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Endophytic Bacterial Community of Leaf Tissues of *Mangifera indica* L. cv. Harumanis Revealed by 16S rRNA Metagenomics

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ABSTRACT

Numerous studies have demonstrated the importance of plant endophytic bacteria in promoting plant growth and fitness, both of which are crucial to agriculture. They have been shown to enhance nutrient uptake and utilisation, disease resistance, and stress tolerance, resulting in higher yields. However, only a few studies have been carried out on *Mangifera indica* cv. Harumanis endophytes, and those focused on fungi. This study therefore aimed to explore the composition, diversity, and potential functions of the endophytic bacterial community in *M. indica* cv. Harumanis, and this, to the best of our knowledge, is the first report. Fresh and asymptomatic leaves were collected for analysis. The bacterial endophytes were investigated by metagenomic analysis of the V3-V4 region of the 16S rRNA. Results showed unclassified bacteria (51-82%), followed by Alphaproteobacteria (5-15%), Gammaproteobacteria (3-8%), and Actinobacteria (2-10%) as the dominant bacterial phyla in the leaf tissues. Dominant genera revealed in the leaf tissues included *Pseudonocardia, Caulobacter, Nocardioides,* and *Achromobacter,* which are generally considered beneficial in environmental and agricultural contexts. These bacteria possess several plant growth-promoting traits such as nutrient cycling, defence against pathogens

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and stress tolerance, indicating their potential role in sustainable agriculture. The study concludes that the leaves of *M. indica* L. cv. Harumanis have a unique endophytic bacterial community that is crucial for plant growth and health and can be explored for agricultural applications, including biofertilizers and biocontrol agents.

Keywords: Bacterial diversity, folia tissues, fruit crops, plant-microbe interactions, 16S amplicon sequencing

INTRODUCTION

Mangifera indica L., commonly known as mango and belonging to the Anacardiaceae family, is a well-known fruit all over the world. It is economically and culturally valuable, especially in Asia, where it is widely farmed. Mango is one of the most treasured and widely cultivated tropical fruits in the world, with popularity spanning more than 100 nations, particularly in Asia. It was discovered in the Himalayan foothills of Northeastern India, Myanmar, and Bangladesh, and is a popular evergreen fruit tree native to Southeastern Asia (Kumar et al., 2021b). The 'Harumanis' variety, often known as the "King of Mangoes," is mostly grown in Malaysia's northern area, notably in the state of Perlis. It is quite popular in Malaysia with a unique sweetness and fragrant scent (Rathakrishnan et al., 2023). This cultivar is commonly used in Malaysia's commercial mango sector, both for local sale and export. Its distinct flavour and scent distinguish it from other varieties, resulting in considerable demand (Azizan et al., 2019).

Only a few studies have been carried out on *M. indica* cv. Harumanis endophytes, and existing studies are predominantly centred on endophytic fungi, particularly in the context of screening for disease potential and identifying biocontrol agents against various diseases (Ahmad et al., 2023; Bin Rosali et al., 2025; Suhanna et al., 2013; Zainudin et al., 2019). There is limited research conducted on the bacterial endophytes of this plant cultivar. Characterising bacterial endophytes of fruit crops via 16S amplicon sequencing is essential for profiling both culturable and unculturable microbial communities (Alibrandi et al., 2020). This technique helps identify beneficial bacteria that enhance plant growth, reduce abiotic stress, and protect against postharvest diseases, thereby supporting sustainable agriculture (Marian et al., 2025). Assessment of bacterial community dynamics is crucial for estimating endophytic community structure and its ecological behaviour, which are vital for understanding the molecular interactions with host plants (Abdullahi et al., 2020; Adhikary et al., 2021). Research on other mango cultivars by Adhikary et al. (2021) and Kumar et al. (2021a) reveals a shared microbiome that can inform our understanding of the Harumanis variety. According to Afzal et al. (2019), plant endophytic bacteria are microorganisms that live within plant tissues without harming their hosts. By developing symbiotic relationships with their host plants, these bacteria significantly improve plant production and health across a variety of environmental contexts (Kandel et al., 2017). They

live within plant tissues, form connections that promote nutrient exchange and enzymatic activities, and are referred to as plant growth-promoting endophytes (PGPE) (Kuźniar et al., 2024). The methods utilised by PGPE are like those of plant growth-promoting rhizobacteria (PGPR). Endophytic bacteria produce and release hormones that promote numerous physiological processes in plant tissues, thereby positively affecting plant development. They also produce a variety of bioactive compounds (such as alkaloids, amines, peptides, terpenoids and flavonoids) with a wide range of biological activities that can be carried out directly or indirectly, such as the solubilisation of mineral phosphate, potassium, and silicate (Biswas et al., 2023; Hassan et al., 2017), production of indole-3-acetic acid (Fouda et al., 2020), production of ammonia, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, nitrogen fixation, and biocontrol activities (Biswas et al., 2023).

The process of nitrogen fixation, which can involve certain endophytic bacteria, is a significant source of nitrogen for agricultural crops. In addition to reducing the need for artificial fertilisers, improving nutrient use efficiency, increasing crop yields, and reducing environmental pollution, endophytic nitrogen-fixing bacteria can be used as biofertilisers (Ng et al., 2024; Puri et al., 2020). Endophytes provide a variety of beneficial signals and metabolites that promote plant growth in various ways, which plants provide in exchange for protective niches (Ek-Ramos et al., 2019). Bacterial endophytes also mitigate the negative effects of abiotic stress such as salinity (Akbaba & Özden, 2023). Most plants harbour endophytes in their tissues; nevertheless, existing knowledge of PGPE and their biological activity does not align with the high prevalence of endophytes. Despite their importance in plant micro-ecosystems, the link between bacterial endophytes and their host plants remains poorly understood (Wang et al., 2024; Wu et al., 2021).

A better understanding of native plant endophytes may help reveal their potential to promote plant growth and support sustainable agricultural production. To date, no published study has examined bacterial endophytes associated with this cultivar. The discovery of PGP endophytic bacteria in this plant cultivar could be of biotechnological relevance in agriculture. Identifying the microbes naturally residing within the plant's tissues will allow the development of eco-friendly biocontrols, natural biofertilizers, and stress-tolerant crops that reduce reliance on synthetic chemicals. Therefore, the study aims to characterise endophytic bacterial diversity in the leaf tissues of Harumanis mango using 16S rRNA sequencing and investigate their potential to enhance plant growth and stress tolerance for sustainable agriculture.

MATERIALS AND METHODS

Sample Collection and Analysis

The leaves of the *M. indica* cv. Harumanis were collected from a Harumanis mango orchard plot in the Institute of Sustainable Agrotechnology (INSAT), Universiti Malaysia

Perlis (UniMAP), Sungai Chucuh Campus, 02100 Padang Besar, Perlis, Malaysia. The leaves were obtained through a randomised sampling method, which randomly selected healthy plants. From each of these plants, three mature leaf specimens that were healthy and asymptomatic, showing no prior damage or disease symptoms, were collected and pooled. The fresh leaf specimens were carefully placed in sterile, sealed bags, then placed inside polyethene boxes filled with ice and transported to the laboratory on the same day. The plant identification was tentatively recorded in the field. A total of four samples were used for the metagenomic analysis.

Surface Sterilisation

Surface sterilisation was performed on the collected leaves using the method described by Ruiz-Pérez & Zambrano (2017). The leaves were washed for 30 minutes under running water to remove any dirt, debris, and attached epiphytes. The leaves were then thoroughly cleaned with sterile distilled water after being washed with a detergent solution in tap water. Afterwards, the leaves were immersed in 90% (v/v) ethyl alcohol for 60 seconds. Then, immersed for 6 minutes in 5% sodium hypochlorite (NaCIO), followed by 30 seconds in 70% ethyl alcohol (v/v). Finally, the leaf samples were washed three times with sterile distilled water, and the final wash (200 µL) was pipetted and spread onto the nutrient agar (NA) plates. The leaves were dried using sterile filter paper before the efficiency of surface sterilisation was further tested by making an imprint on NA to ensure that every isolate comes from an endophytic rather than an epiphytic source and to examine the effectiveness of the procedure. A control plate containing NA media without a sample was used as a negative control. The plates were incubated at 28 ± 2.0 °C for 3 days and checked daily for microbial growth (Archana et al., 2020).

Genomic DNA Extraction and Sample QC

The genomic DNA from the Harumanis leaf sample was extracted using a commercial kit, the SEPA Plant DNA Isolation Reagent Kit, according to the manufacturer's instructions. The quality of the purified DNA was examined on 1% TAE agarose gel (Grobelak & Hiller, 2017; Nair et al., 2021). The concentration of DNA was measured using a spectrophotometer (Implen NanoPhotometer® N60/N50) and fluorometrically quantified with the iQuant™ Broad Range dsDNA Quantification Kit.

PCR Amplification, Library Preparation and Sequencing

The purified gDNA that passed DNA sample QC was amplified using locus-specific primer pairs. Bacterial 16S V3-V4 (5' to 3'): 16S V3-V4 Forward: CCTACGGGNGGCWGCAG; 16S V3-V4 Reverse: GACTACHVGGGTATCTAATCC. All the PCR reactions were carried out with REDiant 2X PCR Master Mix (1st BASE). Dual indices were attached to

the amplicon PCR using Illumina Nextera XT Index Kit v2 following the manufacturer's protocols. The quality of the libraries was measured using the Agilent Bioanalyzer 2100 System with the Agilent DNA 1000 Kit and fluorometric quantification by Helixyte GreenTM Quantifying Reagent. The libraries were normalised and pooled according to the protocol recommended by Illumina and then sequenced on the MiSeq platform with 300 PE. This was carried out by Apical Scientific Sdn. Bhd.

Bioinformatics Analysis

The raw sequencing data were quality-controlled using Trimmomatic to remove short and truncated reads. Subsequent downstream bioinformatics analysis was performed using the Mothur software package. Then, the near-identical clean reads were clustered together, and the chimaera reads were also removed. Subsequently, non-bacterial sequences such as 18S rRNA, chloroplast, and mitochondrial sequences were removed using Classify seqs and remove lineage options. Operational taxonomic units (OTUs) corresponding to the 16S rRNA sequences for each sample were determined using a 97% identity threshold for 16S gene variants at the species level. The taxonomy for each gene was analysed using RDP Classifier against the Silva database with a confidence threshold of 0.7. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) was used to predict the functional potential of microbial communities in the leaf tissues from the 16S rRNA metagenomic data.

Data Deposition and Accession Number

The nucleotide sequences of the endophytic bacterial community from leaf tissues of *Mangifera indica* cv. Harumanis were submitted to the Sequence Read Archive (SRA) database of the National Centre for Biotechnology Information (NCBI) with a BioProject accession number PRJNA1353884.

RESULTS

Overview of 16S rRNA Amplicon Sequence, Richness and Diversity of the Endophytic Bacteria

A total of 126092, 107573, 115396, and 140617 clean reads were generated for samples 1, 2, 3, and 4, respectively. The clean reads were grouped into operational taxonomic units (OTUs) using a 97% similarity threshold. OTUs of each sample and the relative information of the tags were counted, and the most abundant OTU sequences in each cluster were selected as representative sequences for database blast and annotation. The number of OTUs per sample ranged from 100 to 162, with Sample 1 having the highest and Sample 3 the lowest, and a total of 593 OTUs were identified (Table 1). A Venn diagram represents the shared and unique bacterial taxa detected among the four different leaf samples obtained

from the metagenomic analysis (Figure 1). The numbers inside the overlapping regions indicate how many bacterial taxa are shared among the respective samples, while numbers found only within a single oval indicate unique taxa to that sample. Six (6) bacterial taxa are shared by all samples, forming the core bacterial community, indicating that certain bacterial groups are consistently associated with the plant's leaf surface regardless of minor differences in leaf location or sampling conditions. Rank abundance and rarefaction curves were generated and reached a plateau, indicating that the sample distribution and sampling depth were sufficient to determine the species richness in all the study areas, as shown in Figure 2.

Table 1
Sequence characteristics and the α-diversity of endophytic bacteria from the leaf tissues of M. indica *L. cv Harumanis*

Label	Group	Reads	OTU	Chao	Shannon
1	Sample-1	126092	166	9	5.050377
2	Sample-2	107573	165	13	4.781283
3	Sample-3	115396	100	9	4.558349
4	Sample-4	140617	162	9	4.978021

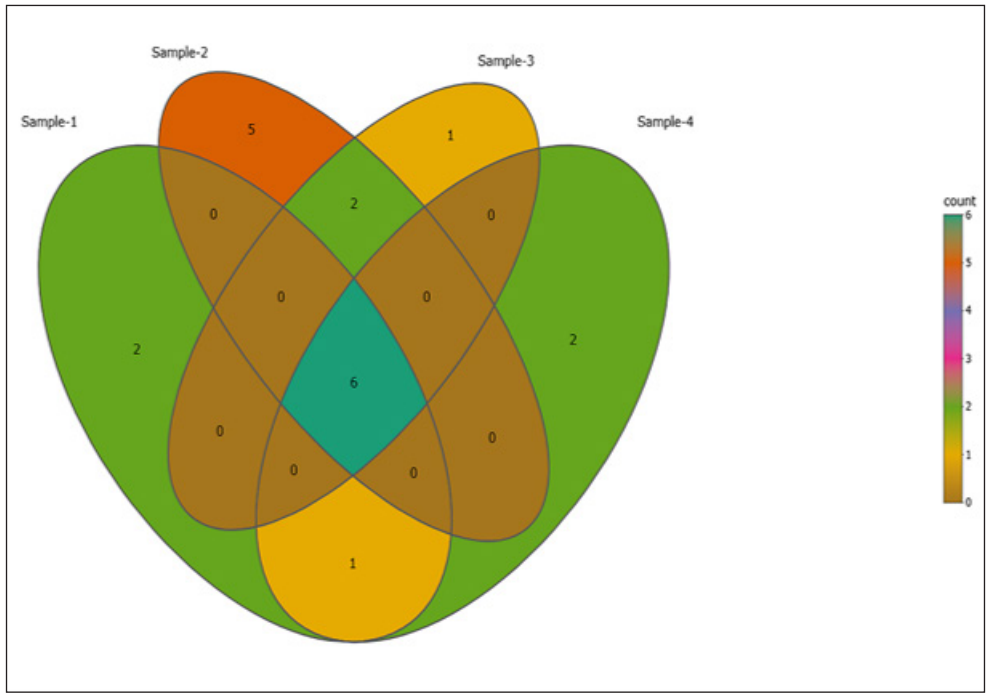

Figure 1. Shared and unique endophytic bacterial taxa detected among four leaf samples from the metagenomic analysis of *M. indica* L. cv. Harumanis

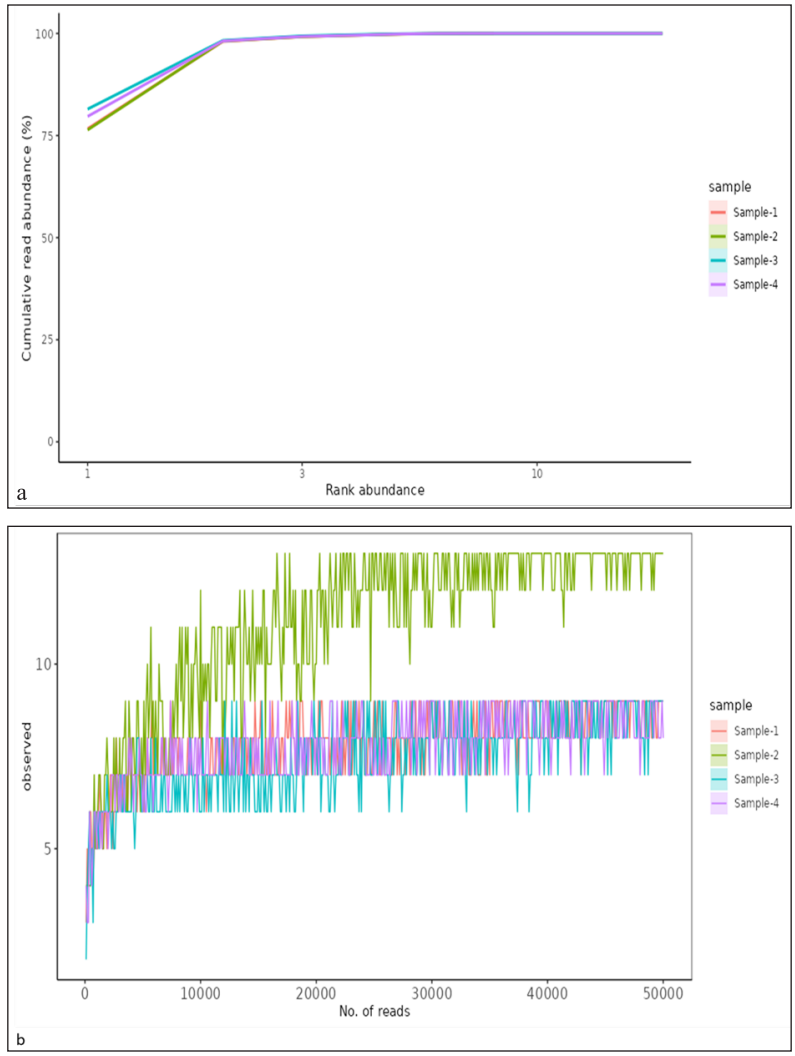

Figure 2. Rank abundance (a) and Rarefaction (b) curves generated from endophytic bacterial communities of *M. indica* cv. Harumanis leaves, indicating that the sample distributions and depth were adequate to assess species richness

The richness and diversity of the bacterial community in the leaves were estimated by computational analysis of alpha-diversity. Chao1 index estimated the number and richness of species. The Chao1 estimate for sample 2 was the highest (13), indicating greater species richness, while samples 1, 3, and 4, which had the lowest values (9 each), had lower species richness (Figure 3). A similar trend was observed in the Shannon diversity index values, with sample 2 again exhibiting the highest value. This indicates that it not only contains more species, but they are more evenly distributed. Sample 3 and Sample 4 have lower values, implying dominance by a few taxa (Figure 3).

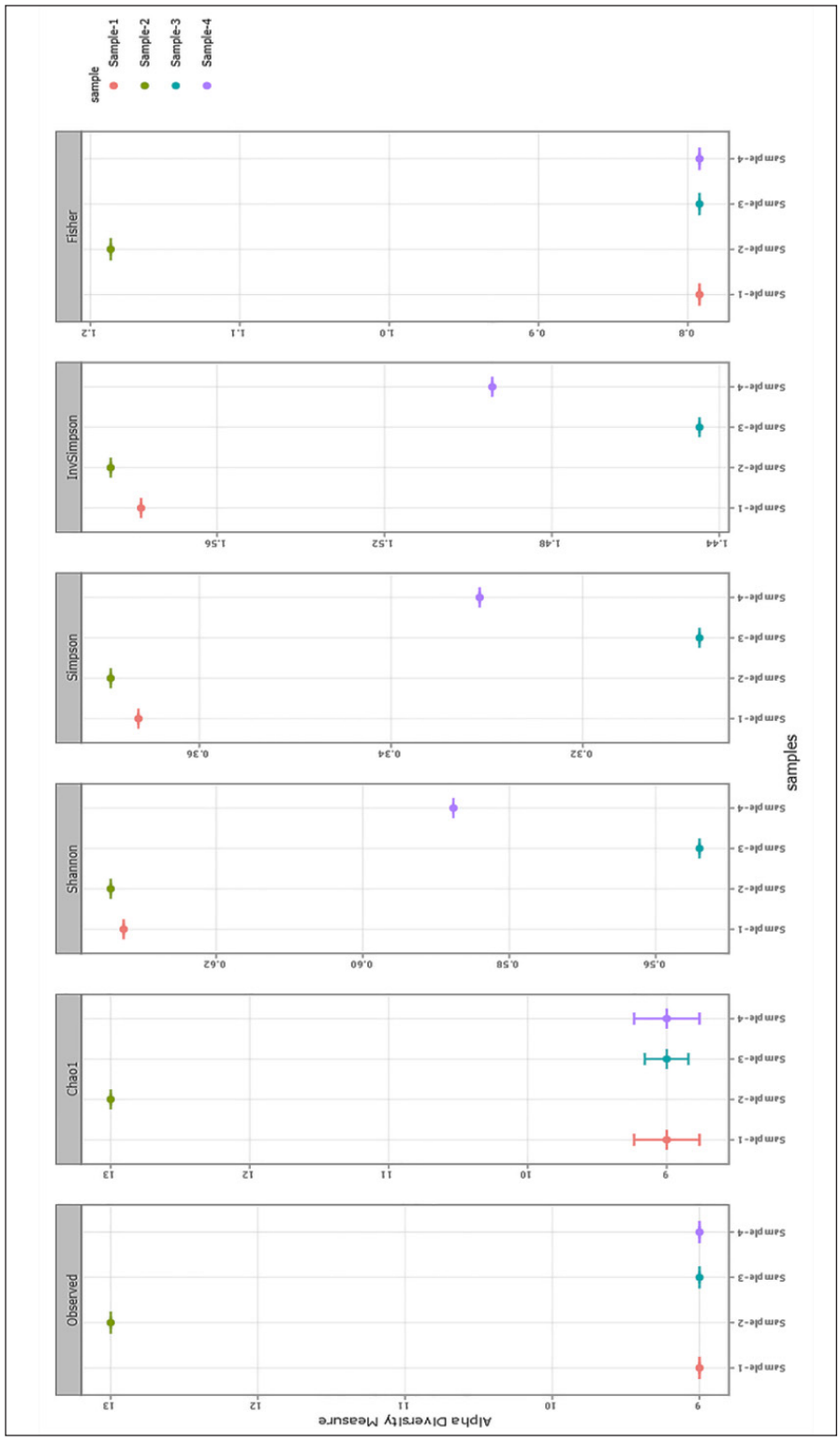

Figure 3. Alpha diversity indices of endophytic bacterial communities of leaves of *M. indica* cv. Harumanis. Sample 2 exhibited the highest bacterial richness and evenness, indicating greater community diversity and ecological stability compared to other samples

Structure of the Endophytic Bacterial Community in the Leaf Tissues of *Mangifera indica* cv. Harumanis

The most represented and dominant phylum among all the bacterial endophytes isolated from the leaf tissues of *Mangifera indica* cv. Harumanis was unclassified bacteria (51-82%), followed by Alphaproteobacteria (5-15%), Gammaproteobacteria (3-8%), and Actinobacteria (2-10%) (Figure 4). Sample 3 had the highest percentage of unclassified bacteria (82%), while Sample 2 had the highest percentages of Alphaproteobacteria and Gammaproteobacteria (15% and 8%, respectively). Actinobacteria were also found at the highest abundance (10%) in Sample 2. The top endophytic bacterial families detected in the leaves are presented in Figure 5. At the family level, the Bacteria-unclassified were also more abundant, with the highest occurrence in Sample 3 (82%). Caulobacteraceae, Sphingomonadaceae, Proteobacteria-unclassified, Bacterioles-unclassified and Enterobacteriaceae were much more abundant in Sample 2 (4% - 5%) than in other samples. The family Burkholderiales were also high in abundance in samples 4 and 1 (3% and 2%, respectively).

The composition of the bacterial community associated with the leaves of *M. indica* cv Harumanis revealed different taxa distributed and clustered across the samples (Figure 6). The relative abundance of each bacterial taxon in each sample is represented by the colour gradient, where red shades indicate high abundance and dominance, and blue shades indicate low abundance. The taxa labelled unknown mitochondria are the most abundant bacterial community across all samples, compared with other genera such as *Pseudonocardia, Caulobacter, Nocardioides, Achromobacter,* and *Ralstonia,* which show lower abundance. The samples and taxa are grouped into two hierarchical clusters based on similarity and patterns of bacterial abundance. On one cluster are samples 1 and 2, while on the other are samples 3 and 4. Samples in each cluster were closely grouped, indicating that they share a similar bacterial community. The two clusters are near but slightly apart, with minor variation likely due to physiological, environmental, or plant-age factors. The presence of unknown KD4-96 (family) in the samples indicates a bacterial community that could not be classified at the genus level. This further showed that the leaves are underexplored.

Endophytic Bacterial Functional Prediction

The predicted functional gene categories of the bacterial taxa in the leaf tissues by PICRUSt2 revealed diverse functions (Figure 7). Predicted functional genes are represented by corresponding colour intensity to indicate abundance, where yellow/red represent higher intensity or abundance of functional genes, and blue represents lower intensity or abundance. Hierarchical clustering by the tree-like structures (dendrograms) shows similarity among samples and among predicted functions. The samples were divided into

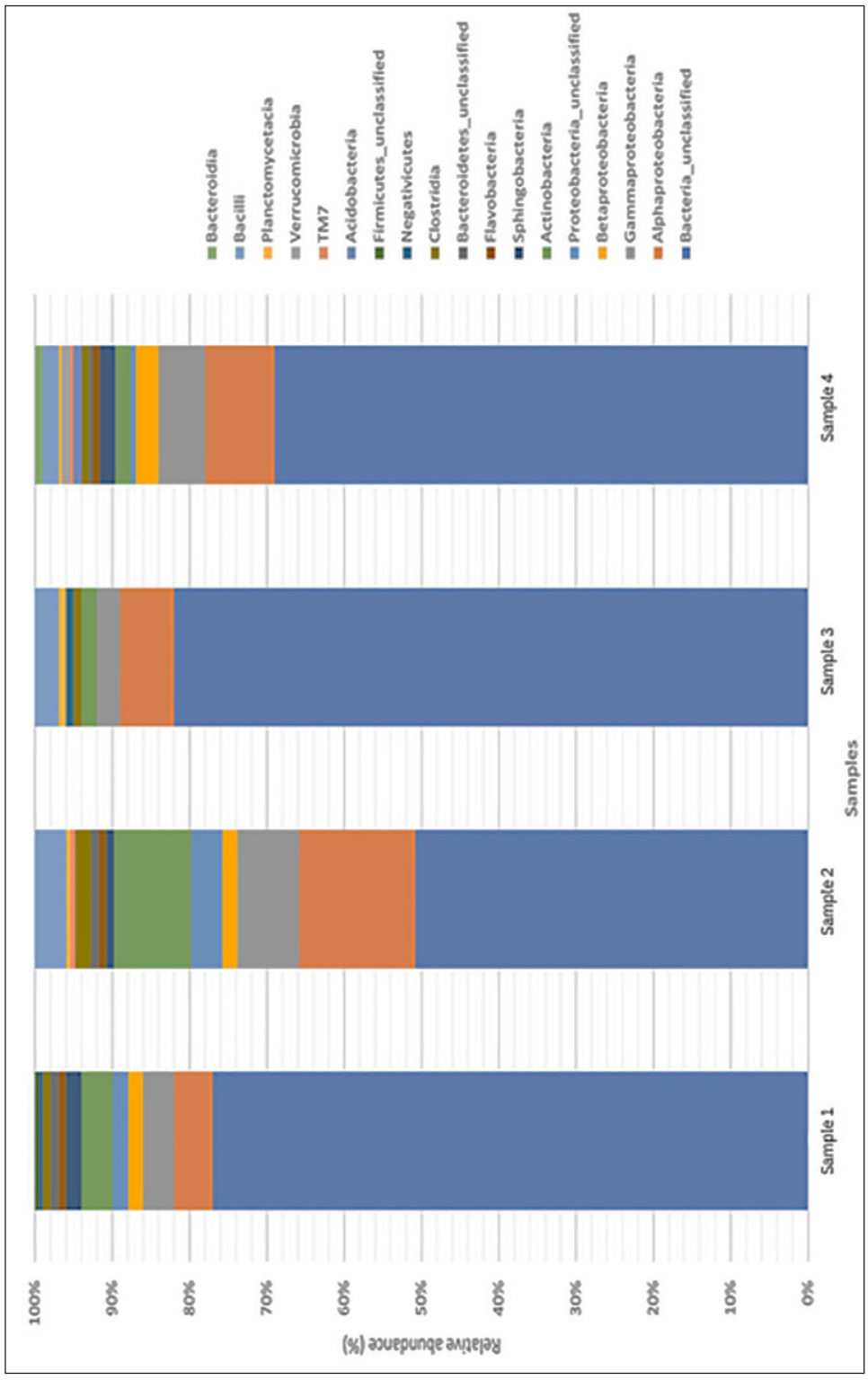

Figure 4. Relative abundance of endophytic bacteria at the phylum level identified from the leaf tissues of *M. indica* L. cv. Harumanis

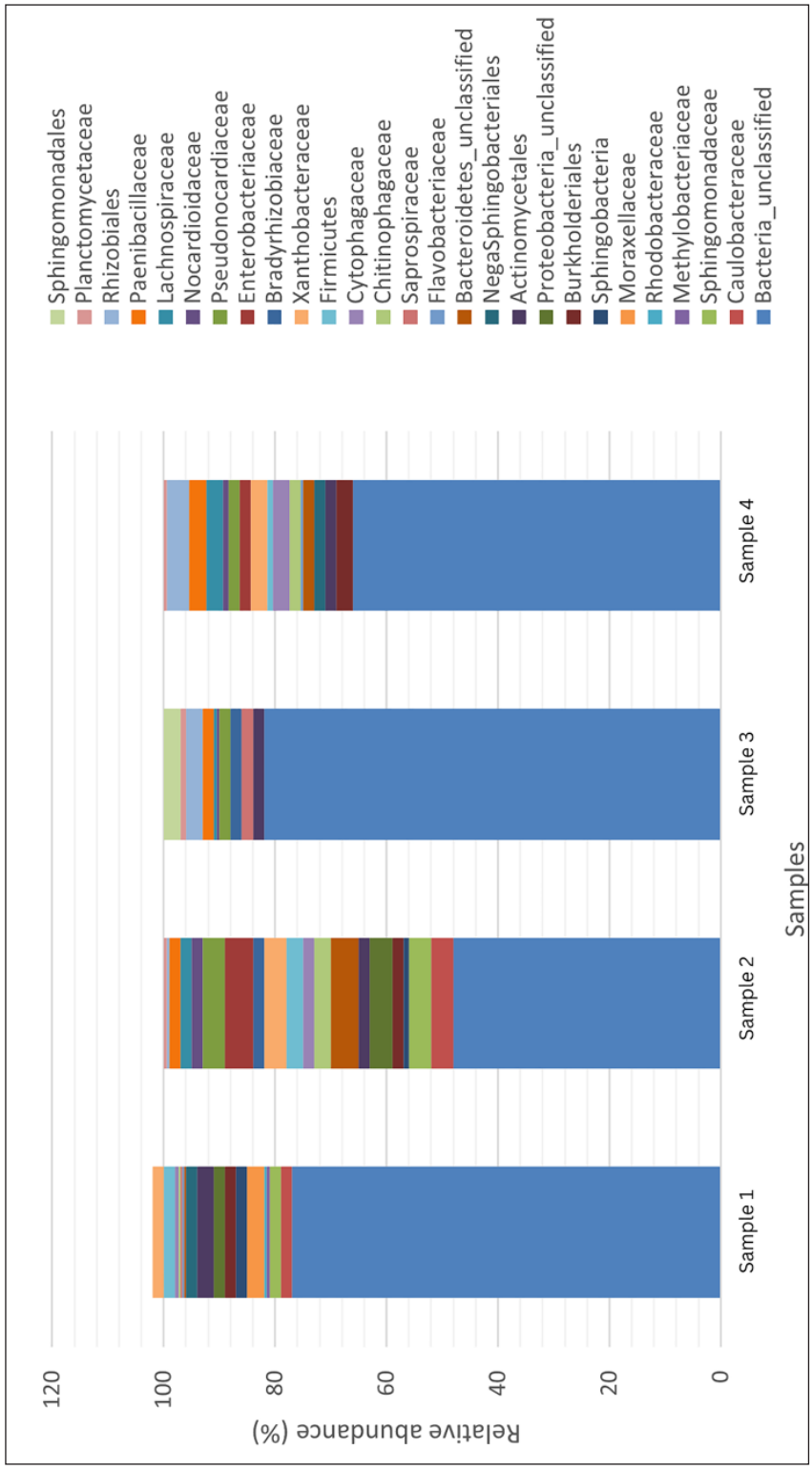

Figure 5. Relative abundance of endophytic bacteria at the family level identified from the leaf tissues of *M. indica* L. cv. Harumanis

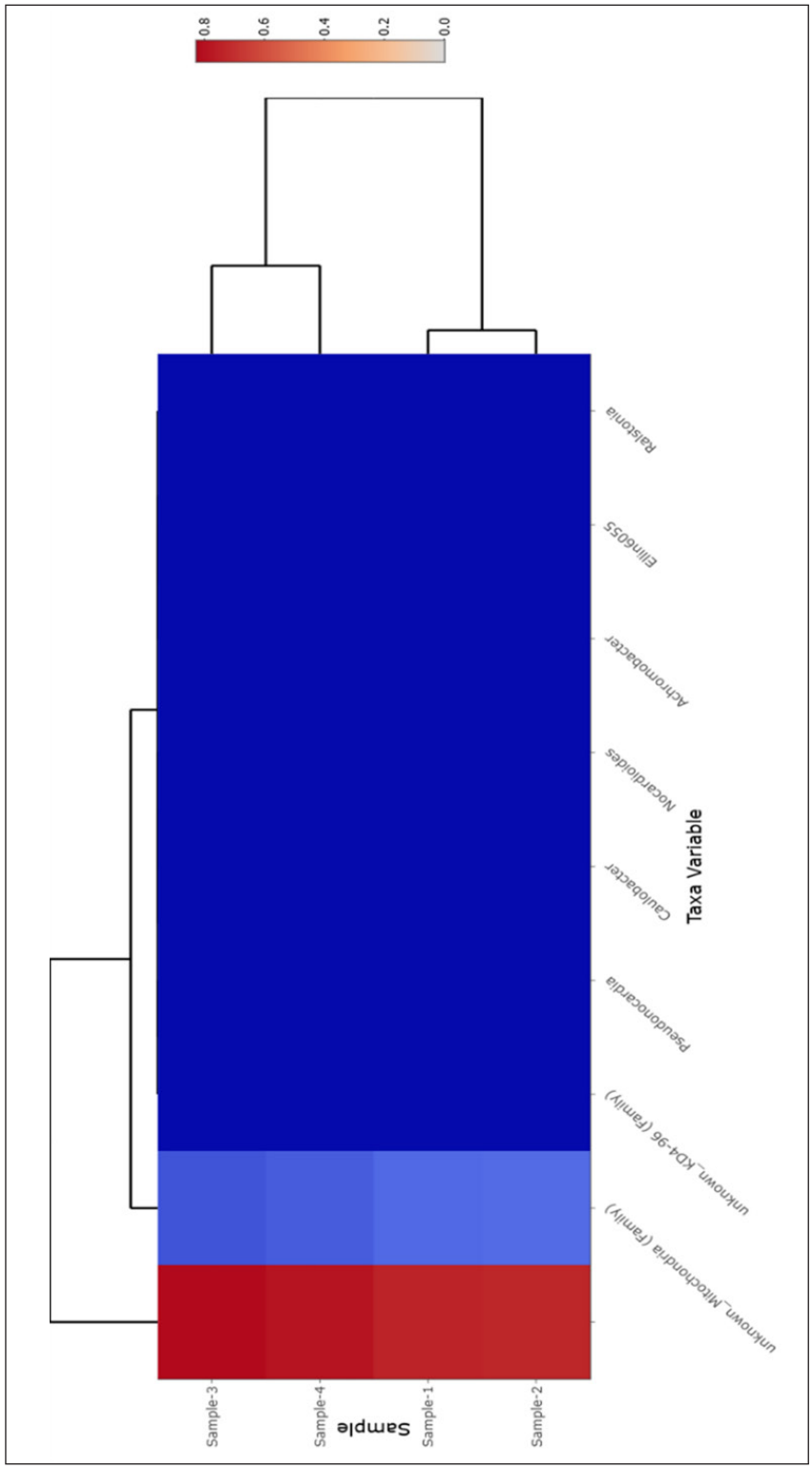

Figure 6. Overview of the taxonomic composition of endophytic bacterial genera associated with the leaf samples of *M. indica* L. cv Harumanis, showing how the bacterial genera are distributed and clustered across the samples

two major clusters: Sample 1, Sample 3, and Sample 4 in one cluster (indicating a similar functional profile), and Sample 2 in a separate cluster, suggesting a distinct functional composition of its bacterial community. The heatmap shows that Sample 2 has a broader region of high functional abundance (yellow/orange) compared to the others, indicating that its bacterial community possesses a greater number or diversity of metabolic genes. Furthermore, the distances between points by the ordination analyses of KEGG Orthologs (KOs) reflect how similar or dissimilar the samples are in their functional gene composition (Figure 8). For example, in CCA, Sample 1 (red) lies far apart, possibly under different environmental or host-associated conditions. NMDS and PCoA reveal functional divergence while maintaining relative distances (Samples 1 and 2 are closer in some plots, while 3 and 4 cluster more tightly). The distinct ordination positions suggest divergent metabolic potentials; e.g., carbohydrate metabolism, nitrogen cycling, or stress response genes may differ among samples. Samples 1 and 2 share more similar bacterial species, abundances, and functions, while Samples 3 and 4 contain more distinct bacterial profiles. This could reflect differences in leaf age, exposure, or microbial colonisation patterns.

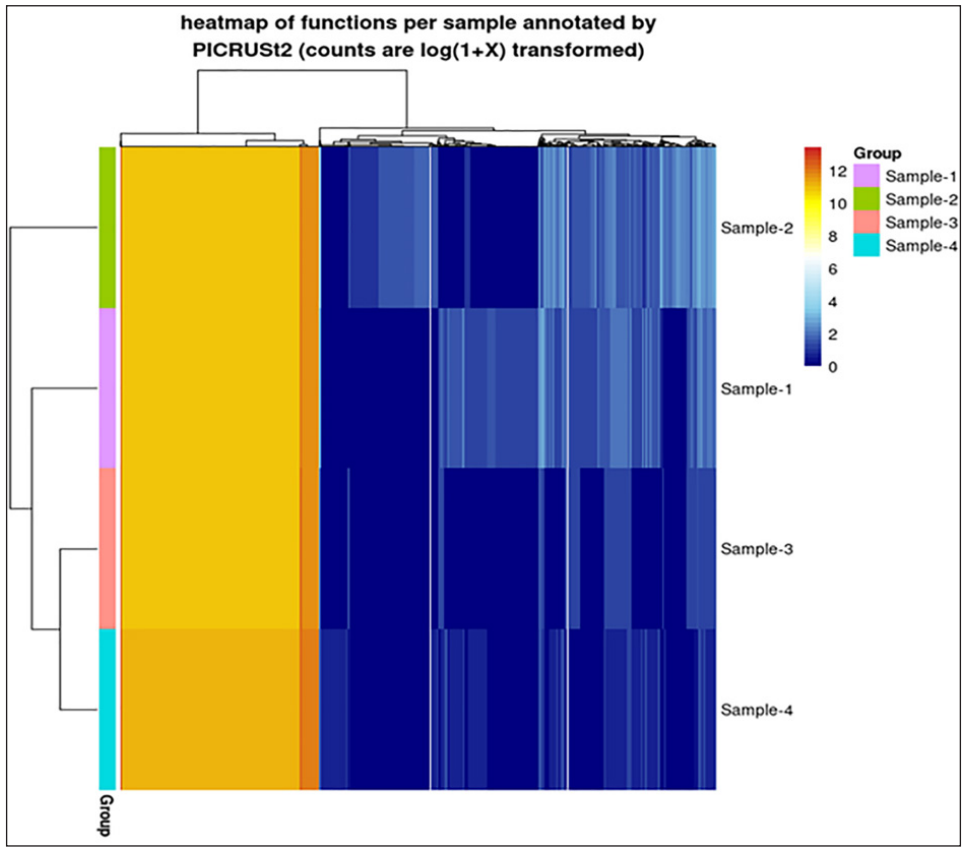

Figure 7. Functional heatmap of endophytic bacterial community in the leaf tissues of *M. indica* L. cv Harumanis predicted by PICRUSt2 based on 16S rRNA metagenomic gene data

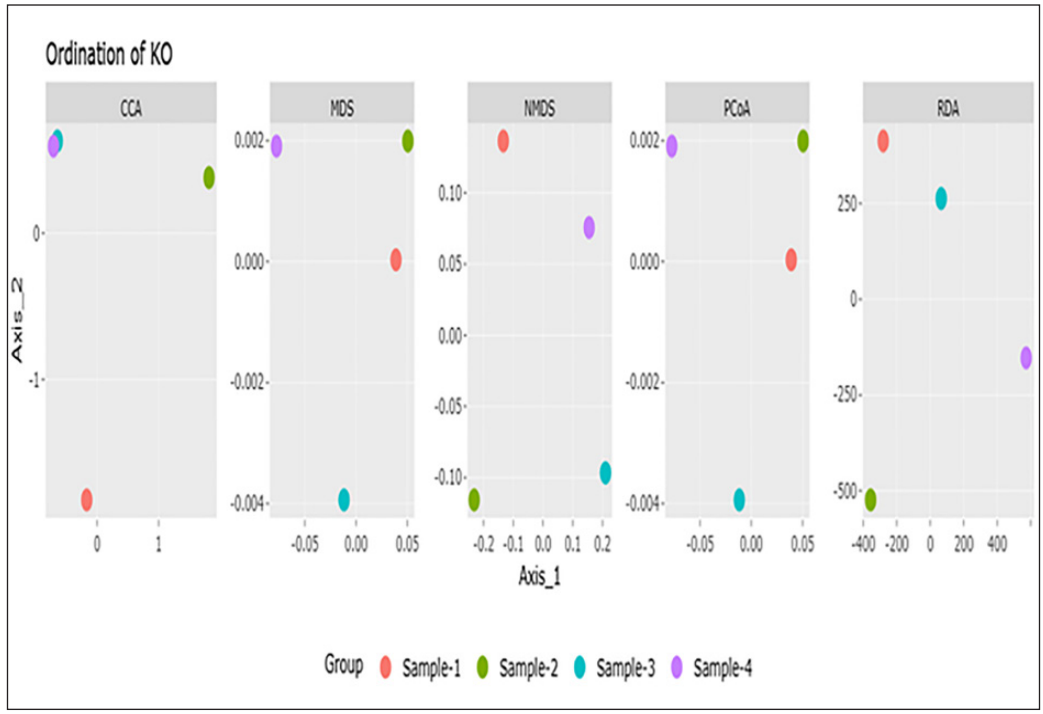

Figure 8. Ordination analyses of KEGG Orthologs (KOs) of endophytic bacterial community in the leaf tissues of *M. indica* L. cv Harumanis. Ordination plots (CCA, MDS, NMDS, PCoA, and RDA) show similarity or dissimilarity among samples based on their functional gene composition

DISCUSSION

In this study, 16S amplicon metagenomic analysis provided information on the composition, diversity, and distribution of endophytic bacteria associated with leaf tissue samples of harumanis mango. Since OTUs are thought to fairly represent community members, they are frequently used to infer diversity and functional features (Lladó Fernández et al., 2019). Six bacterial taxa are shared by all samples, forming the core bacterial community, indicating that certain bacterial groups are consistently associated with the plant's leaf surface regardless of minor differences in leaf location or sampling conditions. In general, any collection of microbial taxa, or the genomic and functional characteristics associated with those taxa, that are typical of a host or environment of interest is referred to as the core microbiome (Lladó Fernández et al., 2019; Neu et al., 2021). The occurrence of bacterial taxa in preferred locations, even when environmental factors strongly influence their distribution, clearly indicates that the bacteria are well adapted to the system (Abdullahi et al., 2021). Such core taxa are typically beneficial bacteria involved in various plant-beneficial functions, such as nutrient mobilisation, stress tolerance, and protection against pathogens (Finkel et al., 2017; Neu et al., 2021). The environment in Perlis is relatively hot

and dry, with distinct seasons directly shaping Harumanis mango's endophytic bacteria. Extreme temperatures, soil chemistry, and seasonal shifts determine which internal bacteria thrive, shaping the tree's defence mechanisms against diseases such as anthracnose and stem-end rot (Galsurker et al., 2020). The specific hot and dry conditions in northern Malaysia encourage the plant to produce higher levels of specialised secondary metabolites, which act as chemical filters and select for specific internal microbiota (Peron et al., 2025). Harsh, dry weather generally decreases overall microbial richness but increases the proportional abundance of resilient, stress-adapted bacterial communities inside the leaves and stems.

Higher community diversity and evenness in sample 2, as revealed by the computational analysis, could indicate a more stable and supportive environment in the leaf tissues. A more stable or nutrient-rich leaf environment that supports diverse bacteria could also indicate healthy phyllosphere conditions (Sivakumar et al., 2020). The alpha diversity metrics indicate that Sample 2 harbours the richest and most balanced bacterial community, while Samples 3 and 4 show reduced diversity, possibly reflecting environmental stress or host-specific selection. Environmental stress, leaf senescence, or antimicrobial compounds might have reduced bacterial colonisation (Bai et al., 2025). Even though leaves appear to be a harsh habitat due to their exposure to sunlight, dryness, and temperature fluctuations, many bacteria can live and even flourish there (Moitinho et al., 2020). These microbes may affect nutrient cycling, disease resistance, and plant growth (Niu et al., 2026). While some bacteria defend the plant from diseases, others generate hormones that promote development. These results highlight how leaf microenvironments strongly influence bacterial richness and evenness, shaping the structure and function of the leaf tissues' microbiome. Several variables, including plant species, leaf age, environmental factors, and agricultural techniques, affect the makeup of bacterial communities on leaves (Zhang et al., 2022). Gaining an understanding of these cultures will aid in developing environmentally friendly farming practices and increasing crop yields.

The most represented and dominant phyla among all the bacterial endophytes from the leaf tissues of *Mangifera indica* cv. Harumanis were bacteria-unclassified, Alphaproteobacteria, Gammaproteobacteria, and Actinobacteria. Other studies also reported similar results; for example, Li et al. (2025) showed that Proteobacteria were the dominant bacterial phylum in mango leaves. Adhikary et al. (2021), in their study exploring endophytic bacterial communities and their dynamics in the pre-flowering and post-flowering seasons in mango and its hemiparasites using 16S rRNA gene metagenomics, found that the most abundant genera belonged to the phyla Firmicutes, Proteobacteria, and Actinobacteria. Proteobacteria and Actinobacteria are key players when it comes to plant growth and health. Proteobacteria are involved in nutrient cycling, especially nitrogen fixation, while Actinobacteria are known for direct plant growth enhancement through the

production of hormones and indirectly through the production of antibiotics, solubilization of nutrients, and antagonistic activities against pathogens (Franco-Correa, 2016; Saberi Riseh, 2022). The higher percentages for unknown sequences at both the phylum and family levels is an indication that there are many potential new isolates to be discovered. The majority of Earth's microbial diversity remains uncharacterized in laboratory cultures, with many unidentified microorganisms, termed "microbial dark matter" (Lloyd et al., 2018), being numerically dominant across major environments. Additionally, about 25% of the microbial cell population belongs to phyla with no known relatives, suggesting these organisms are vital for ecosystem functions (Lloyd et al., 2018).

The study highlighted *Pseudonocardia, Caulobacter, Nocardioides, Achromobacter,* and *Ralstonia* as the most common genera associated with harumanis mango leaves. The genus *Pseudonocardia* is a member of the Pseudonocardiaceae family of actinomycetes. Research has shown that *Caulobacter* strains can increase plant biomass across a variety of plant species, aligning with agroecological relevance and biotechnological advancements (Berrios, 2022). Species of the genus produce secondary metabolites, some of which have antibacterial, antifungal, and antitumor properties, making them valuable in biotechnology (Riahi & Heidarieh, 2022). A study by Ghodhbane-Gtari et al. (2019) revealed two plant-growth-promoting actinobacteria of the genus *Nocardia*, strains BMG51109 and BMG111209, to induce root nodule formation in *Casuarina glauca.* Species of *Achromobacter,* such as *Achromobacter piechaudii,* are known to produce ACC deaminase, which has been shown to alleviate drought stress in plants by increasing biomass and reducing ethylene levels (Yang et al., 2009). The unknown genus (unknown KD4-96) present in the uncultured bacterial lineage may represent a novel taxon in the inner leaf tissues of the harumanis mango. It also highlights the potential of metagenomic studies to reveal unexplored microbial diversity that traditional culture-based approaches couldn't. Deciphering the plant microbiome through next-generation sequencing of the 16S rRNA is a good method to identify phyla but not very suitable to classify species or genus (Sharma et al., 2020). The leaf tissues serve as a home to many uncultivated microorganisms (Moitinho et al., 2020). Also, because of interspecies molecular dependence, it is essential to comprehend the molecular underpinnings of the social networks in this habitat to obtain fresh perspectives on the ecology of the enigmatic community members (Moitinho et al., 2020).

The predicted functional gene categories of the bacterial taxa in the leaf tissues were divided into two major clusters, with Sample 1, Sample 3, and Sample 4 in one cluster (indicating a sharing of similar functional profiles) and Sample 2, on the other hand, in a separate cluster, suggesting a distinct functional composition of its bacterial community. The relationship between real taxa and molecular taxa appears to be far more nuanced. Only a few genes (the core genome) are usually shared among strains of the same bacterial

species (preferably belonging to the same OTU), other genes are strain-specific and distinct (Lladó Fernández et al., 2019). Even though leaves appear to be a tough habitat due to their exposure to sunshine, dryness, temperature fluctuations, UV radiation, and nutrient availability, many bacteria may live and even flourish there. These microbes may have an impact on nutrient cycles, disease resistance, and plant growth (Dastogeer et al., 2020; Sivakumar et al., 2020). Various environmental factors and conditions may account for variation among samples, including sunlight, humidity, temperature, age, and nutrient availability, which are known to affect microbial gene functions. The microbial community with increased metabolic activity, possibly involved in the food cycle, secondary metabolite generation, or stress tolerance, may be reflected in Sample 2 with enrichment in metabolic functions. This may indicate that a metabolically active microbiome is supported by the physicochemical conditions of Sample 2 (younger leaf, increased nutrient exudation, or more moisture). The key microbial processes necessary for plant-microbe interactions are likely represented by the core functions shared among the other samples, such as nitrogen metabolism, stress resistance and antioxidant mechanisms, phytohormone biosynthesis (auxins, cytokinins), plant exudate degradation, and carbon utilisation. Numerous significant anticipated biosynthetic gene clusters for plant phytohormones, secondary metabolites, and novel natural products were found in the genome of Nocardia (Ghodhbane-Gtari et al., 2019). The distinct ordination positions suggest divergent metabolic potentials; for example, carbohydrate metabolism, nitrogen cycling, or stress response genes may differ among samples.

CONCLUSION

The leaves of *Mangifera indica* cv. Harumanis host a diverse community of beneficial endophytic bacteria, primarily belonging to the phyla Alphaproteobacteria (5-15%), Gammaproteobacteria (3-8%), and Actinobacteria (2-10%). Dominant genera revealed in the leaf tissues included *Pseudonocardia, Caulobacter, Nocardioides,* and *Achromobacter,* which are generally considered beneficial in environmental and agricultural contexts. These bacteria possess several plant growth-promoting traits such as nutrient cycling, defense against pathogens and stress tolerance, indicating their potential role in sustainable agriculture. Additional research may provide a more thorough understanding of the functional genes and interactions between the bacteria and the Harumanis mango. By utilising these natural microbial communities, it may be possible to promote healthier plants and lessen reliance on artificial fertilisers.

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