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Bacteriome Diversity Inside the Cemetery Soils: A Metagenomic Approach

Triastuti Rahayu[1,*], Erma Musbita Tyastuti[1], Yasir Sidiq[1], Ambarwati[1], Azmi Zaki Waliudin Althaf[2], Nazia Jamil[3]

[1]Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta, Surakarta 57162, Indonesia; [2]Department of Biotechnology, Graduate School of Universitas Gadjah Mada, Yogyakarta, Indonesia; [3]Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590, Pakistan

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Abstract

Cemetery soil represents a distinctive environment characterised by elevated microbial degradation activity associated with decomposing organic matter, which significantly influences the bacterial community (bacteriome). Despite their significance for numerous purposes, data on the bacteriome of cemetery soil in Indonesia are scarce. This research sought to explore the bacteriome of cemetery soil in Surakarta, Indonesia, obtained from three different sampling locations at depths of 20 cm (Sample A) and 140 cm (Sample B). Soil samples were subjected to chemical characterisation, after which total DNA was extracted and sequenced via next-generation sequencing on the Illumina HiSeq 2500 PE250 platform. The results revealed that Sample B (140 cm depth) exhibited a higher number of operational taxonomic units (OTUs) and greater diversity compared to Sample A (20 cm depth). Proteobacteria was the dominant bacterial phylum at 20 cm depth, whereas Actinobacteriota predominated at 140 cm depth. Within the Proteobacteria phylum, the genera *Pseudomonas*, *Sphingomonas*, and *Microvirga* were consistently present across all samples. The phylum Actinobacteriota consistently showed the presence of the genera *Gaiella*, *Pseudoarthrobacter*, *Streptomyces*, and *Micromonospora*. According to Principal Component Analysis (PCA), the composition of bacterial communities in cemetery soil was found to be affected by the depth at which sampling took place. Biomarker analysis discovered *Actinomadura* sp. and *Nocardioides* sp. to be the predominant discriminating genera at depths of 20 cm and 140 cm, respectively. Research in this area provides valuable insights into the diversity of the bacteriome in cemetery soil across both shallow and deeper levels.

Keywords: Endophytic bacteria, Enterobacter, Bacillus, 16S rRNA gene, IAA

1. Introduction

Burial sites in cemeteries are designated areas for the interment of human corpses. In Indonesia, bodies are usually buried at depths ranging from 1.5 to 2 metres. Following a burial, decomposition starts, transforming cemeteries into highly active sites for organic decomposition (Keenan et al., 2018). The decomposition process in cemeteries involves a diverse array of organisms including bacteria, fungi, and insects, primarily those from the order Diptera (Goff, 2010; Horenstein et al., 2012). Factors influencing decomposition include external elements like temperature, oxygen levels, and humidity, as well as internal factors such as soil acidity, the time and cause of death, and the presence of diseases within the deceased body (Ioan et al., 2017; Javan et al., 2019).

In cemetery soil, the primary decomposers are lipolytic and proteolytic bacteria. About 20% of human remains are composed of proteins, approximately 10% are lipids, around 5% are minerals, 4% are water, and roughly 1% are carbohydrates (Janaway et al., 2009; Dent et al., 2004; Ioan et al., 2017). The decomposition process yields a range of organic compounds, such as amino acids, fatty acids, ammonia, ammonium, methane, mercaptans, sulphates, nitrates, and calcium. The decomposition process also enhances electrical conductivity (Fineza et al., 2014; Taylor et al., 2023) and leads to the emission of heavy metals (Egbimhanlu et al., 2020; Franco et al., 2022). Research using culture-dependent methods on cemetery soil in Surakarta has isolated proteolytic and lipolytic bacteria, with a proteolytic index (IP) of 4.2 (Salsabilla et al., 2024) and a lipolytic index (IL) of 5.43 (Rini et al., 2023).

Temporal fluctuations in the bacterial community of cemetery soil occur during corpse decomposition, with such changes being influenced by the unique decomposition environment (Garcia et al., 2020; Singh et al., 2018; Keenan et al., 2018). In the initial phases of decomposition, bacterial families like Actinomycetaceae, Aerococcaceae, Bifidobacteriaceae, Campylobacteriaceae, Corynobacteriaceae, Lactobacillaceae, Micrococcaceae, and Staphylococcaceae are commonly found, whereas families such as Alcaligenaceae, Planococcaceae, and Pseudomonadaceae prevail in later stages (Garcia et al., 2020).

Extensive research on bacterial communities associated with corpse decomposition has been conducted globally,

* Corresponding author. e-mail: tr124@ums.ac.id.

primarily for forensic purposes (Wei et al., 2022; Garcia et al., 2020; Javan et al., 2019; Singh et al., 2018), alongside investigations into the risks of pathogenic contamination (Finley et al., 2015; Abia et al., 2019). In contrast, such studies are notably scarce in Indonesia. Existing research in this domain has primarily employed culture-dependent approaches, isolating bacteria from various soil depths and identifying potential proteolytic (Całkosiński et al., 2015; Salsabilla et al., 2024) and lipolytic (Rini et al., 2023; Mandiri et al., 2023) bacterial species. Proteolytic bacteria produce proteases, which play significant roles in industries such as food processing, waste management, detergents, biofilm degradation, leather processing, silver recovery, dairy, baking, beverages, and pharmaceuticals (Saputri et al., 2023; Matter et al., 2023). Similarly, lipases produced by lipolytic bacteria have applications in treating oily wastewater, oil degradation, leather processing, detergent production, biodegradation of oil and organic waste, food processing, oil manufacturing, biodiesel production, and the medical industry (Solanki et al., 2021; Ali et al., 2023; Chandra et al., 2020).

Bacteriome metagenomic data from the cemetery soils were described by Yao et al. (2021). This study is necessary to obtain the cemetery soil bacteriome data that can be utilised in multiple biotechnological sectors. This study sought to examine a bacterial community (bacteriome) isolated from cemetery soil in Surakarta, Central Java, Indonesia.

2. Materials and methods

2.1. Sample collection and DNA extraction

The selected sites for soil sample collection in Surakarta, Central Java, Indonesia, were two cemeteries: Pracimaloyo (P) and Bonoloyo (B) (See Figure 1). Soil was excavated to a depth of 20 cm at each site for Group A and 140 cm for Group B. Six samples were collected in total: two from the P site at depths of 20 cm and 140 cm, labelled as P12A and P12B, and four from the B site, also at depths of 20 cm and 140 cm, labelled as B18A, B23A, B18B, and B23B. Figure 1 illustrates the sampling locations. Comprehensive chemical and physical analysis was conducted on the collected soil samples (Dauletkul et al., 2025), and the total genetic material was isolated from the samples using the ZymoBIOMICS DNA Miniprep Kit, following the manufacturer's protocol (Rakhmawati et al., 2024).

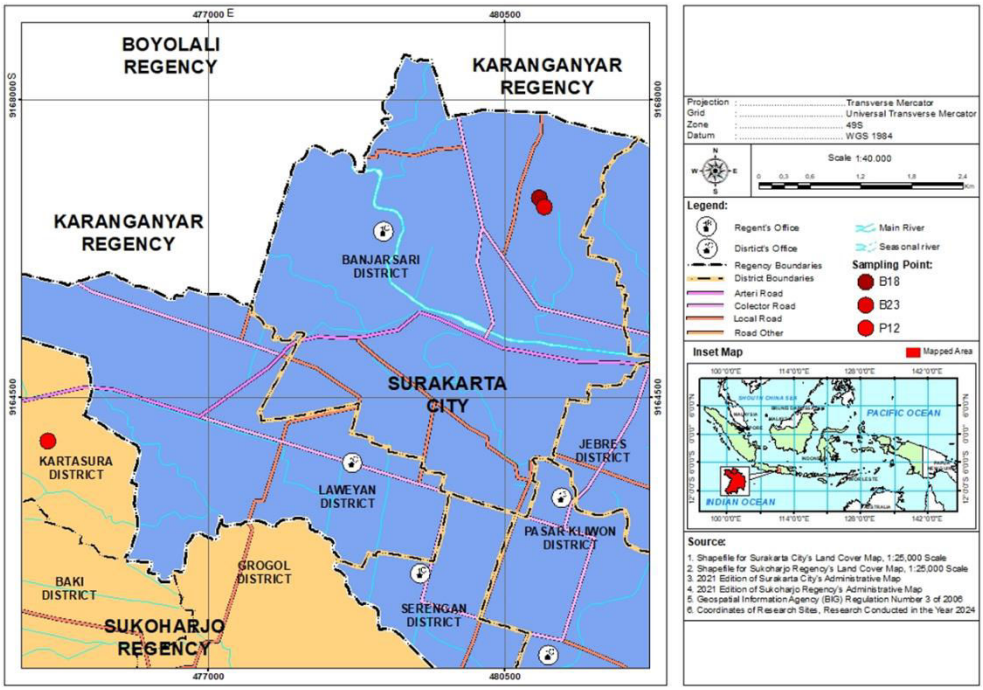

Figure 1. Sampling sites of cemetery soils in Surakarta City, Indonesia

2.2. Library Construction, Quality Control and Sequencing

PCR amplification was conducted on the target regions, V3-V4 regions, using the following primer sequences 341F 5'-CCTAYGGGRBGCASCAG-3' and 806R 5'-GGACTACNNGGGTATCTAAT-3' (Asriatno et al., 2023; Rahayu et al., 2024; Rakhmawati et al., 2024). The correct size of the PCR products was identified through agarose gel electrophoresis using a 2% gel. PCR products from each sample were then combined, end-paired, A-tailed, and further linked with Illumina adapters in equal amounts. The resultant libraries were sequenced using a paired-end Illumina system to produce 250bp paired-end unprocessed reads. The library's quality was verified using Qubit and real-time PCR quantification, along with bioanalyzer analysis to determine its size distribution. Quantified libraries will be grouped together and sequenced on the Illumina platform, with the sequencing adjusted in accordance with the effective library concentration and the desired amount of data.

2.3. Sequencing Data Processing

Paired-end reads were selected based on their distinct barcodes, and subsequent trimming involved cutting off the barcode and primer sequences, after which they were merged using FLASH (V1.2.7) (Rahayu et al., 2024), a tool capable of rapid and accurate analysis, intended for

merging paired-end reads with overlapping sequences from the opposing end of the same DNA fragment, which were then referred to as raw tags. Raw tags underwent quality control under specific filtering conditions to produce high-quality clean tags, as described in the QIIME (V1.2.7) (Bokulich et al., 2013) quality control process (Magoč and Salzberg, 2011). The tags were compared with the reference database via the UCHIME algorithm (Caporaso et al., 2010).

2.4. OTU Cluster and Taxonomic Annotation

Sequence analysis was carried out utilizing Uparse software, which processed all relevant tags. Sequences with a similarity of 97% or higher were grouped into the same operational taxonomic unit (OTU). Sequences corresponding to each operational taxonomic unit were chosen for further annotation. The QIIME platform, which utilises the Mothur method, was employed to annotate each chosen sequence against the SSUrRNA database from SILVA138, with species annotation undertaken at each taxonomic rank, using a threshold of 0.8–1. The phylogenetic relationships among all OTU representative sequences were determined using MUSCLE (Version 3.8.31), a program renowned for its high accuracy and processing speed. The OTU abundance data were normalised relative to the sample with the fewest sequences. The subsequent analyses of alpha and beta diversity were conducted using the normalized dataset.

2.5. Alpha Diversity

The complexity of biodiversity in a sample was analyzed using 6 indices, specifically Observed-species, Chao1, Shannon, Simpson, ACE, and Good-coverage, as per the methods outlined by Thakur et al. (2022). All of these indices in our samples were calculated using QIIME (Version 1.7.0) and visualised with R software (Version 2.15.3).

2.6. Beta Diversity

An assessment of beta diversity was conducted to evaluate the differences in species diversity among samples. The QIIME software, version 1.7.0, calculated beta diversity using both weighted and unweighted unifrac methods. Prior to cluster analysis, principal component analysis (PCA) was conducted using the FactoMineR and ggplot2 packages in R software (version 2.15.3) to reduce the dimensionality of the original variables. For obtaining principal coordinates and simplifying visualisation of intricate, multi-dimensional data, Principal Coordinate Analysis (PCA) was utilised. A previously collected set of samples was transformed, with a distance matrix of weighted or unweighted UniFrac being converted into a new set of orthogonal axes, the first principal coordinate of which represents the greatest variation, the second the next highest, and so on. Permutational multivariate analysis of variance (PcoA) analysis was depicted with the aid of the WGCNA package, statistical software, and the ggplot2 package in R software, version 2.15.3. The Unweighted Pair-group Method with Arithmetic Means (UPGMA) hierarchical clustering, using average linkage, was employed with the QIIME software (Version 1.7.0) to analyze the distance matrix.

2.7. Biomarker Analysis

Linear Discriminant Analysis Effect Size (LEfSe) is a statistical approach used for biomarker analysis, combining non-parametric tests with linear discriminant analysis (LDA). Firstly, the Kruskal-Wallis test is used to identify significant differences among microbial taxa, and subsequently pairwise Wilcoxon tests are employed to verify consistency across subclasses. Ultimately, LDA calculates the effect size of each taxon, identifying those with the greatest discriminatory power as biomarkers (Marcos-Zambrano et al., 2021). Taxa with LDA scores exceeding a specified threshold (usually above 2.0) and having statistically significant p-values are considered reliable indicators that can differentiate between sample groups.

3. Results

3.1. Taxonomic Composition Analysis.

The programme QIIME classified all sequences from phylum to genus using its default settings. The sequences were categorised into 30-39 phyla, 67-87 classes, 143-190 orders, 197-395 families, and 270-516 genera. The different samples shared similar bacterial compositions but had different phylum distributions, as indicated in Table 1.

Table 1. Number of identified taxa inside the cemetery soils

Taxon	P12A	P12B	B18A	B18B	B23A	B23B
Phylum	30	37	33	39	38	34
Classis	67	80	71	87	79	84
Order	143	183	164	180	190	187
Familia	197	395	248	271	292	296
Genus	270	429	397	397	477	516

The phyla in sample A, which measures 20 cm, are ranked by their relative abundance as follows: Proteobacteria at 8.9%, Actinobacteriota at 6.5% and Firmicutes at 5.8%. In contrast to sample A, Actinobacteriota is the most prevalent phylum in sample B, making up 10.2% of the total, whereas Proteobacteria account for 6.6% and Firmicutes 5% (Figure 2). The phylum Crenarchaeota was found in almost all samples, accounting for between 1-7% of the total relative abundance. In the two samples, *Bacillus* (a type of Firmicutes phylum) dominated, with a range of OTU numbers extending from 4631 to 14392. The dominant genera isolated from sample B18B were *Marmoricola* and *Nocardioides* in the phylum Actinobacteriota, corresponding to OTU numbers 13206 and 12789, respectively. Species like *Gaiella*, *Pseudoarthrobacter, Streptomyces,* and *Micromonospora* were found in nearly all samples within the same phylum. Proteobacteria phylum genera including *Pseudomonas, Sphingomonas,* and *Microvirga* were predominantly found in all the samples. The predominant phylum Proteobacteria genera in the B18A sample were Wolbachia and Buchnera, comprising OTUs 7371 and 6658, as shown in Figure 3.

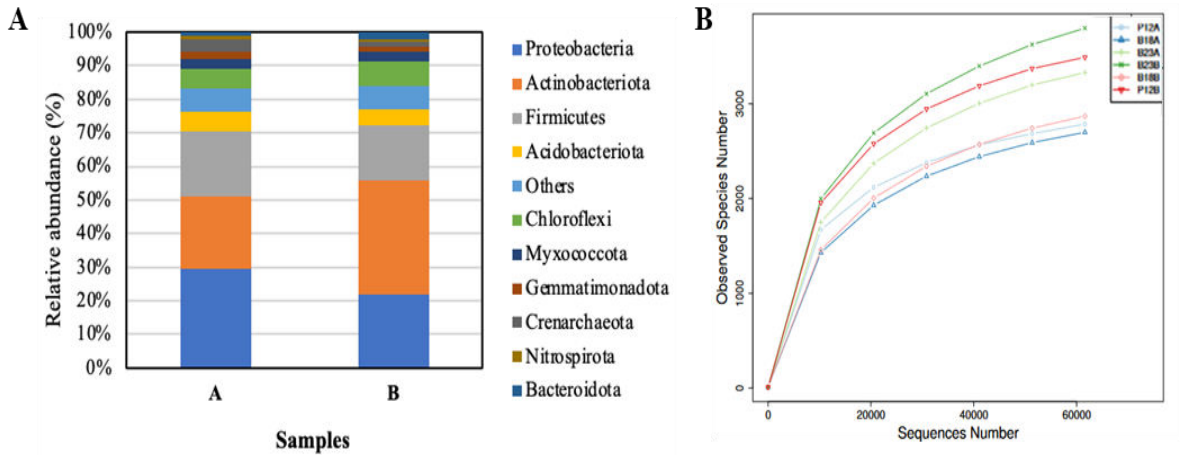

Figure. 2. Relative abundance and the number of observed species. **A.** The relative abundance of soil cemetery bacterial community within phyla. B samples were soil from140 cm-depth, while A samples were 20 cm-depth. **B**. Rarecurve of observed species number in six sampling sites.

3.2. Alpha Diversity Analysis

The average numbers for effective tags, taxon tags, and observed OTUs from the six samples were 109,942, 95,613, and 3,456, respectively, as per Table 2. In all 6 samples, the rarefaction curves typically trended towards the saturation plateau, as shown in Figure 2B. The rarefaction curves demonstrated the variability in the OTU abundance across multiple samples. Notably, the OTU numbers for samples P12B, B18B, and B23B at a depth of 140 cm (3788, 3285, 4162) exceed those for samples P12A, B18A, and B23A at a depth of 20 cm (2785, 2992, 3724) (Table 2).

Figure 3. Heatmap of relative abundance of cemetery soil bacterial community within genera. Red color indicates higher relative abundance compared to white and blue. The numbers designate the number of OUT

Tax_detail	P12A	P12B	B18A	B18B	B23A	B23B
p__Firmicutes;c__Bacilli;g__*Bacillus*;	4631	4911	9577	14392	11338	6670
p__Actinobacteriota;c__Actinobacteria;g__*Marmoricola*;	148	308	132	13206	150	280
p__Actinobacteriota;c__Actinobacteria; g__*Nocardioides*;	250	1162	344	12789	452	980
p__Proteobacteria;c__Alphaproteobacteria;g__*Wolbachia*;	0	504	7371	0	597	813
p__Proteobacteria;c__Gammaproteobacteria;g__*Buchnera*;	0	2528	6658	0	1	6
p__Proteobacteria;c__Gammaproteobacteria;g__*Pseudomonas*;	3	503	1442	4769	1201	1830
p__Crenarchaeota;c__Nitrososphaeria;g__*Nitrososphaeraceae*;	1157	556	81	1045	4709	1346
p__Proteobacteria;c__Gammaproteobacteria;g__*Escherichia-Shigella*;	20	184	2605	8	115	217
p__Actinobacteriota;c__Actinobacteria;g__*Pseudarthrobacter*;	72	2233	242	1243	771	2454
p__Actinobacteriota;c__Thermoleophilia;g__*Gaiella*;	1383	1734	2136	2366	1635	1616
p__Firmicutes;c__Bacilli; g__*Paenibacillus*;	263	163	2332	689	405	417
p__Actinobacteriota;c__Actinobacteria;g__*Agromyces*;	40	407	179	2040	199	297
p__Firmicutes;c__Bacilli; g__*Lactobacillus*;	0	1243	1963	393	1466	1711
p__Proteobacteria;c__Alphaproteobacteria;g__*Asticcacaulis*;	1	11	1900	0	4	17
p__Firmicutes;c__Clostridia;g__*Romboutsia*;	23	1018	1647	47	1150	1387
p__Proteobacteria;c__Gammaproteobacteria;g_*Achromobacter;*	0	0	1615	1	10	3
p__Proteobacteria;c__Alphaproteobacteria;g__*Sphingomonas*;	325	795	578	1174	587	1608
p__Proteobacteria;c__Alphaproteobacteria;g__*Microvirga*;	311	1061	354	379	784	1582
p__Firmicutes;c__Desulfotomaculia;g__*Desulfurispora*;	2	0	0	1535	0	0
p__Proteobacteria;c__Gammaproteobacteria;g__*Pseudoxanthomonas*	2	42	1530	0	81	85
p__Proteobacteria;c__Alphaproteobacteria;g_*Devosia*;	16	107	572	10	750	1452
p__Verrucomicrobiota;c__Verrucomicrobiae;g__*Candidatus*;	84	113	402	1388	141	102
p__Actinobacteriota;c__Actinobacteria;g__*Intrasporangium*;	65	395	138	1339	132	403
p__Proteobacteria;c__Alphaproteobacteria;g__*Pedomicrobium*;	585	251	153	59	1327	623
p__Actinobacteriota;c__Thermoleophilia;g__*Solirubrobacter*;	198	1204	398	982	414	733
p__Firmicutes;c__Clostridia;g__*Sedimentibacter*;	0	1160	1	5	0	2
k__Archaea;p__Crenarchaeota;g__*Candidatus_Nitrososphaera*;	454	255	7	128	1143	532
p__Nitrospirota;c__Nitrospiria;g__*Nitrospira*;	1134	539	254	269	726	886
p__Firmicutes;c__Bacilli;g__*Ammoniphilus*;	255	104	297	1059	210	170
p__Proteobacteria;c__Alphaproteobacteria;g__Allorhizobium;	29	327	258	8	837	1005
p__Acidobacteriota;c__Vicinamibacteria;g__Vicinamibacteraceae;	952	693	232	946	998	733
p__Actinobacteriota;c__Actinobacteria;g__Microlunatus;	94	548	118	0	568	889

p__Actinobacteriota;c__Actinobacteria;g__*Streptomyces*;	841	327	142	863	689	740
p__Myxococcota;c__Myxococcia;g__*Anaeromyxobacter*;	325	161	118	824	115	194
p__Actinobacteriota;c__Rubrobacteria;g__*Rubrobacter*;	89	247	103	83	312	799
p__Proteobacteria;c__Alphaproteobacteria;g__*Pelagibacterium*;	0	0	0	0	429	796
p__Proteobacteria;c__Alphaproteobacteria;g__*Dongia*;	793	433	189	68	417	634
p__Firmicutes;c__Bacilli;g__*Domibacillus*;	155	461	206	131	784	591
p__Actinobacteriota;c__Actinobacteria;g__*Micromonospora*;	569	678	325	757	485	772
p__Actinobacteriota;c__Actinobacteria;g__*Sinomonas*;	27	142	31	707	42	54
p__Proteobacteria;c__Gammaproteobacteria;g__*Steroidobacter*;	212	330	124	13	549	701
p__Proteobacteria;c__Gammaproteobacteria;g__*Enterobacter*;	0	698	69	171	26	76

Data on alpha diversity indicate that the soil in cemeteries has a high level of bacterial diversity. The Shannon diversity index (H') for all the samples is greater than 8 (H'>8), indicating a high level of diversity. The Simpson dominance index indicates a correlation with H' values for sample A at a low diversity level (Table 2).

Table 2. OTU numbers and alpha diversity of cemetery soil bacterial community

Samples	Effective Tags	Taxon tags	Observed OTUs	Alpha diversity		
				Shannon	Simpson	Chao1
P12A	77,857	61,592	2,785	9.38	0.10	3.13
P12B	95,022	80,044	3,788	9.53	0.10	3.85
B18A	97,677	88,278	2,992	8.13	0.98	2.95
B18B	164,563	150,070	3,285	8.27	0.99	3.25
B23A	105,118	90,139	3,724	8.98	0.99	3.62
B23B	119,412	103,555	4,162	9.64	0.10	4.48
Average	109,942	95,613	3,456			

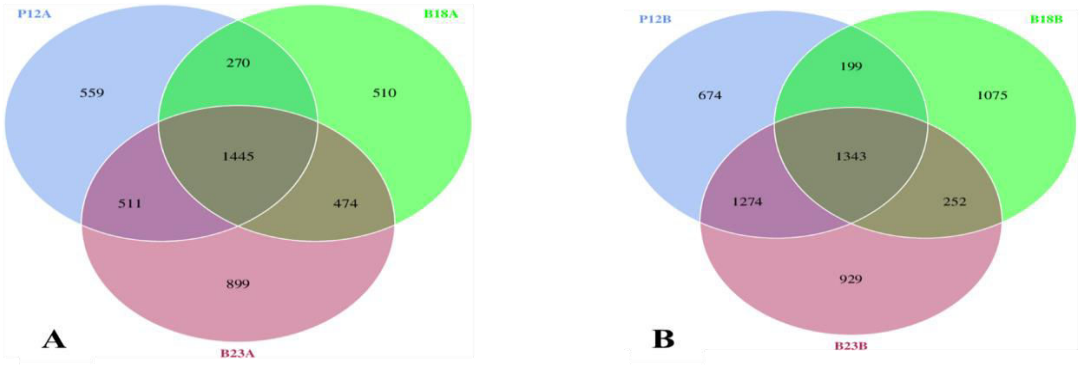

Figure 4. Venn diagram of cemetery soil bacterial community collected from 20 cm-depth (A) and 140 cm-depth (B)

Furthermore, 911 OTU are identified as the core genera for all samples, primarily consisting of *Marmoricola*, *Bacillus*, *Pseudoarthrobacter* and *Escherichia coli* were present with relative abundances of 4.55% and 4.0%, respectively, while *Pseudoarthrobacter* and *Escherichia coli* also had relative abundances of 3.6% and 3.0% respectively. Eleven phyla exhibited relative abundances exceeding 0.2% (Figure 5). Within the Phylum Actinobacteriota, 15 core genera were identified as having relative abundances greater than 0.3%. The genus *Candidatus_Nitrososphaera* within the phylum Crenarchaeota in Kingdom Archaea had a relative abundance of 1.0% (Figure 3).

3.3. Beta Diversity Analysis

The bacterial community composition of cemetery soil was found to be affected by sampling depth, as shown by PCA. Figure 6A shows that bacterial communities collected at a depth of 140 cm (Group B) are distinct from those collected at a depth of 20 cm, with the exception of samples B18B and P12A. This finding is supported by the UPGMA cluster tree, which was constructed using Weighted UniFrac distance, as shown in Figure 6B.

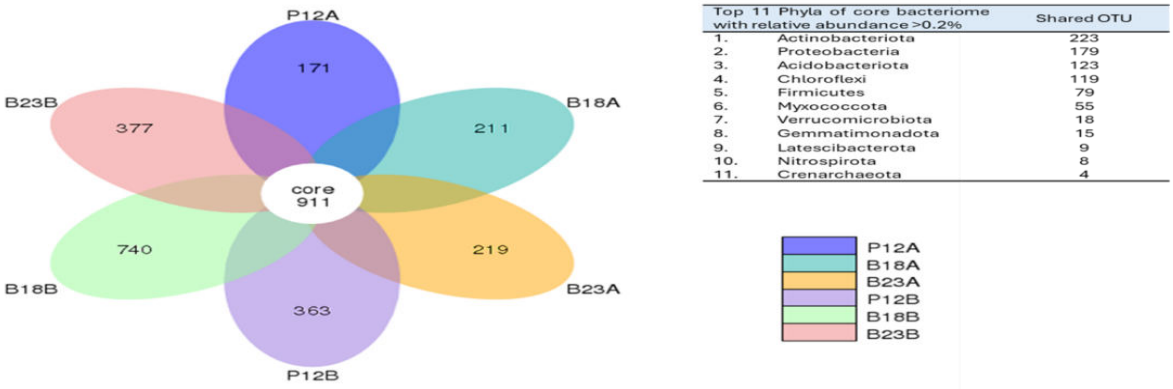

Figure 5. Distribution of genera as the main bacterial community of cemetery soil

3.4. Identification Based on the 16S rRNA Gene

The identification of two potential isolates, T5 and S6, was based on the 16S rRNA gene following extraction and amplification of their DNA genome, which comprises 1500 base pairs of the 16S rRNA gene. The findings indicated that T5 displayed the highest similarity to the genus *Streptomyces*, with a 99.86% identity percentage, comparable to *Streptomyces sp.* VEL17. In contrast, the S6 strain showed an 83.50% identity with *Streptomyces sp*. strain ADE 004.

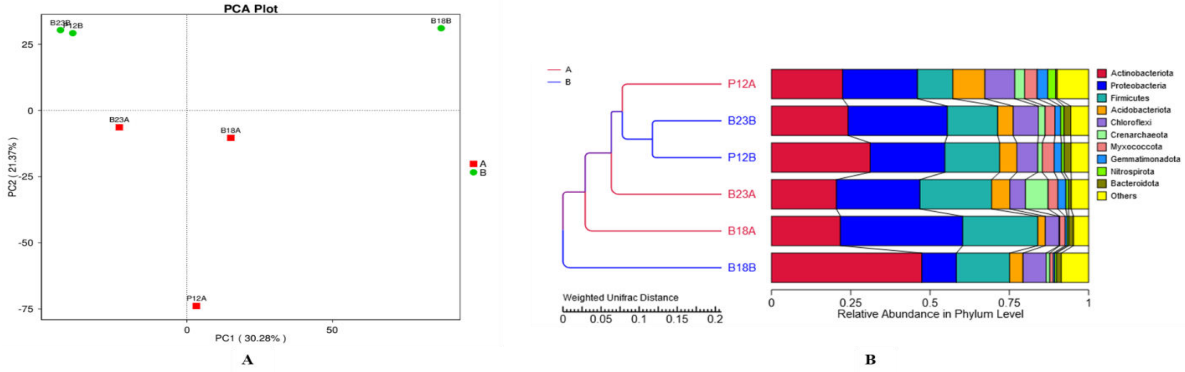

Figure 6. Principal component analysis (PCA) describes the different bacterial community in the cemetery soils in deep and shallow sites (A). UPGMA cluster tree based on Weighted Unifrac distance showed the genetic distance among samples (B).

3.5. Biomarker Analysis

The LEfSe analysis identified 26 bacterial genera as biomarkers at soil depths of 20 cm and 140 cm, as shown in Figure 7. The genera *Actinomadura sp.* and *Lysinibacillus sp.* were more abundant at 20 cm depth, whereas the other 24 genera were more prevalent at 140 cm. *Actinomadura sp.* and *Nocardioides sp.* were notably the organisms that yielded the highest Linear Discriminant Analysis (LDA) scores, 2.97 and 4.61 respectively. These two genera were predominant at different depths: *Actinomadura sp.* at 20 cm and *Nocardioides sp.* at 140 cm.

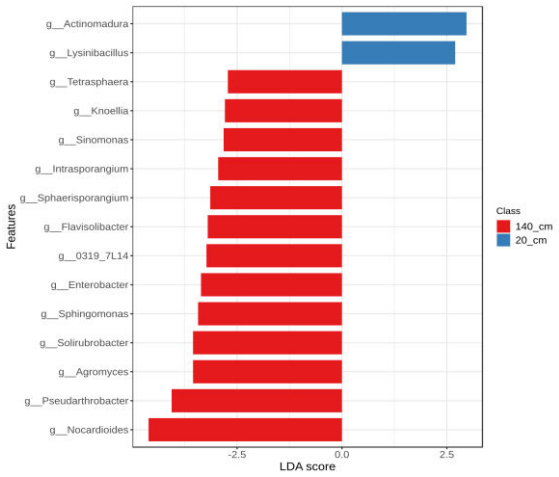

Figure 7. LEfSe analysis results of soil samples at 20-cm and 140-cm depths

4. Discussion

Samples taken from a depth of 140 cm showed greater quantities of operational taxonomic units and higher Shannon Index values than those from a depth of 20 cm, as evident in Tables 1 and 2. Previous research by Garcia et al. (2020), Keenan et al. (2018), and Singh et al. (2018) supported these findings. In Indonesia, bodies are buried at a depth of about 140 cm (Edgar et al., 2011). The bacterial abundance at this depth can be attributed to the availability of organic substances produced from active corpse decomposition (Fineza et al., 2014; Taylor et al., 2023). Fluorescence diacetate (FDA) hydrolysis and dehydrogenase activity also demonstrated active decomposition in cemetery soil (Lumombo, 2017). Evidence has shown that these organic substrates facilitate bacterial metabolism (Emmert et al., 2021; Aya et al., 2022; Baskaran et al., 2020; Huang et al., 2020; He et al., 2020). It was also discovered that electrical conductivity significantly impacts the growth of bacteria that are positively correlated, a connection backed by the higher electrical conductivity values documented for samples collected from 140 cm depth (Table 3), according to Song et al. (2022)..

The phylum Proteobacteria predominated at 20 cm depth, whereas Actinobacteriota was dominant at 140 cm depth (Figure 2). The prevalence of Actinobacteriota at burial sites has similarly been reported in prior studies (Abia et al., 2019; Singh et al., 2018). Unique OTUs were more abundant at 140 cm depth than at 20 cm depth (Figure 4), likely due to limited oxygen availability at the greater depth despite nutrient abundance (Fineza et al., 2014). This oxygen limitation acts as a constraining factor for certain bacterial communities, thereby promoting the emergence of distinct assemblages at each sampling depth. In contrast, shared OTUs were more prevalent at 20 cm depth than at 140 cm depth (Figure 4), reflecting sufficient growth factors for bacteria without substantial variation. At 20 cm depth, the bacterial community is predominantly influenced by exogenous factors in addition to corpse decomposition.

In Sample B, the phylum Actinobacteriota is dominant and comprises one of the phyla that contribute to the shared operational taxonomic units (OTUs) across the six samples examined (Figure 5). This phylum consists of widely distributed genera that are vital in breaking down various organic materials, such as cellulose, polysaccharides, proteins, lipids, and organic acids (Ghare et al., 2023). Members of Actinobacteriota can thrive in extremely challenging environments, such as deserts, caves, deep-sea sediments, and salt lakes, thanks to their capacity to generate spores or conidia for reproduction (Mahapatra et al., 2020). This group consists of specific

taxa that have developed specialised adaptations. Thermophilic genera like *Thermomonospora, Streptomyces, Microbispora, Saccharomonospora*, and *Saccharopolyspor*a can survive temperatures ranging from 45°C to 65°C (Fu et al., 2021). Acidophilic genera such as *Kitasatospora* and *Streptomyces* can endure pH levels between 3.5 and 6.5, as stated by Anandan et al. (2016). Halophilic genera, comprising *Rhodococcus*, *Micromonospora*, and *Streptomyces*, have adapted to salinity levels spanning 0.5 to 4 M, as noted by Hui et al. (2021). In addition, some of these species act as endophytes (Liu et al., 2020), symbionts (González et al., 2024), or endosymbionts (Matroodi et al., 2020).

The phylum Actinobacteriota contains several genera with notably high relative abundance, and *Gaiella, Pseudoarthrobacter, Streptomyces,* and *Micromonospora* stand out as prominent examples (Figure 3). Research indicates that *Gaiella* thrives in soils contaminated with polycyclic aromatic hydrocarbons (PAHs), as reported by Zahra et al. (2020). Additionally, studies have shown a positive correlation between *Gaiella* and higher concentrations of nitrate (NO_3-N), ammonium (NH_4^+-N), and soil organic carbon (SOC) in cemetery soil, both within the soil structure and intracellularly (Meynier et al., 2020). Furthermore, the genus *Streptomyces* encompasses a range of thermophilic, halophilic, and acidophilic species. The presence of Actinobacteriota, particularly *Streptomyces*, in cemetery soil underscores its capability for tasks like decomposing organic substances (including lipids, proteins, and cellulose) and producing antibiotics (Goudjal et al., 2018; Zhang et al., 2023). This genus is recognized for producing a wide variety of valuable secondary metabolites (Li et al., 2022; Ambarwati et al., 2023; Ayuningrum et al., 2022).

Research revealed a significant relative abundance in the genera *Wolbachia* and *Buchnera.* Insects and arthropods are home to symbiotic organisms, which often use decomposing remains to lay eggs or form cocoons (Wulandari & Rahayu, 2015; Arab & Lo, 2021; Kaur et al., 2021; Moran, 2021; Porter & Sullivan, 2023), a trend also observed by Sanaei et al. (2021), Hu et al. (2020). The bacterial community composition in cemetery soil appears to be impacted by the depth at which the sampling site is collected (Figure 5). Studies of soil samples, identified as Sample A and Sample B, revealed no significant differences, although electrical conductivity data showed considerable statistical significance (Table 3). The electrical conductivity has been identified as a crucial factor impacting changes in soil bacterial populations (Pittner et al., 2020; Han et al., 2021; Wang et al., 2020).

Table 3. The results of chemical analysis of cemetery soils

Paremeters	Unit	Block and deep (cm)						Method
		B18		B23		P12		
		20 cm	140 cm	20 cm	140 cm	20 cm	140 cm	
pH (H_2O)		7.29	7.95	7.84	7.90	8.10	7.51	pH meter 1:5 IK. 5.4.c
pH (KCl)		6.69	6.27	6.54	6.87	7.39	6.45	pH meter 1:5 IK. 5.4.c
DHL	(µs/cm)	87	28	13	18	9	42	Conductometer 1 : 5
C-organic	%	1.23	1.03	1.13	0.99	0.79	0.73	Walkley & Black IK 5.4.d
N-total	%	0.08	0.07	0.04	0.05	0.06	0.03	Kjeldahl IK 5.4.e
K availability	Ppm	106	91	129	124	147	171	Morgan-Wolf
P_2O_5	ppm	2	1	NA	NA	41	93	Olsen IK.5.4.h

The microbial genera represented by *Actinomadura* sp. and *Nocardioides* sp. serve as contrasting ecological indicators of different soil horizons. *Actinomadura* is primarily located in surface soils, due to its ability to adapt to oxygen-rich areas rich in organic matter (Ay et al., 2017; Lahoum et al., 2016). The organism's capacity to break down complex plant polymers like lignin and cellulose (Shi et al., 2019), as well as its production of antimicrobial compounds (Ay, 2021), gives it a competitive advantage within the diverse microbial populations of topsoil. These characteristics establish *Actinomadura* as a robust indicator of biologically active, nutrient-rich surface layers, especially in areas affected by plant residues or agricultural activities. In contrast to other species, *Nocardioides* species are more common in deeper soil layers, where their physiological adaptations allow them to survive under oligotrophic, low-oxygen, and high-pressure conditions. Their metabolic versatility allows them to survive in microaerophilic or intermittently anaerobic environments with limited nutrient availability (Sui et al., 2024; Ma et al., 2023). Their robust cellular structures and stress-tolerance mechanisms improve survival in subsoil conditions, including compaction and lower moisture levels. As a result, Nocardioides is consistently identified as a vital taxon within microbial communities found in deep soils, indicating ecological niches that are both stable and resource-limited.

This study provides important insights into the bacteriome of cemetery soils, identifying the most abundant phyla across both shallow and deep sampling sites. Future research should focus on isolating and characterising potentially significant bacterial groups found in cemetery soil.

5. Conclusion

This study found that sample B contains a greater number of OTUs and greater diversity compared to sample A. At a depth of 20 cm, Proteobacteria is the predominant phylum, whereas at 140 cm depth, Actinobacteria takes over. Genera of the phylum Proteobacteria that were consistently found in all samples included *Pseudomonas, Sphingomonas,* and *Microvirga*. Concurrently, the equally well-represented genera within the phylum Actinobacteriota include *Gaiella, Pseudoarthrobacter, Streptomyces* and *Micromonospora*. Principal component analysis revealed that bacterial community composition in cemetery soil is affected by soil depth. Biomarker analysis actually showed that specific genera were present, including *Actinomadura* sp. at a depth of 20 cm and *Nocardioides* sp. at 140-cm deep. This discovery offers important insights into the variety of the bacteriome in both deep and shallow regions of the cemetery. A more in-depth examination of the detected biomarker genera within

deep and shallow soils could be a vital area of study in the near future.

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