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Bacterial community profiles of leaves of wild and cultivated *pohpohan* (*Pilea melastomoides* (Poir.) Wedd.) plants based on a metagenomic analysis

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Abstract. *Yonantiko E, Astuti RI, Budiarti S. 2024. Bacterial community profiles of leaves of wild and cultivated* pohpohan *(*Pilea melastomoides *(Poir.) Wedd.) plants based on a metagenomic analysis. Biodiversitas 25: 2996-3004.* Recent studies show that fresh vegetables serve as hosts for diverse endophytic bacterial communities. In Indonesia, certain tropical plants are consumed fresh without cooking, which may raise concerns regarding human health and food safety. *Pilea melastomoides* (Poir.) Wedd. is a traditional food plant consumed fresh as a vegetable by the Sundanese people in Indonesia. This plant grows in the wild and can be cultivated in a standard plantation, thereby raising the question of the endophytic bacterial communities in both planting systems. Thus, this study aimed to profile the bacterial communities in wild and cultivated *P. melastomoides* via a metagenomic approach targeting the 16S rRNA gene. Data analysis revealed that the leaf samples collected from the cultivated plants exhibited the highest diversity, as indicated by the diversity indices of species richness based on Chao1 (0.0244), Shannon (0.0291), and species evenness based on Pielou (0.0282). The most abundant bacterial phyla identified were Firmicutes and Proteobacteria. In cultivated plants, the predominant endophytic bacteria included: *Limosilactobacillus* (5.38%), *Escherichia/Shigella* (4.96%), *Bacillus* (4.76%), *Salmonella* (3.57%), and *Staphylococcus* (3.57%). In contrast, certain genera of pathogenic bacteria, such as *Escherichia/Shigella* (15.59%), *Bacillus* (10.48%), *Limosilactobacillus* (9.00%), *Salmonella* (7.78%), and *Listeria* (5.94%), were dominant among the wild plants. The findings of pathogenic bacteria are dominated by Gram-negative bacteria, which play a role in the nitrogen, carbon, and sulfur cycles. The stable and consistent presence of endophytic bacteria in plant tissues provides opportunities for long-term studies for food safety and sustainable agricultural practice strategies. Further analysis should be conducted to confirm the virulence of these pathogens in both wild and cultivated *P. melastomoides* plants.

Keywords: Endophyte bacteria, microbiome, next generation sequencing, *pohpohan*, *Pilea melastomoides*, 16S rRNA

Abbreviations: OTU: Operational Taxonomic Unit

INTRODUCTION

In Indonesia, tropical plants are widely used as functional food sources and raw materials for herbal medicines. An ethnobotanical study carried out by Kodir et al. (2022) revealed that the Sundanese people of West Java, Indonesia in particular, regularly consume fresh vegetables as part of their daily *'lalapan'* menu. The *lalapan* menu is generally based on the leaves and fruit of plants. The leaves in the lalapan menu that are frequently consumed by Sundanese people include lettuce (*Lactuca sativa* L.), basil (*Ocimum americanum* L.), *kenikir* (*Cosmos caudatus* Kunth), and pohpohan (*Pilea melastomoides* (Poir.) Wedd.). *Pilea melastomoides* (Poir.) Wedd (*pohpohan*), a native aromatic vegetable, has been traditionally consumed by the Sundanese people in the highlands of West Java since ancient times (Adhiningsih et al. 2022). The Sundanese people in West Java have consumed *pohpohan* as a traditional ethnic food (Cita 2020; Kodir et al. 2022).

Consuming fresh vegetables can benefit human intestinal health by modulating the diversity of intestinal microbiota (Sakkas et al. 2020). Gut microbiota play vital roles in maintaining the digestive and immune systems and can even influence mental health (Illiano et al. 2020; Xiang et al. 2020). Studies show that consuming raw vegetal foods, such as salads, can contribute to gut microbial community structure, leading to healthy conditions (Swain et al. 2014; Mantegazza et al. 2023).

However, consuming unhygienic fresh vegetables can lead to disease. Fresh vegetables may become contaminated with pathogenic microorganisms such as *Listeria monocytogenes*, *Salmonella* sp., and *Escherichia coli* O157 throughout the stages of cultivation, harvest, and postharvest processing (Sant'Ana et al. 2012; Abass et al. 2016; Luna-Guevara et al. 2019; Sarré et al. 2023). Data from the West Java Provincial Health Service (2023) revealed that diarrhea cases in West Java, particularly in the Bogor regency, were considered high in 2016-2022 (Open Data Jabar 2023). A possible cause is the regular habit of consuming unhygienic fresh vegetables. Foodborne pathogens like *L. monocytogenes* have been detected in ready-to-eat vegetable packages (Sant'Ana et al. 2012) and fresh lettuce

(Miceli and Settanni 2019). Similarly, leafy vegetables such as mint, parsley, coriander, and lettuce are frequently contaminated with pathogenic *Salmonella* species (Yang et al. 2020; Nguyen et al. 2021; Sarré et al. 2023) and *E. coli*.

Cultivation practices, including the application of biofertilizers or chemical fertilizers, can modify the bacterial communities within the rhizosphere and phyllosphere. This alteration subsequently influences the composition and dynamics of endophytic bacteria within the plant (Zhang et al. 2017). It is suspected that such fertilizer treatment may interfere with the soil pH and biogeochemical cycles, thereby creating a specific environment for bacterial communities to grow and inhabit plant tissues (Pu et al. 2022; Hu et al. 2024). The utilization of organic fertilizer and manure that is not well managed has been reported to cause the outbreak of certain pathogenic bacteria in the soil, which can inhabit plant tissues (Black et al. 2021). This issue is critical for ensuring the food safety of freshly eaten vegetables, such as *P. melastomoides*.

Although numerous studies have identified microbial communities in fresh vegetables and salads (Sant'Ana et al. 2012; Miceli and Settanni 2019), there is a lack of research on the endophytic bacteria in *P. melastomoides*, a leafy vegetable that grows both wild and cultivated in the highlands of West Java. Such data are essential to determine the safety of consuming raw vegetables. The stable and consistent existence of endophytic bacteria in plant tissue allows long-term research to be validated by other researchers to advance science. Thus, this study investigated the community profiles of endophytic bacteria in wild and cultivated *P. melastomoides*. A metagenomic analysis was performed, targeting the 16S rRNA gene, to reveal the bacterial community profiles in the two varieties of *P. melastomoides*.

MATERIALS AND METHODS

Sample collection

Leaf samples of *P. melastomoides* were obtained from the forest area of Mount Halimun Salak National Park, Tamansari Sub-district, Tamansari District, Bogor District, West Java, Indonesia. Using the cluster random sampling technique, leaf samples were obtained from cultivated (6°41'33.53"S 106°44'23.45"E) and wild (6°40'2.64"S 106°44'50.81"E) *P. melastomoides* plants (Figure 1) and divided into three plots. Samples were collected via sample pooling in each plot by combining 10 samples from the cultivated and wild sites.

Young and healthy leaves of *P. melastomoides*, specifically from the first to third shoots, ensuring they were fresh, disease-free, and undamaged by pests were collected and samples and then stored in sterile zipper-lock plastic bags, kept cool in a cooler box at 4°C during field collection, and then frozen at -80°C until further analysis.

Each sample was authenticated for species identification and was confirmed by a plant taxonomist at Herbarium Bogoriense, National Research and Innovation Agency (sample number: B-1046/II.6.2/IR.01.02/5/2023). All the samples were identified as *P. melastomoides* (local name: *pohpohan*) belonging to the family of Urticaceae.

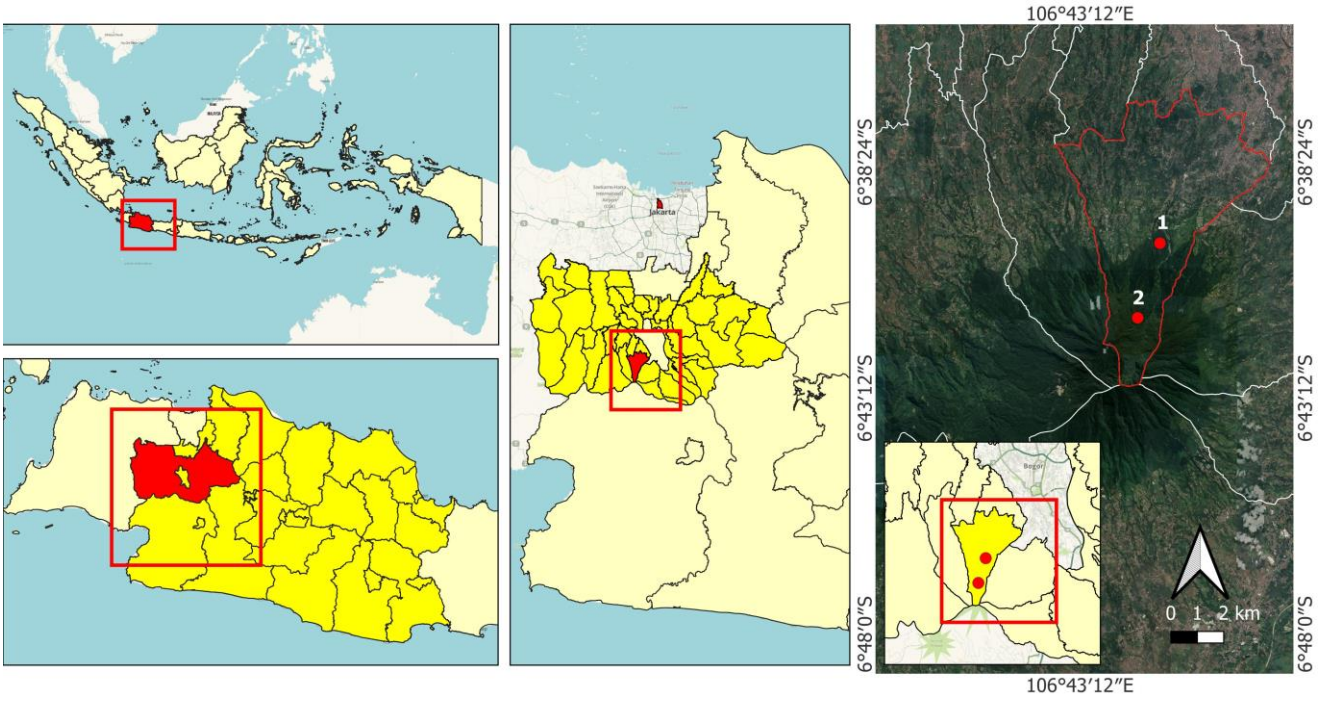

Figure 1. Map of forest area of Mount Halimun Salak National Park, Tamansari Sub-district, Bogor District, West Java Province, Indonesia. The sampling sites of the cultivated and wild *Pilea melastomoides* (*pohpohan*) were indicated Point 1: Cultivated *pohpohan* and; Point 2: Wild *pohpohan*

Sample sterilization

Sample sterilization was done by following previous method (Maela and Serepa-Dlamini 2022). The leaves were washed under running tap water to remove adhering contaminants and rinsed using sterile distilled water prior to surface sterilization. The samples were washed sequentially by soaking in 70% ethanol for 1 min, soaking in 2.5% sodium hypochlorite for 5 min, soaking in 70% ethanol for 30s, and rinsing five times using sterile distilled water to remove residue from the solution. Afterward, to determine if the sterilization was successful, the final sample (100 µL) was inoculated on nutrient agar (NA) plates and incubated at 28°C for 24-72 h. Microbial growth on the NA plate indicated that the sterilization was unsuccessful, and the sterilization process was repeated. Small sections of the leaves were inoculated on NA plates and incubated at 28°C for 72 h to confirm that the epiphytes had been removed. No microbial growth was observed on the NA plates, indicating a successful sterilization.

Metagenome DNA extraction

Metagenome was extracted using the Quick-DNA Magbead Plus Kit (Zymo Research, D4081) according to the manufacturer's protocol. Leaves sample (50 mg) was added to 750 µL DNA/RNA Shield to a bead beating tube. The sample was then mechanically homogenize using vortex at maximum speed for one minute. Sample was then centrifuged at 10.000× g for 1 minute. The resulted lysate (400 µL) was then transferred to a new tube. The corresponding digested sample was further purified using Quick-DNA MagBinding Buffer (400 µL). MagBinding Beads was added at volume 33 µL and mixed the solutions by pipette mixing for 10 minutes. The sample was transferred to the magnetic stand until beads had separated from the solution. The resulted supernatant was removed. The remaining sample was further transferred off the magnetic stand for washing using DNA Pre-Wash Buffer. The sample was again transferred to the magnetic stand until beads had separated from the solution. Supernatant was then discarded and the remaining sample was again transferred off the magnetic stand for purification using g-DNA Wash Buffer. The magnetic bead-based DNA separation and purification was repeated for 12 times. To dry the beads, the sample was transferred to a heated element and incubated at 55°C for 10 minutes. Elution buffer was then added to elute the DNA. The quality of the extracted DNA was measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA), and genome integrity was confirmed using 1% agarose gel electrophoresis.

Amplification of 16S rRNA gene

The amplification of the DNA isolate samples was conducted using the polymerase chain reaction (PCR) technique targeting the V3-V4 amplification region using two universal bacterial primers, namely primers 341F and 806R (Takahashi et al. 2014). Region V3-V4 of 16SrRNA gene was targeted for analysis because of its effectiveness in differentiating bacterial taxa, the availability of extensive reference databases and sequencing data, and its optimal balance between sequencing depth and taxonomic resolution (Mansoor et al. 2019; Alibandri et al. 2020). Amplification was performed using MyTaqHS Red Mix, 2X (Bioline, BIO-25048). Qualitative analysis was conducted via 1% agarose gel electrophoresis.

Library preparation and sequencing

The metagenome of the endophytic microbial DNA samples was sent for sequencing to NovogeneAIT Genomics Singapore Pte Ltd. A metagenome sequencing library was prepared with genomic DNA (up to 100 ng) using the NEB Next® UltraTM II FS DNA PCR-free Library Prep Kit (New England Biolabs, USA, catalog #E7430L) according to the manufacturer's protocol, including DNA fragmentation, end conversion, adapter ligation, cleanup of adapter-ligated, library amplification, and library quantitation. The library quality was assessed using a Qubit@ 2.0 Fluorometer system (Thermo Scientific) and Agilent Bioanalyzer 2100. Finally, metagenome libraries were sequenced using an Illumina NovaSeq 6000 pair-end sequencing platform (2×250 bp) (Modi et al. 2021).

Metagenomic analysis

Data processing and quality control

A dataset resulting from raw reads was generated from paired-end reads and filtered to obtain quality sequences. First, data was processed through the sequence merging stage using the Flash software (version 1.2.11) (Magoč et al. 2011), and paired-end reads were combined to cut the primary sequence. Afterward, the tag filtration stage was executed by filtering the quality of the raw tags under default filtering conditions according to QIIME (Version 1.7.0) in the quality control process (Bokulich et al. 2013). Thereafter, the chimeras removal stage was performed, using the VSEARCH software (version 2.16.0) by comparing the tags to the SILVA 16S rRNA bacterial reference database (https://www.arb-silva.de/) to remove non-biological sequences or sequences formed from errors during the PCR process to obtain effective tags (Quast et al. 2013; Rognes et al. 2016).

OTU cluster and species annotation

Operational taxonomic unit (OTU) production was conducted for taxonomic profiles and diversity analysis and processed using the UPARSE software (v7.0.1001) (Edgar 2013). The OTUs were determined using a threshold level of 97% similarity between the sequences. Each representative sequence was aligned to the SILVA database (http://www.arb-silva.de/) based on the Mothur algorithm for annotating taxonomic information (Quast et al. 2013).

Alpha and beta diversity analysis

Alpha diversity, including species richness (Chao1 and Shannon indices) and evenness (Pielou and Simpson indices), was calculated for each sample of wild and cultivated *P. melastomoides*. The statistical analysis of the alpha diversity data was performed using t-test analysis. This was performed using the R program (version 4.2.1) (R Core Team 2022) with the vegan package (version 2.6.4) (Oksanen et al. 2022) and ggplot2 (version 3.4.2) (Wickham

2016). Statistical calculations were performed using the t-test by processing the OTU data, which had been normalized using the rarefying method. Beta diversity was conducted to reveal differences in the overall microbial profiles of the wild and cultivated *P. melastomoides*, which was estimated by conducting principal coordinate analysis (PCoA) using the Bray-Curtis distance. Thereafter, the statistical significance of β diversity was determined using the permutational multivariate analysis of variance (PERMANOVA) test.

Core feature identification and differential abundance analysis

Differences in the taxonomic metagenome abundance of endophytic bacteria of the wild and cultivated *P. melastomoides* were statistically examined using the linear discriminant analysis (LDA) effect size (LEfSe) method using the LEfSe software (version 1.1.2) (Segata et al. 2011). LEfSe uses the nonparametric Kruskal-Wallis rank-sum test to detect feature abundances that differ between groups. The biological consistency was examined with a series of paired tests using the unpaired Wilcoxon rank-sum test. Finally, the effect size of the significantly abundant features was estimated using the LDA test. Here, LEfSe parameters, such as the LDA score and Wilcoxon p-value, were set to 2.5 and 0.05, respectively. The results were visualized in bar graphs using ggplot2 (version 3.4.2) (Wickham 2016).

Taxonomic composition profile

Taxonomic annotations were made using the R program (version 4.2.1) with OTU abundance data resulting from the OTU production. Thereafter, each sample was determined by the top taxa at the phylum and genus level. These selections were used to create distribution histograms that depict the relative abundance of the taxa. This approach facilitates the visualization of taxa with high relative abundance and proportions at various classification levels in each sample or group. The results were visualized in bar graphs using ggplot2 (version 3.4.2) (Wickham 2016).

RESULTS AND DISCUSSION

General information on metagenome dataset

A total of 1085273 raw sequence reads from six metagenome libraries were generated using the Illumina NovaSeq 6000 platform. The average sequence length of the paired reads was 2×250 bp with an average Phred score above 30 for the Q30 value. After quality filtering, namely host decontamination and trimming of the essential quality, 1002372 high-quality sequences were obtained for further analysis (Table 1).

Alpha diversity

Alpha diversity was calculated using a diversity index to determine the bacterial species richness (Chao1 and Shannon indices) and evenness (Pielou and Simpson indices) in each sample of wild and cultivated *P. melastomoides*. Based on the t-test analysis of the Chao1 and Shannon indices, the alpha diversity of the endophytic bacterial communities of *P. melastomoides* in each cultivated and wild group was significantly different ($p<0.05$) (Figure 2). The data suggested that the species richness of the endophytic bacteria in each sample group was significantly different. This aligned with the species evenness in the sample groups of the cultivated and wild *P. melastomoides*, which significantly differed ($p<0.05$) based on the Pielou index. However, based on statistical tests, the Simpson index was not significantly different ($p>0.05$) (Table 1) between the sample groups.

Based on the alpha diversity analysis, the endophytic bacterial community of the cultivated *P. melastomoides* exhibited higher species richness and evenness than those of the wild variety. This might be due to the agricultural practices that are applied by the farmers. Organic farming techniques using organic fertilizers from rice husks and dry chicken manure were applied in the cultivated *P. melastomoides* plantation. Such high endophytic microbial diversity has been observed in the leaves of wheat plants (Sun et al. 2021) and lettuce (Wang et al. 2023), which were subjected to organic fertilizers.

In this regard, organic fertilizers may provide nutrition, including carbon and nitrogen sources, leading to the microbial enrichment of soil (Lazcano et al. 2021; Hu et al. 2024). A study stated that the addition of organic fertilizer could promote microbial diversity and increase the relative abundance of soil-beneficial species, such as G*lomeromycota*, *Mortierellomycota*, *Humicola*, and *Bacillus* in *Panax notoginseng* (Burkill) F.H.Chen ex C.H.Chow (Pu et al. 2022). Such a rich microbial community in the soil may support further plant tissue internalization by these microbial species. The internalization of plant tissue by soil-inhabitant bacteria has been reported earlier in the internalization of *Escherichia coli* in lettuce and spinach (Wright et al. 2017), as well as that of *Stenotrophomonas* and *Sphingomonas* in potato tuber and spring barley (Kracmarova et al. 2020).

Table 1. Statistical data of the quality of sequence reads generated by Next Generation Sequencing using the Illumina NovaSeq 6000 platform of bacterial 16S rRNA genes from the leaves of cultivated and wild *P. melastomoides*

Sample type	Sample name	Statistics									
		RawPE	Combined	Qualified	Nochime	Base(nt)	Avglen(nt)	GC	Q20	Q30	Effective%
Cultivated	C1	160170	157691	153898	148849	60524640	406.62	55.51%	98.05%	93.81%	92.93%
	C2	183035	180452	175992	169740	69024761	406.65	55.26%	98.11%	93.93%	92.74%
	C3	183461	180809	176432	165073	67205484	407.13	55.09%	98.13%	93.93%	89.98%
Wild	W1	181967	179157	174929	167202	67992071	406.65	55.10%	98.10%	93.88%	91.89%
	W2	194656	191191	186345	179297	72853160	406.33	55.34%	97.96%	93.57%	92.11%
	W3	181984	179670	175733	172211	69942354	406.14	55.31%	98.09%	93.86%	94.63%

Beta diversity

Beta diversity analysis was employed to analyze differences in the microbial composition of the samples from the two habitats. Here, the beta diversity was analyzed using a distance matrix and visualized as a PCoA plot, representing the distance value between each sample in the dataset. The distance index used in this study was the Bray-Curtis distance index. This index can reveal differences in the species composition of the microbial communities between the cultivated (C) and wild (W) *P. melastomoides*.

The PCoA plot shows the grouping pattern between the microbial communities in the W and C groups. Axis 1 shows a variance percentage of 51.4%, whereas axis 2 shows a variance percentage of 22.3%; This explains the similarity between the endophytic bacteria profiles of the cultivated and wild *P. melastomoides* (Figure 3). Interestingly, based on the PERMANOVA test, the compositions of endophytic bacteria of the cultivated and wild *P. melastomoides* were not significantly different (Pr>F had a value of 0.2). These findings indicate that cultivated and wild environments exhibit similar microbial balances in terms of the endophytic bacterial community profile of *P. melastomoides*. This was likely due to the tissue properties of the plant host, *P. melastomoides*, which are relatively similar to the wild or cultivated types. In addition, the abiotic factors of the wild and cultivated environments were similar in terms of the average daily temperature (23-30°C).

Dominant bacterial endophytes observed in cultivated and wild *P. melastomoides*

Based on the LEfSe analysis, the bacterial community structure significantly differed between the cultivated and wild *P. melastomoides*. The LEfSe histogram analysis resulted in LDA scores above 2.5 with p-value below 0.05, indicating significant taxonomic differences between the cultivated (positive scores) and wild (negative scores) varieties (Figure 4). Based on this analysis, 26 endophytic bacterial taxon groups exhibited differences in their relative abundance in the cultivated and wild *P. melastomoides*.

The taxon nomenclature of each sample of cultivated and wild *P. melastomoides* was identified using the Silva database (http://www.arb-silva.de/) (Quast et al. 2013). The dominant bacterial endophytes of the sample of the cultivated *P. melastomoides* were identified as one class, six orders, two families, and three genera. Contrarily, the dominant bacterial endophytes of the wild *P. melastomoides* sample comprised one class, two families, and ten genera.

At the genus level, the dominant endophytic bacteria in samples of the cultivated *P. melastomoides* were identified as species of *Sphingorhabdus* and *Calorithrix*. In the wild group, the dominant endophytic bacteria were *Apilactobacillus, Arthrobacter, Blautia, Collinsella, Dialister, Candidatus* or *Portiera, Pseudomonas, Listeria, Salmonella*, and *Escherichia*/*Shigella*. However, further analysis should be conducted to identify certain taxa, including those identified as uncultured bacteria, MSBL5 and B2M28 (Figure 3).

The LEfSe output results combined with the SILVA database identification only provide unique codes without clear or specific taxa names. This can occur if the reference database only has limited information at the genus level. Taxa with unique codes belonging to unculturable microbes are typically detected using DNA methods. Thus, their functions have not been extensively studied and remain unknown.

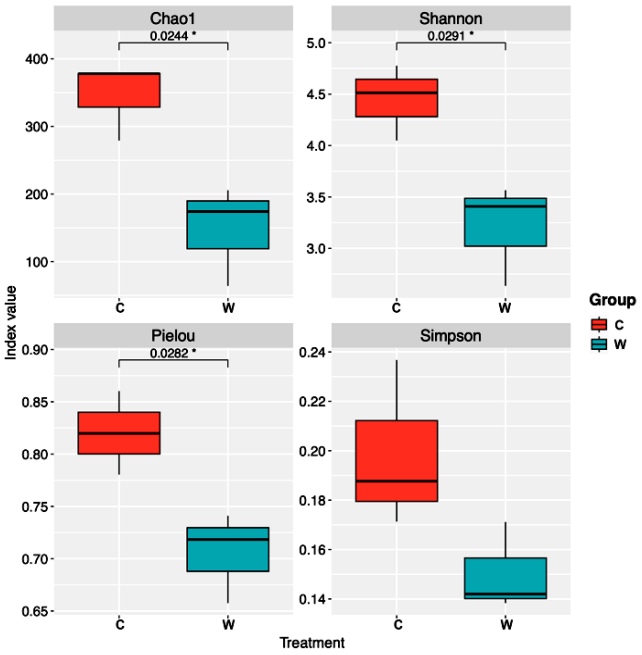

Figure 2. Alpha diversity of endophytic bacterial communities in cultivated (C) and wild (W) *P. melastomoides* evaluated by Chao1, Shannon, Pielou, and Simpson indices. The T-test analysis was conducted to evaluate the difference between the values in each index. The data were significantly different if $p<0.05$ and not significantly different if $p>0.05$ (indicated by *)

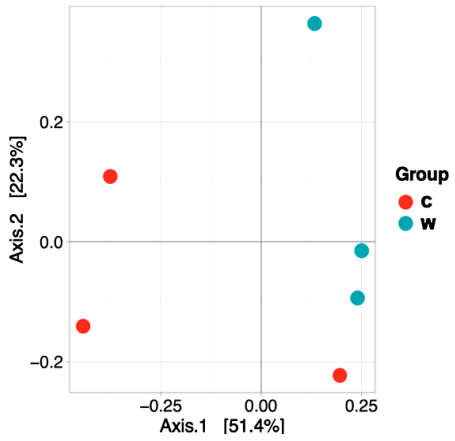

Figure 3. Beta diversity of endophytic bacterial communities in the cultivated (C) and wild (W) *P. melastomoides*, represented in the principal coordinate analysis (PCoA) plot based on the Bray-Curtis index

Taxonomic composition of endophytic bacteria observed in cultivated and wild *P. melastomoides*

Based on the metagenomic analysis, the diversity and taxonomic composition of endophytic bacteria communities in the samples of the cultivated and wild *P. melastomoides* varied at different levels. The distribution of taxa abundance at the phylum level (Figure 5.A) and genus level (Figure 5.B) were visualized in a histogram.

Firmicutes and Proteobacteria were the most dominant phyla of endophytic bacteria in both cultivated and wild *P. melastomoides*. However, Firmicutes exhibited relatively higher abundance in the wild (58.24%) than in the cultivated (34.30%) *P. melastomoides.* Similar results were observed for *Proteobacteria*. Contrarily, *Chloroflexi* exhibited higher abundance in the cultivated *P. melastomoides* than in the wild variety. The top five abundant phylum level endophytic bacteria in the cultivated *P. melastomoides* group were in the following order: *Firmicutes* (34.30%), *Proteobacteria* (20.22%), *Chloroflexi* (11.04), *Acidobacteriota* (7.44%), and *Bacteroidota* (1.72%). The wild *P. melastomoides* was dominated by the bacterial phyla of Firmicutes (58.24%), Proteobacteria (32.60%), Bacteroidota (2.20%), Actinobacteriota (0.52%), and Chloroflexi (0.41%).

The bacterial community profiles of the cultivated and wild *P. melastomoides* were dominated by the Firmicutes and Proteobacteria phyla. This has been reported in a study on the dominant bacterial community in green olives (Soto-Giron et al. 2021), common beans (Costa et al. 2012), and lettuce (Lee et al. 2023). At the genus level, the cultivated *P. melastomoides* leaves were rich in *Limosilactobacillus* (6.27%), *Bacillus* (5.24%), *Escherichia/Shigella* (4.96%), *Salmonella* (3.57%), and *Staphylococcus* (3.57%). However, in the wild *P. melastomoides*, the genera of *Escherichia/ Shigella* (15.64%), *Bacillus* (10.57%), *Limosilactobacillus* (9.90%), *Salmonella* (7.78%), and *Listeria* (5.94%) were identified as the most abundant.

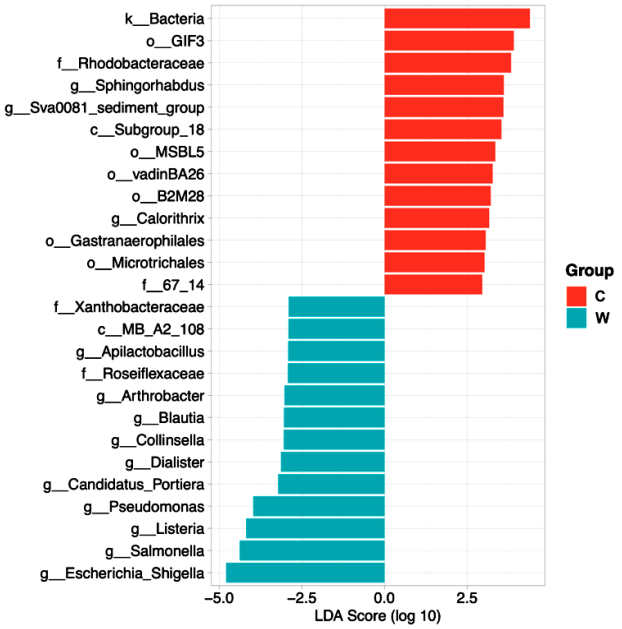

Figure 4. Histogram of LefSe based on linear discriminant analysis (LDA) score (>2.5) of dominant endophytic bacteria in cultivated (C) and wild (W) *P. melastomoides*

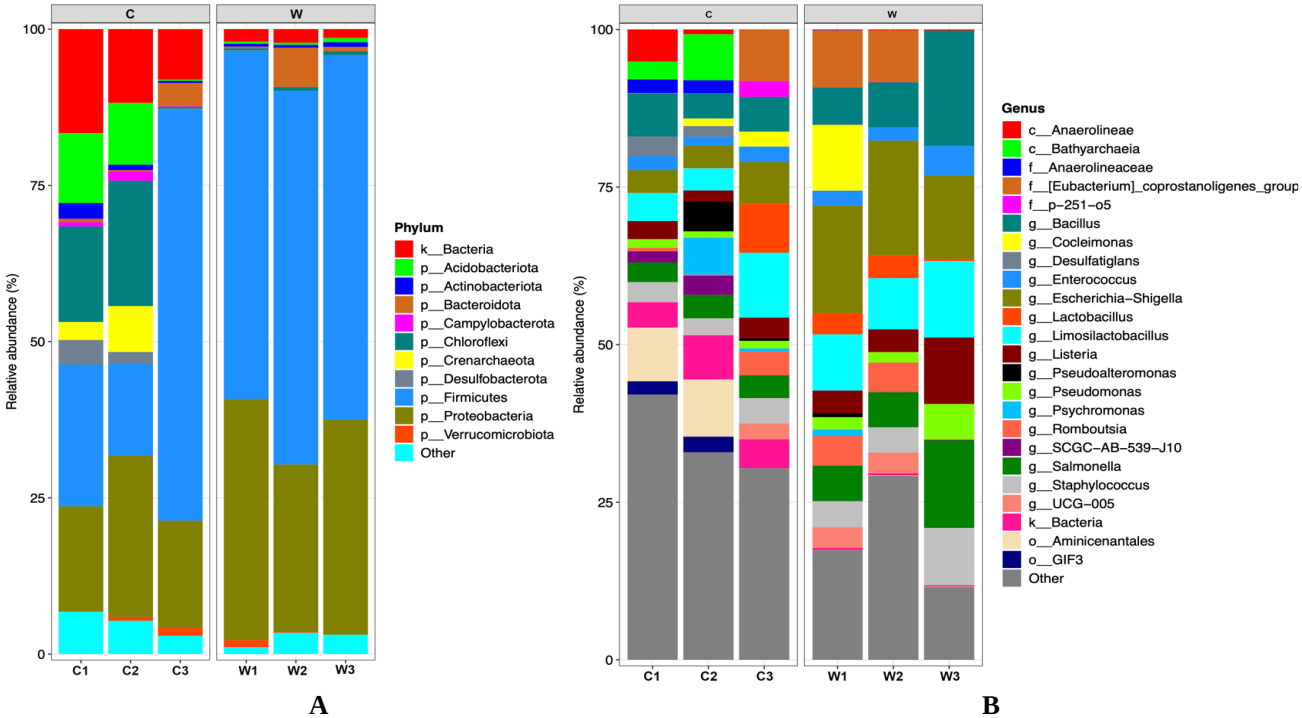

Figure 5. Histogram of the microbiota of each bacterial group in cultivated (C) and wild (W) *P. melastomoides* at the A. Phylum; B. Genus levels

Firmicutes, including members of the genera (Lee et al. 2021), are integral members of plant microbiomes. This study revealed the presence of Bacillus in the samples of cultivated and wild *P. melastomoides* leaves. This bacterial group was reported to elicit plant growth-promoting activity that can control plant diseases. For instance, *Bacillus niacini, Solibacillus silvestris*, and *Bacillus luciferensis* were reported to antagonize pathogenic *Rhizoctonia solanacearum* in tomato plants (Lee et al. 2021). Interestingly, the bacterial group of Choroflexi was dominant in the leaves of the cultivated *P. melastomoides*. Chloroflexi is a filamentous bacterium that is actively involved in waste degradation and nutrient cycling. Thus, the presence of Chloroflexi in cultivated *P. melastomoides* leaves might have been due to organic fertilizer application, which facilitates the enrichment of this group in the soil (Zhang et al. 2021). Chloroflexi has been observed to be dominant in the soil of a paddy plantation treated with rice straw and biochar (Tang et al. 2021).

The Proteobacteria phylum is known to be dominant in both cultivated and wild *P. melastomoides* groups. The study's results identified the presence of the species of *Escherichia* and *Salmonella* in the leaves of cultivated and wild *P. melastomoides*. The study showed that *Salmonella* spp. and *Escherichia coli* are common bacteria in fresh vegetables and fruits (Rincón and Neelam 2021). Previous studies have also revealed that *Salmonella enterica* is found in carrots (Nithya and Babu 2017), spinach, radishes, tomatoes, melons, and cucumbers (Verma et al. 2018). Meanwhile, *Escherichia coli* is also found in lettuce (Araújo et al. 2017), leeks, radishes, basil, and spinach (Shakerian et al. 2016). The Proteobacteria phylum is a member of Gram-negative bacteria that plays a role in the nitrogen, carbon, and sulfur cycles (Osman et al. 2019). Proteobacteria, including copiotrophs, proliferate in nutrient-rich environments, primarily carbon (Gumiere et al. 2019). Field observations show that *P. melastomoides* plantation soil is fertile because organic fertilizers are consistently applied. Zhou et al. (2022) proved that organic fertilizers can increase soil carbon and nitrogen content, increasing soil fertility and plant productivity.

Among the potentially beneficial endophytic microbes such as Firmicutes on the growth of *P. melastomoides*, interestingly, some potential microbes that are pathogenic to humans were found in the leaves of cultivated and wild *P. melastomoides*. However, the presence of microbes considered pathogenic was relatively higher in *Escherichia*/*Shigella* (15.64%), *Salmonella* (7.78%), and *Listeria* (5.94%), in wild *P. melastomoides* compared to cultivated. Wild *P. melastomoides* may utilize pathogenic microbes due to the internalization of these bacteria from the soil. Wild environments will likely be a breeding ground for pathogens through anthropogenic activities such as domestic wastewater and animal activities. Indeed, previous studies have shown the internalization of pathogenic *Escherichia coli* in leafy vegetables from various plants, including soil and hydroponic plants (Riggio et al. 2019). Potential human pathogens in cultivated *P. melastomoides* leaves raise significant concerns in fresh consumption. Plantation strategies can be applied to minimize pathogenic microbial contamination of fresh plants, including mulching, soil solarization, fertilization, irrigation and application of biocontrol fertilizers (Lenzi et al. 2021).

In conclusion, the dominant endophytic bacterial phylum of cultivated and wild *P. melastomoides* were identified as *Firmicutes* and *Proteobacteria*. The increased presence of *Chloroflexi* in cultivated *P. melastomoides* suggests that agricultural practices, such as the use of organic fertilizers, may facilitate the enrichment of this phylum. The leaves of wild *P. melastomoides* appear to harbor more human pathogenic bacteria than the leaves of cultivated *P. melastomoides*. To minimize pathogenic contamination in *P. melastomoides*, good agricultural practices should be applied to the cultivation of this plant, including crop rotation, proper irrigation management, organic fertilizer substitution, and proper handling and storage.

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