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# Tropical Soil Bacterial Diversity in Sabah, Malaysia

(Kepelbagaian Bakteria Tanah Tropika di Sabah, Malaysia)

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ABSTRACT

*Bacteria are an essential biological component of soil function that plays fundamental roles in biogeochemical cycling, soil quality improvement, habitat-shaping, and ecosystem conservation. It is therefore important to have a good record of soil bacteria in the tropics in order to monitor future changes that may occur due to global warming and other factors. However, extremely limited data are available on the diversity of bacteria in soils in some tropical Borneo regions such as Sabah, Malaysia. This research, therefore, was undertaken to determine the bacterial diversity of soils from various locations in Sabah, Malaysia. Ten soil samples (n=10) were collected around Sabah. 16S rDNA of bacterial DNA extracted from soils were amplified and analysed using the Denaturing Gradient Gel Electrophoresis (DGGE). A total of 100 dominant and well-defined DNA fragments observed in the DGGE gel were extracted, sequenced, and aligned. The results indicated that 93 different bacterial operational taxonomic units (OTUs) representing bacteria from 8 different phyla were present. The most abundant phyla in the analysed Sabah soils were* Proteobacteria *followed by* Acidobacteria, Firmicutes, Actinobacteria, Planctomycetes, Verrucomicrobia, Chloroflexi, *and* Bacteroidetes. *The examined soils of Sabah and Peninsular Malaysia had similar dominant phyla in general, except that the most dominant phylum in Peninsular Malaysia soils is the* Acidobacteria *instead of* Proteobacteria*. These baseline data generated from this work are important and can be used to track bacterial diversity shifts due to soil or environmental changes in the future.*

*Keywords: 16S rDNA; bacterial diversity; DGGE; Sabah; tropics*

ABSTRAK

*Bakteria adalah komponen biologi penting dalam fungsi tanah yang memainkan peranan asas seperti pengitaran biogeokimia, peningkatan kualiti tanah, pembentukan habitat dan pemuliharaan ekosistem. Oleh itu, rekod yang baik mengenai bakteria tanah di kawasan tropika adalah penting untuk diperoleh bagi memantau perubahan pada masa akan datang yang mungkin berlaku akibat pemanasan global dan faktor lain. Walau bagaimanapun, jumlah data tentang kepelbagaian bakteria dalam tanah dari kawasan tropika Borneo tertentu seperti Sabah, Malaysia adalah sangat terhad. Oleh itu, projek ini dijalankan untuk menentukan kepelbagaian bakteria tanah dari pelbagai lokasi di Sabah, Malaysia. Sebanyak 10 sampel tanah (n=10) dikumpulkan dari sekitar Sabah. 16S rDNA daripada DNA bakteria yang diekstrak diamplifikasikan dan dianalisis menggunakan Penyahaslian Gradien Gel Elektroforesis (DGGE). Sebanyak 100 fragmen DNA yang dominan dan jelas dicerap dalam gel DGGE telah diekstrak, dijujuk dan dijajarkan. Keputusan menunjukkan bahawa 93 unit operasi taksonomi (OTU) bakteria yang berbeza mewakili bakteria daripada 8 filum yang berbeza telah dijumpai. Filum yang paling banyak dalam tanah dari Sabah yang telah dianalisis adalah* Proteobakteria *diikuti oleh* Asidobakteria, Firmikutes, Aktinobakteria, Planktomisetes, Verrukomikrobia, Klorofleksi *dan* Bakteroidetes*. Tanah di Sabah dan Semenanjung Malaysia yang diperiksa mempunyai filum dominan yang serupa pada umumnya, kecuali filum yang paling dominan dalam tanah di Semenanjung Malaysia iaitu* Asidobakteria *dan bukannya* Proteobakteria*. Data asas yang direkodkan dalam kajian ini adalah penting dan boleh digunakan untuk mengesan perubahan kepelbagaian bakteria yang disebabkan oleh perubahan tanah atau persekitaran pada masa akan datang.*

*Kata kunci: 16S rDNA; DGGE; kepelbagaian bakteria; Sabah; tropika*

## INTRODUCTION

Soil bacteria are the essential biological component that plays pivotal roles in improving soil quality and maintaining a balanced ecosystem. Some bacteria affect water movement in soil by producing substances that bind soil particles into small aggregates where stable

aggregates improve water infiltration and its water containing ability. Through nitrification, nitrogen fixation and denitrification, bacteria are able to recycle the nutrients by converting the waste to the preferred forms useful to other organisms (Hayat et al. 2010). Given the importance of soil bacteria to community and niche as the major player in biogeochemical cycling, it is important to keep a good record of soil bacterial diversity for conservation purposes or to track changes due to various environmental factors such as global warming. Sabah is one of the 13 states within Malaysia located at the northernmost part of Borneo which shares its borders with Sarawak on the south west region and East Kalimantan of Indonesia in the south (Hashim 2003). Sabah has an equatorial climate, hot and humid throughout the year with an average annual temperature of 28 °C where daily temperatures hover between 23 and 34 °C year round.

Global warming and other environmental factors that are reported to affect the diversity of microbes in the Sub-arctic region heath ecosystem (Rinnan et al. 2007) are also affecting those from other regions including the tropics. Since the introduction of 16S rRNA gene-based molecular analysis by Woese in 1987, many analyses have been carried out to determine soil microbial community fingerprints of many countries around the world. Soil bacterial diversity data are now available for many regions such as the polar region (Foong et al. 2010; Teo & Wong 2014) and humid sub-tropical climate such as North and South America (Fierer & Jackson 2006; Janssen 2006; Xue et al. 2008). Nevertheless, the geographical locations covered are still relatively limited. There are only a few that addressed the bacterial diversity in forest soil of Kashmir, India (Ahmad et al. 2009) and Peninsular (West) Malaysia (Kerfahi et al. 2016; Kim et al. 2014). Knowledge on the diversity of terrestrial tropical bacteria is scarce, for example, in Sabah, East Malaysia. The objective of this project was therefore to determine the diversity of soil bacteria in Sabah, Malaysia, the second largest country in the twelfth largest mega-diverse country in the world.

## Materials and Methods

### Soil Sampling and Storage

Ten soil samples were collected around Sabah between September and October 2015. Approximately 70 g of soil with five replicates at each location was collected with sterile 50 mL centrifuge tubes from the depth of 2 to 10 cm. The sampling locations were S1 (N06°08.314' E116°19.320'), S2 (N05°56.701' E116°138.568'), S3 (N06°01.168' E116°29.890'), S4 (N06°00.243' E116°32.577'), S5 (N05°48.458' E116°02.172'), S6 (N05°46.917' E115°58.873'), S7 (N05°40.942' E115°56.365'), S8 (N06°07.570' E116°08.973'), S9 (N06°02.650' E116°06.718') and S10 (N06°03.413' E116°07.411') as illustrated in Figure 1.

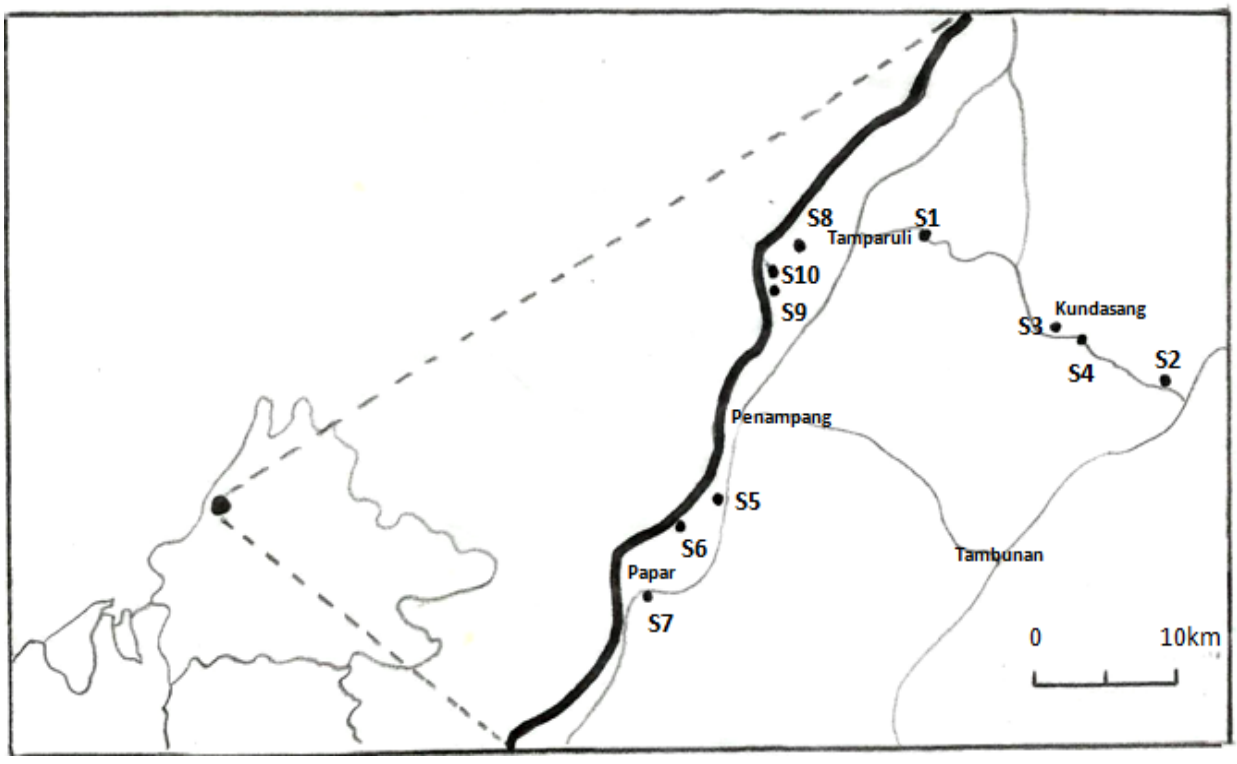


FIGURE 1. Sampling locations; S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10 of Sabah

### TOTAL DNA EXTRACTION

Total DNA was extracted using the PowerLyzer® PowerSoil® DNA Isolation Kit. Equal volumes of five independent extractions from soils from each location were pooled. The integrity of DNA was verified by agarose gel electrophoresis and quantified using NanoVue nano spectrophotometer.

### AMPLIFICATION OF PARTIAL 16S RRNA GENE

Primers, GC S-D-Bact-0341-b-S-17 (5'-GC*CCT ACG GGN GGC WGC AG-3') and S-D-Bact-0785-a-A-21 (5'-GAC TAC HVG GGT ATC TAA TCC-3') targeting the V3-V4 region of the 16S rRNA (Klindworth et al. 2013) were used. A 40 bp GC clamp (5'- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G) was added to the 5' end of the forward primer. All PCR amplifications were performed in a 50 µL mixture containing 4 ng DNA template, 2.5 mM magnesium chloride, 0.5 µM forward and reverse primers, 0.2 mM deoxynucleotide triphosphates, 2.0 U Taq polymerase and 1× GoTaq PCR buffer (Promega). The amplification conditions were an initial denaturation of 94 °C for 5 min, 20 cycles of touchdown (94 °C for 1 min, annealing for 40 s with a 0.5 °C decrement per cycle from 64 °C and extension at 72 °C for 50 s) followed by 15 regular cycles of 94 °C for 1 min, 54 °C for 40 s, 72 °C for 50 s, and a final extension of 72 °C for 10 min. The PCR products (approximately 464 bp) were verified by agarose gel electrophoresis.

### DGGE

PCR amplicons were loaded onto a 6% (w/v) 16 × 16 cm polyacrylamide gels (ratio of acrylamide to bis-acrylamide, 37:1) with 35-75% denaturant (100% denaturant consist of 7 M urea and 40% (w/v) formamide) and electrophoresis was performed using the D-Code Universal Mutation Detection System (Bio-Rad). Electrophoresis was conducted at 60 °C in 0.5 × TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA at pH 8.0) at a fixed voltage of 100 V for 16 h. After electrophoresis, the gel was stained with 1 × SYBR® Gold Nucleic Acid Gel Stain (Molecular Probe, Invitrogen) for 45 min. Subsequently, the gel was visualiSed under UV illumination and photographed using the Alpha Imager System (Alpha Innotech).

### ANALYSIS OF DGGE BANDING PATTERNS

The DGGE DNA banding patterns were analysed using the GelCompar II software (Applied Maths) in which the Shannon-Wiener index was calculated. Hierarchical cluster analysis was performed using UPGMA (Unweighted Pair Group Method with Mathematical Averages) dice coefficient of similarity. The band strengths (peak height) were estimated visually in order to calculate Shannon-Wiener index by assigning weak bands with a value of 1, intermediate with 2 and strong with 3 according to the procedure described by Gafan et al. (2005). Shannon-Wiener index (H') = - $(Ni/N)$ ln $(Ni/N)$, where $Ni$ is the peak height, $N$ is the sum of all peak heights and ln is the natural log.

### SEQUENCING AND ANALYSIS OF SELECTED DGGE BANDS

A total of 100 DNA bands were excised and sequenced using a Sanger sequencer according to the manufacturer's protocol. The partial 16S rDNA sequence was aligned using BioEdit Sequence Alignment Editor, and assessed for sequence similarity using MegAlign software (DNAStar). Sequences with less than 97% similarity were grouped into different operational taxonomic units (OTUs). The taxonomic identity of the OTUs was obtained using the Classifier tool in the Ribosomal Database Project II (RDP)-Release 10 (http://rdp.cme.msu.edu/) and Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Compositions of the bacterial phyla from all locations were tabulated and depicted graphically in a pie chart.

### NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

The nucleotide sequences referred in this study were deposited in the NCBI nucleotide sequences database under accession numbers, KY435838 - KY435885.

## RESULTS

Total DNA of the 10 samples was extracted directly from the soil without enrichment and culturing steps yielded between 15.48 and 23.68 µg of genomic DNA per gram of soil. The genomic DNA had $A_{260/280}$ and $A_{260/230}$ ratios range between 1.82 and 2.02 and 1.66 and 2.02 respectively (data not shown). Extracted DNA was diluted to 4 ng/L prior to PCR amplification to minimise and eliminate the inhibitory effect of humic acid and other contaminants that co-purified with the genomic DNA.

### DGGE PROFILING

The DGGE banding patterns of 16S rDNA fragments of 10 soil samples (Figure 2) showed that each sample had unique DNA fingerprint. In general, each location had

more than 13 DNA bands with S1 (31) showing the highest number of DNA bands and followed by S2 (29) and S10 (21). S4 (13) and S6 (13) had the least DNA bands and thus had lower Shannon-Wiener diversity indices (H') of 3.34 and 3.43, respectively, compared to S1 (5.48), S2 (5.15) and S10 (4.21). The Shannon diversity indices of other samples were shown in Table 1.

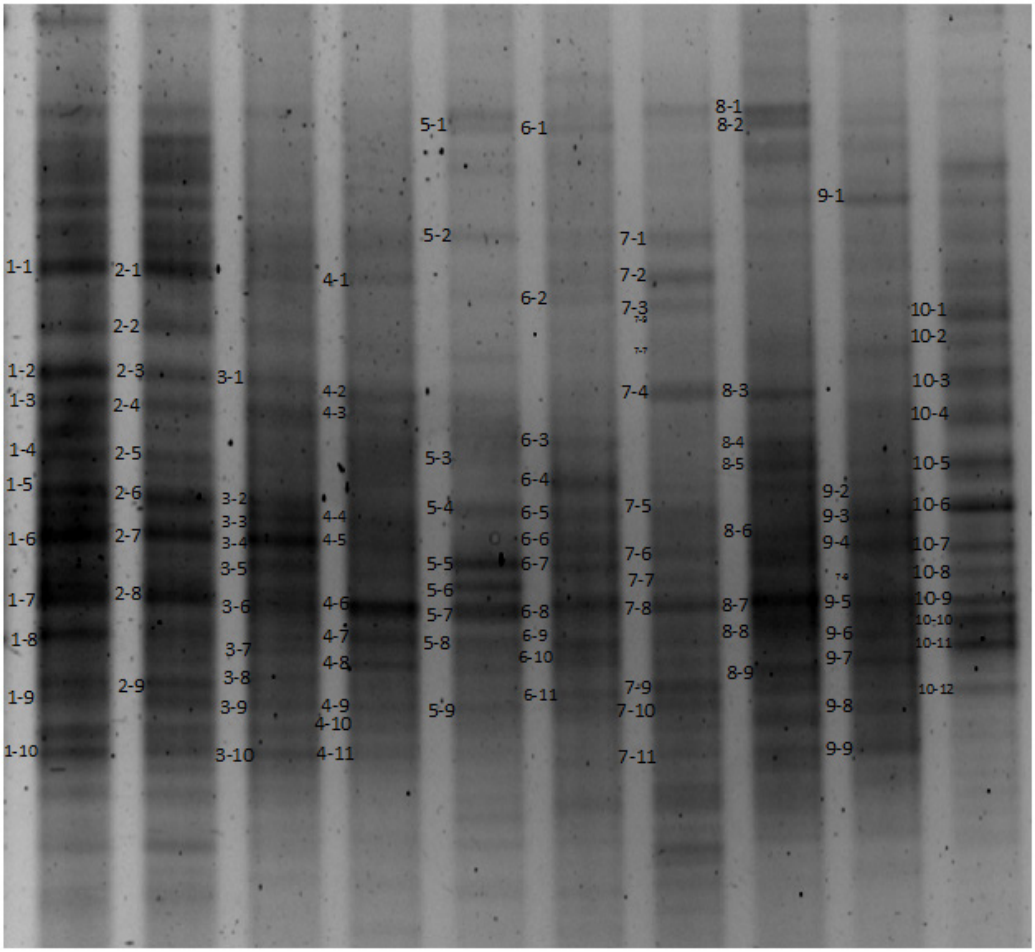


FIGURE 2. 16S rDNA fragments banding patterns on a DGGE gel. Sources of the soil samples: S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10. Positions of the 100 excised DNA bands of each sample are indicated with numbers

TABLE 1. DNA concentration, Shannon-Wiener diversity index, banding pattern information, number of sequenced clones, OTUs and phyla for all locations (S1 to S10)

| Sample | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 |
|---|---|---|---|---|---|---|---|---|---|---|
| DNA concentration (µg/g) | 22.44 | 22.72 | 23.68 | 22.08 | 19.80 | 20.20 | 19.76 | 22.36 | 16.72 | 15.48 |
| Shannon-Wiener diversity index (H') | 5.48 | 5.15 | 3.68 | 3.34 | 3.51 | 3.43 | 3.91 | 3.96 | 3.57 | 4.21 |
| No of bands | 31 | 29 | 19 | 13 | 14 | 13 | 17 | 17 | 15 | 21 |
| No of excised and sequenced bands | 10 | 9 | 10 | 11 | 9 | 11 | 11 | 9 | 9 | 11 |
| No of OTUs | 9 | 7 | 9 | 11 | 8 | 11 | 11 | 9 | 6 | 12 |
| No of phyla | 5 | 4 | 5 | 3 | 5 | 5 | 6 | 5 | 2 | 5 |

A dendrogram representing the relatedness of the bacterial community between samples was generated using Dice-coefficient of similarity and UPGMA algorithm in the GelCompare II software (Figure 3). The dendrogram gave an overview of the bacterial distribution in the soils of Sabah. S1 and S2 had the highest similarity (74.19%) and thus were clustered in the same branch as shown in Figure 3. S6 and S9 (64.29%) and S4 and S7 (51.85%) were also clustered together due to high similarity among the mentioned samples. On the other hand, S1 and S4 were extremely different (19.05%), grouping the two samples in different clades. The similarity matrices between all other samples were tabulated in Table 2. Samples with higher similarity were more likely to share the same composition of bacteria and vice versa.

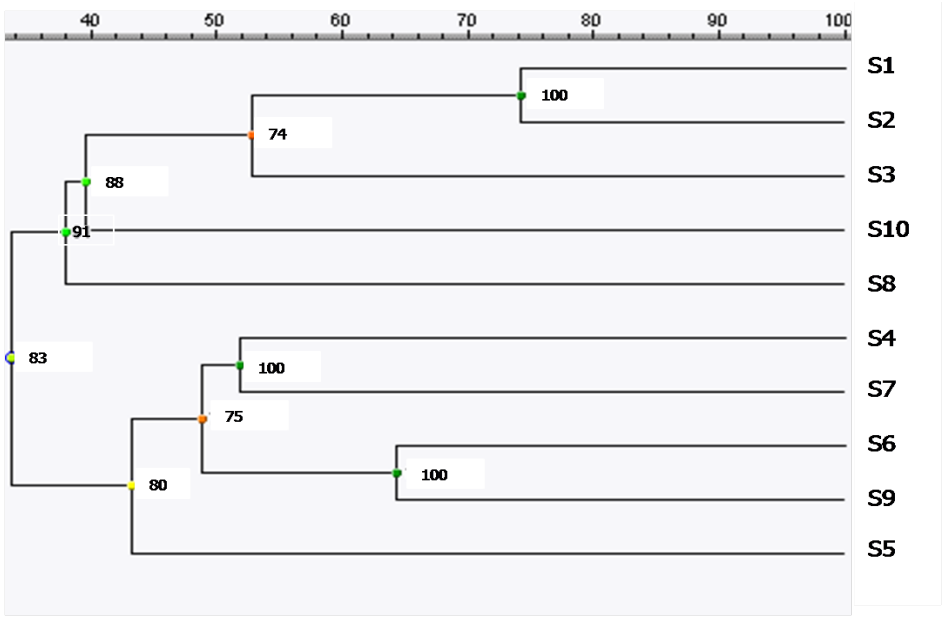


FIGURE 3. Cluster analyses of ten samples using GelCompare II (UPGMA, Dice coefficient of similarity). Figures next to the branches are the cophenetic correlations values

TABLE 2. Similarity matrix of ten soil samples (S1 to S10)

| | S1 | S2 | S3 | S10 | S8 | S4 | S7 | S6 | S9 | S5 |
|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 100.00 | | | | | | | | | |
| S2 | 74.19 | 100.00 | | | | | | | | |
| S3 | 41.67 | 63.83 | 100.00 | | | | | | | |
| S10 | 37.74 | 43.14 | 37.84 | 100.00 | | | | | | |
| S8 | 37.50 | 38.30 | 40.00 | 35.90 | 100.00 | | | | | |
| S4 | 19.05 | 30.00 | 37.04 | 32.26 | 35.72 | 100.00 | | | | |
| S7 | 32.65 | 34.05 | 30.31 | 42.11 | 40.00 | 51.85 | 100.00 | | | |
| S6 | 22.73 | 32.56 | 40.00 | 29.41 | 32.26 | 52.17 | 46.67 | 100.00 | | |
| S9 | 25.53 | 31.82 | 45.17 | 38.89 | 48.49 | 40.00 | 56.25 | 64.29 | 100.00 | |
| S5 | 25.53 | 26.67 | 37.50 | 38.89 | 30.31 | 40.00 | 50.00 | 42.86 | 40.00 | 100.00 |

DNA SEQUENCE ANALYSIS

A total of 100 dominant DNA bands from 10 soil samples were extracted and sequenced. Positions of excised DNA bands in the gel were shown in Figure 2. The 16S rDNA sequences were aligned to those in the NCBI genebank. Results indicated that 93 different bacterial OTUs from 8 different phyla were present. One hundred dominant and well-defined 16S rDNA bands were analyzed. The identities of the OTUs were resolved using Classifier tool in RDP II and NCBI database showing 93 different OTUs from eight phyla with different relative abundance. In general, the dominant phyla from the Sabah soil samples were *Proteobacteria* (33%) followed by *Acidobacteria* (24%), *Firmicutes* (12%), *Actinobacteria* (11%), *Planctomycetes* (6%), *Verrucomicrobia* (5%), *Chloroflexi* (2%), and *Bacteroidetes* (1%). The remaining 7% of the OTUs were not grouped into any of the known phyla. Figure 4 shows the complete phyla compositions of the excised DNA bands. Six phyla were identified in soils from S7 which had the highest number of phyla. It was followed by S1, S3, S5, S6, S8 and S10 with 5 phyla each.

*Proteobacteria* being the most prevalent phylum was found in all locations, dominating the soils of 5 out of 10 samples. Alpha*Proteobacteria* was the most dominant sub-division followed by Delta*Proteobacteria*, Beta*Proteobacteria,* and Gamma*Proteobacteria*. *Proteobacteria* covered up to 54% of total DNA bands sequenced for location S4, the highest representative across all the samples. *Acidobacteria* was the second most dominant phylum and was present in all location as well, representing 24% of total bands sequenced. *Acidobacteria* was mostly found in S9, which covered 66.6% of DNA bands sequenced in S9 soils. The least dominant phylum was *Bacteroidetes* which was only found in S6 soil sample representing 9.1% of DNA bands of the sample. Locations S2 and S3 had two unclassified phyla while S6 and S7 had one each. The details of the relative abundance of different bacterial phylum in all ten soils were tabulated in Table 3.

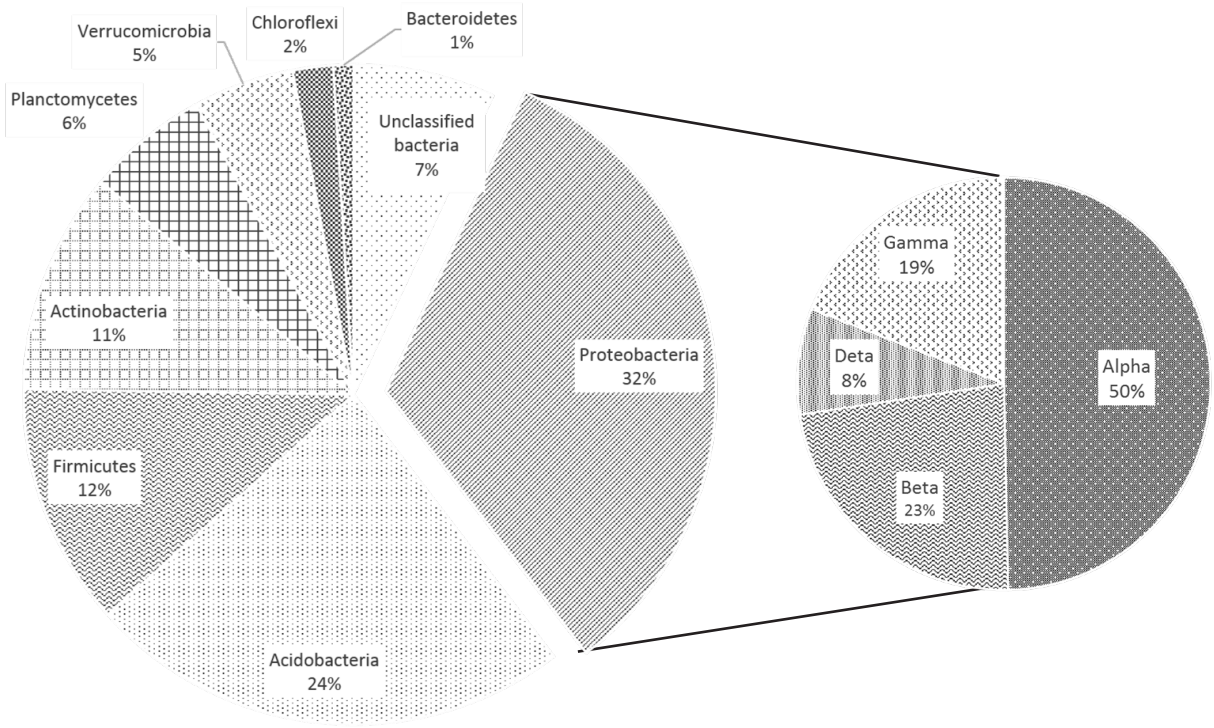


FIGURE 4. Overall compositions of dominant phyla for the ten locations (S1 to S10)

TABLE 3. Relative abundance of different bacterial phyla for all ten locations (S1 to S10)

| Sample | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Proteobacteria* | 4 | 1 | 1 | 6 | 1 | 5 | 2 | 4 | 4 | 5 | 33 (33%) |
| Alpha | 1 | | 1 | 3 | | 3 | | 1 | 1 | 3 | |
| Beta | | 1 | | 2 | | 1 | 1 | 1 | | | |
| Delta | | | | 1 | | 1 | 1 | 2 | 2 | | |
| Gamma | 2 | | | | 1 | | | | 1 | 2 | |
| | 1 | | | | | | | | | | |
| *Acidobacteria* | 2 | 4 | 1 | 3 | 2 | 1 | 3 | 2 | 5 | 1 | 24 (24%) |
| *Firmicutes* | | | 4 | | 2 | 2 | 2 | 1 | | 1 | 12 (12%) |
| *Actinobacteria* | 2 | | | | 3 | 1 | 1 | 1 | | 3 | 11 (11%) |
| *Planctomycetes* | 1 | 1 | 1 | 2 | | | 1 | | | | 6 (6%) |
| Verrucomicobia | 1 | 1 | 1 | | | | 1 | | | 1 | 5 (5%) |
| Chloroflexi | | | | | 1 | | | 1 | | | 2 (2%) |
| *Bacteroidetes* | | | | | | 1 | | | | | 1 (1%) |
| Unclassified | | 2 | 2 | | | 1 | 1 | | | 1 | 7 (7%) |
| Total | 10 | 9 | 10 | 11 | 9 | 11 | 11 | 9 | 9 | 11 | 100 (100%) |

Threshold value 80% was selected as default in CLASSIFIER RDP II

## DISCUSSION

### DGGE GEL BANDING PATTERN-BASED ANALYSIS

The banding patterns of 10 soil samples (Table 1) showed that each sample had unique DNA fingerprint on the gel, illustrating that each sample harbored different dominant bacterial species. The Shannon-Wiener indices of these 10 tropical soil samples ranged between 3.34 and 5.48, a higher value compared to polar samples which had indices of 1.48 to 3.0 (Foong et al. 2010; Teo & Wong 2014). Tropical soils are expected to be richer in diversity as the environment is more accommodating for the survival of microorganism in contrast with polar samples from the extreme environment with limited source and nutrients which proved to be challenging for microorganism survival. Dunbar et al. (2000) and Xue et al. (2008) reported that Shannon-Wiener diversity index of non-polar soils ranged between 2.8 and 7.0 which were within the range of the diversity index calculated in this study. Each sample had different diversity index due to distinctive environmental conditions giving rise to different dominant bacterial community. The bacterial diversity is known to be influenced by multiple interacting parameters between biotic and abiotic factors (Kuramae et al. 2012; Schutter et al. 2001; Yergeau & Kowalchuk 2008).

### SEQUENCE-BASED ANALYSIS

The dominant phyla found in soil samples around Sabah in descending order were *Proteobacteria*, *Acidobacteria*, *Firmicutes*, *Actinobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Chloroflexi,* and *Bacteroidetes*. The remaining 8% of the OTUs were not grouped into any of the known phyla in the database. *Proteobacteria* (33%), *Acidobacteria* (24%), *Firmicutes* (12%), and *Actinobacteria* (11%) were the most dominant phyla found in Sabah soil samples. The results were consistent with those reported by Kerfahi et al. (2016) and Kim et al. (2014). According to these studies, soil samples from Peninsular Malaysia were mainly dominated by *Acidobacteria*, *Proteobacteria,* and *Actinobacteria*.

Although soils in Sabah and Peninsular Malaysia were dominated by similar phyla, they had different relative abundance. In Peninsular Malaysia, almost 40% were dominated by *Acidobacteria* while in Sabah, only 24% were found. On the other hand, Sabah soil was mainly dominated by *Proteobacteria*. The differences of relative abundance of *Firmicutes* and *Acidobacteria* between soil in West and East Malaysia were probably due to differences in biotic and abiotic characteristics of the soil environment or the geographical locations.

Whilst both Peninsular and East (Sabah) Malaysia were dominated by *Proteobacteria*, *Acidobacteria,* and *Actinobacteria* with different relative abundance, Sabah was also dominated by *Firmicutes* which was a less dominant phylum in Peninsular Malaysia. Studies on soil microbial communities including those from North America and South America typically found Alpha*Proteobacteria*, *Acidobacteria,* and *Actinobacteria* to be the most dominant groups (Janssen 2006; Kobabe et al. 2004; Tripathi et al. 2014) as opposed to *Firmicutes*, which was categorised as a less abundant member. However, *Firmicutes* had been reported to dominate the bacterial communities in forest soil of Kashmir, India (Ahmad et al. 2009) and soils of pasture and grassland of Netherland (Kuramae et al. 2012), proving that *Firmicutes* was considered as a dominant phylum in the soil. Fierer and Jackson (2006) collected 71 unique soil samples from across North America and identified *Firmicutes* as one of the 6 dominant bacterial phyla. Studies over the past years also showed that although there are more than 100 bacterial phyla, fewer than 10 are abundant in soil and *Proteobacteria*, *Acidobacteria,* and *Actinobacteria* are generally more prevalent in soil (Aislable et al. 2013; Janssen 2006).

Different types of environment favor different phyla depending on their tolerance and ability of each phylum to utilise the surrounding resources for their survival (Roszak & Colwell 1987). Unlike the tropics, environment such as desert favored the growth of *Actinobacteria* and *Bacteroidetes* (Makhalanyane et al. 2015; Vásquez-Dean et al. 2020) that were identified as the minority in the tropical soil. Polar region, on the other hand, was dominated by *Acidobacteria* and *Proteobacteria*, similar with the tropics. However, various different phyla that were found in polar region were absent in the tropical soil including Cyanobacteria, Deinococcus, Nitrospira and BRC1 (Foong et al. 2010; Teo & Wong 2014).

The *Proteobacteria* is a major phylum which includes a wide variety of metabolically diverse group. They are known to be able to degrade wide range of toxic compound, function as nitrogen fixers such as *Rhizobium* and *Mesorhizobium* and play key role in carbon cycle (Aislable et al. 2013). The group is divided into six sub-divisions where four of which, Alpha-, Beta-, Gamma- and Delta- are detected in Sabah soil, consistent with the results reported by the previous study (Janssen 2006; Miyashita 2015). Alpha*Proteobacteria*, accounting 44.94% of total *Proteobacteria* was the most dominant sub-division followed by Delta*Proteobacteria* (22.58%), Beta*Proteobacteria* (19.35%) and Gamma*Proteobacteria* (16.13%). Members of *Proteobacteria* are considered to be copiotrophs, where they survive better in environments rich in nutrients such as tropical soil, justifying the reason *Proteobacteria* being the most dominant phylum found in Sabah soil.

*Acidobacteria* is widespread in soil and often reported as one of the major phyla found in soil. This phylum is highly diverse where more than 20 different subgroups can be detected in soil. However, members of subgroups 1, 2, 3, 4 and 6 were reported to be predominant in soil (Jones et al. 2009), similar with the results in this study where all *Acidobacteria* detected were from the subgroups mentioned. Their functions are not well studied as they mostly cannot be cultured except for members from subdivision 1 (Janssen 2006). However, sequencing results from three cultured soil *Acidobacteria* from previous study by Ward et al. (2009) suggested that members in this phylum may be oligotrophs, which are able to grow under apparent optimal conditions. Their ability to grow in low nutrient environment, bear fluctuations in soil moisture, and perform nitrate and nitrite reduction enable them to survive in most types of soil from different environment including tropical soil making it as one of the most prevalent phylum found in Sabah soil.

Members of *Firmicutes* are microbes with Gram-positive cell membrane that are more readily cultured compared to other phyla (Aislable et al. 2013). Some of the *Firmicutes* identified in this study were among the best-known genera, *Bacillus* and *Clostridium* which were acknowledged as the prevalent members of the soil bacterial community. They were the endospore-forming bacteria and their ability to produce endospore was suggested to be associated with long term survival in soil during dry periods (Aislable et al. 2013). *Firmicutes* were minor soil bacterial communities in certain habitats (Janssen 2006; Kobabe et al. 2004; Tripathi et al. 2014) but were abundant in other environments such as grassland in Netherland (Kuramae et al. 2012) and forest soil of Kashmir, India (Ahmad et al. 2009), inferring that *Firmicutes* is an important phylum in some soils.

Similar to *Firmicutes*, *Actinobacteria* are Gram-positive bacteria that tend to have abundant soil culture collections (Aislable et al. 2013). The *Actinobacteria* contains three common subphyla in soil namely *Actinobacteridae*, *Acidimicrobidae,* and *Rubrobacteridae* which are among the *Actinobacteria* identified in this study. Members of *Actinobacteria* were known to exhibit antimicrobial properties (*Streptomyces*) (Aislable et al. 2013), resist ionising radiation (*Rubrobacter*) (Holmes et al. 2000) and function as acid-tolerant ferrous iron oxidiser (*Acidimicrobium*) (Clark & Norris 1996). According to previous study by Janssen (2006), *Actinobacteria* make up on average 13% of soil bacterial communities, identical to the results in this study where 12% of total sequenced bands were identified as *Actinobacteria*.

*Planctomycetes* (6%), *Verrucomicrobia* (5%), *Chloroflexi* (2%), and *Bacteroidetes* (1%) were also detected in the Sabah soil samples. Similar phyla were reported by Kerfahi et al. (2016) and Kim et al. (2014) in samples from Peninsular Malaysia. They reported that *Verrucomicrobia*, Chloroflexi, *Bacteroidetes,* and *Planctomycetes* were the less abundant members. The ecology, physiology, and genetics of Plactomycetes, *Verrucomicrobia*, *Chloroflexi* and *Bacteroidetes* remains poorly understood as they are under-represented in soil culture collections (Janssen 2006). The major group found in soil were class Planctomycetacia of *Planctomycetes* (Garrity et al. 2004) class Spartobacteria of *Verrucomicrobia* (Bergmann et al. 2011) class Thermomicrobia of *Chloroflexi* (Hugenholtz et al. 1998; Rappé & Giovannoni 2003) and class Sphingobacteria of *Bacteroidetes* (Janssen 2006). Previous studies by Janssen (2006) and Aislable (2013) showed that members of *Planctomycetes*, *Verrucomicrobia*, *Chloroflexi,* and *Bacteroidetes* made up an average of 2, 7, 3 and 5% of soil bacterial communities, respectively, consistent with the results in this study. However, due to poor understanding of these phyla, conclusion of why these phyla were less prevalent cannot be made. Further characterisations of bacteria from these phyla are needed before deduction is possible.

## Conclusion

In conclusion, the results of this study gave information and an overview of the dominant bacterial phyla that were present in soil samples around Sabah. The most significant finding was that the bacterial population from Sabah which differed only slightly from Peninsular Malaysia, where *Firmicutes* was among the most abundant phylum. Although both East and Peninsular Malaysia were dominated by similar phyla, they have different relative abundance, with *Acidobacteria* and *Proteobacteria* being the most dominant phylum in Peninsular Malaysia and Sabah, respectively. In future, it would be interesting to investigate how environmental parameters such as temperature determine the bacterial diversity and composition at a specific location. Given the importance of soil microbes to community and niche as the major player in biogeochemical cycling, it is of great interest to determine their responses to climate change as their changes may lead to dysfunctional and disruption of the ecosystem structure. The findings of this study suggested that efforts should be made to ensure consistent isolation and conservation of beneficial bacterial species to prevent the loss of vulnerable beneficial bacterial species.

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