamination or not. This happened in especially for the members from the *Bacillus cereus sensu lato* group. Although *Bacillus* is usually treated as a common bacterium that lives in moderate environment, but it has been reported to be found in various extreme environments associated with copper contamination (Hassen *et al*., 1998; Marandi, 2011; Oves *et al*., 2013; Kumar *et al*., 2015). As mentioned in Low *et al*. (2018a; 2018b), a *Bacillus thuringiensis* strain MCMY1 isolated from Mamut Copper Mine previously tested for its resistance towards simulated copper induced condition and whole genome sequencing analysis revealed that this strain relied on copper homeostasis genes family, *copABCDZ*, the repressor *csoR* and other genes such as the one responsible for the Cytoplasmic Copper

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Homeostasis Protein (*cutC*) to adapt in copper contaminated conditions.

It is undeniable that by using culture-dependent method, the number of species analyzed may have been underestimated as compared to metagenomic approaches. However, by executing the culturable methods, it is possible to isolate, describe and characterize the selected strains up to the species level (Gonzalez-Rocha *et al*., 2017). Our results showed six bacterial strains with as low as 96% similarities with the available 16S rRNA sequences from NCBI GenBank database; these have also opened up a possibility of new species descriptions isolated from an extreme environment. Soil bacterial community is often used as an indicator to evaluate the quality and ecological function of soil (Janssen, 2006; Roesch *et al*., 2007; Kolton *et al*., 2011; Liu *et al*., 2016). Apparently, there is no report or microbial diversity study made previously at Mamut Copper Mine ever since the establishment up until it was abandoned in its current state. Our current data serve the best as the benchmark for future comparisons in order to understand the evolution of the soil bacterial complex in this area over a certain period of time. Subsequently, this study has resolved several gaps of knowledge in regards to the microbiological studies at the abandoned Mamut Copper Mine site. The biodiversity study based on non-culturable method such as the Next Generation Sequencing (NGS) can be done in future to identify the total diversity and compare it to the findings of the current study.

CONCLUSION

The high concentration of copper limited the growth of bacteria, bacteria from the phyla Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria were found to have adapted to this man-made ecological niche. The members of *Bacillus* were the most dominant among the culturable bacteria isolated from these sites. The copper concentration detected from the soil samples collected revealed a positive correlation with the Shannon diversity index and the richness, but not the evenness.

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