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Diversity of root bacterial community associated with seven orchid species from Mount Merbabu National Park, Central Java, Indonesia

ARTINI PANGASTUTI[1,♥], ARI PITOYO[1], ARI SUSILOWATI[1], RIZKA MEISARI[1], IRVY YULIANA[1], KHATSANATUL AULIA[1], LIANA PUTRI PUSPITASARI[1], RUSIANI[2], HENDRO PRASOJO[2]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./Fax.: +62-271-663375, ♥email: artini_p@staff.uns.ac.id
[2]Mount Merbabu National Park. Jl. Merbabu 136, Boyolali 57316, Central Java, Indonesia

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Abstract. *Pangastuti A, Pitoyo A, Susilowati A, Meisari R, Yuliana I, Aulia K, Puspitasari LP, Rusiani, Prasojo H. 2023. Diversity of root bacterial community associated with seven orchid species from Mount Merbabu National Park, Central Java, Indonesia. Biodiversitas 24: 5676-5684.* Indonesia has a high diversity of wild orchids. One of the orchid habitats that has not been widely explored is Mount Merbabu National Park (TNGMb), Central Java, Indonesia. The dependence of orchids on specific microorganisms for survival and growth, especially during early development, has not been widely studied. Endophytes, such as bacteria, can help the host plant grow, tolerate stress, resist disease, acquire nutrients, or form symbiotic relationships. However, bacterial endophytes associated with orchids have not been extensively explored. This research examined the diversity of bacteria associated with orchid roots in Mount Merbabu National Park using a culture-independent method. Seven orchid species were sampled for root bacterial community analysis, that identified as *Spathoglottis plicata*, *Dendrobium sagittatum*, *Malaxis kobi*, *Apendicula alba*, *Pholidota carnea*, *Dendrobium tenellum*, and *Bulbophyllum compressa*. Bacterial diversity in all orchid roots was high, with several dominant bacterial species. Proteobacteria, Firmicutes, Bacteriodota, and Actinobacteriota were the four most abundant phyla, with Proteobacteria having the highest abundance (37 to 90%) in all samples. The top ten bacteria genera were *Pseudomonas*, *Serratia*, *Rhodanobacter*, *Acinetobacter*, *Escherichia*, *Bifidobacterium*, *Clostridium*, *Parabulkholderia*, *Faecalibacterium*, and Muribaculaceae family that could not be identified at the genus level. Overall, 61, 42, 838, 98, 78, 973, and 1383 OTUs were unique to *S. plicata*, *D. sagittatum*, *M. kobi*, *A. alba*, *P. carnea*, *D. tenellum*, and *B. compressa*, respectively, whereas all seven species shared 335 OTUs. Our research suggested that environmental factors and the host plant's genetics affect the plant microbiome's composition and diversity. These elements might be essential for ecosystem function and conservation.

Keywords: Culture-independent method, Mount Merbabu National Park, orchid, root-associated bacteria

INTRODUCTION

Orchids belong to Orchidaceae, one of the largest and most diverse families of flowering plants, with over 25,000 species distributed in almost all terrestrial ecosystems (Fiallo-Olivé et al. 2020). Orchids have evolved several remarkable adaptations to cope with environmental conditions (Kaur and Sharma 2021). Indonesia has many wild orchids, with around 5,000 species spread across various habitats (Sjahril et al. 2013; Kurniawan et al. 2021; Purba and Chasani 2021). Many wild orchid species are endemic to specific regions, and most have yet to be thoroughly investigated. Mount Merbabu National Park (Taman Nasional Gunung Merbabu/TNGMb), located in Central Java Province, Indonesia, is a conservation area with high biodiversity, including orchids. However, its potential has yet to be widely explored. Previous research found 18 epiphytic and terrestrial orchids growing in this area (Nugroho et al. 2018).

One of the most striking features of orchids is their dependence on specific microorganisms for their survival and growth, especially during their early developmental stages (Bhatti and Thakur 2022). Orchids interact with endophytes, microorganisms residing within the plant's tissues, without exhibiting any discernible adverse effects or pathogenicity. Endophytes include bacteria, fungi, or algae, and they provide various benefits to the host plant, such as plant growth promotion, stress tolerance, disease resistance, nutrient acquisition, or symbiotic associations (Herrera et al. 2022). However, the role of endophytic bacteria in orchids has yet to be widely studied. Information on orchid-associated bacteria is even more limited in wild orchids.

Plants establish intricate interactions with diverse microorganisms in the soil. Root-associated bacteria and mycorrhizal fungi promote plant health and enhance overall performance. Several studies have demonstrated that some bacteria associated with plant roots are crucial in strengthening plant resistance, promoting plant diversity, and facilitating plant cohabitation. These beneficial effects are achieved by bi- or tripartite interactions involving the bacteria, the plant hosts, and mycorrhizal fungi (Kaur and Sharma 2021). According to Taniguchi et al. (2012), some bacteria associated with plant roots can enhance mycorrhizal colonization and function by producing signaling molecules, enzymes, or hormones. Bacteria linked with plant roots might also influence interactions between plants and mycorrhizal fungi by inducing changes in plant root shape, physiology, or gene expression (Ujvári et al. 2021).

In addition, some root-associated bacteria can mediate plant-plant interactions by affecting plant competition, facilitation, or allelopathy (Utami et al. 2022). Root-associated bacteria can influence the soil's physical and chemical attributes, including but not limited to soil temperature, moisture content, structural composition, pH levels, and nutrient availability (Nwachukwu et al. 2021). These bacteria also produce phytohormones, siderophores, enzymes, antibiotics, or volatile organic compounds that enhance nutrient uptake, root development, plant health, or mycorrhizal colonization (Vocciante et al. 2022). Microbes associated with orchids have the potential for various biotechnology applications, such as Plant Growth Promoting Bacteria (PGPR), due to the interdependent relationship between host and microbes. Bacterial endophytes may play an essential role in the adaptation and survival of wild orchids in their natural environments.

Overall, orchids and bacteria interact in a complicated and dynamic way, affecting many orchid biology and ecology. This research aimed to assess the diversity of bacteria associated with orchids utilizing culture-independent approaches and Next Generation Sequencing (NGS) technology. There has never been a report on the diversity of the microorganisms associated with orchids in Indonesia. The study will provide new insights into the ecology and evolution of orchids and their bacterial partners and contribute to the conservation and cultivation of these valuable plants.

MATERIALS AND METHODS

Orchid roots sampling

Orchid roots were collected from Mount Merbabu National Park (TNGMb), Central Java, Indonesia. The data collection in this study utilized the cruising method. The observations were carried out along a path with a radius of 10 meters on both the right and left sides. The presence of each orchid species was recorded at the time of observation. Orchid roots were collected, put in sterile plastic, and transported using an ice box. Sampled orchids were documented and identified. Root samples were cleaned entirely from the soil using sterile water. The root surface underwent a sterilization process involving a 20 second exposure to 95% ethanol, followed by immersion in a 5% sodium hypochlorite solution for 3 minutes. Subsequently, the surface was subjected to seven washes using sterile physiological saline to eliminate non-associative microorganisms (Alibrandi et al. 2020). Five root samples from the same orchid species were pooled together. DNA extraction firstly by immersing the sample in liquid nitrogen, then pulverization using a mortar and pestle. Isolation of genomic DNA using the ZymoBIOMICS^{TM} DNA Mini Kit protocol (Zymo Research). The quality and quantity of DNA were assessed using a BioPhotometer Plus instrument (Eppendorf).

Amplicon sequencing

Sequencing was carried out at PT Genetics Science Jakarta. The analysis used 16S rRNA gene amplicons in V3 and V4 (for bacteria). The 16S rRNA gene was amplified using specific primers 341 (5'CCTAYGGGRBGCAS CAG'3) and 806R (5'GGACTACNNGGGTATCTAAT'3). Sequencing was performed using Illumina Novaseq 6000 in paired-end read with a read length of 250 bp.

Bioinformatics analysis

Paired-end sequence reads were combined using the Flash program version 1.2.7. Filtering of raw tags quality was done with Qiime program version 1.7.0. The tags were subsequently compared to the reference database using the Uchime method to identify and eliminate chimeric sequences. The Uparse software version v7.0.1001 was employed for sequence analysis. Sequences with a similarity percentage greater than or equal to 97% were allocated to the identical Operational Taxonomic Unit (OTU). Representative sequences from each OTU were screened for further annotation. Annotation of species at each taxonomic level (with a threshold of 0.8~1) ranging from kingdom, phylum, class, order, family, genus, and species was performed using Mothur software for each representative sequence based on the SSU (Small Subunit) rRNA database from the Silva database. Then, to obtain the phylogenetic relationship of all OTU sequence representatives, the multi sequences were compared using the Muscle program version 3.8.31. OTU abundance was normalized using a standard from the number of matching sequences to the sample with the shortest sequence. Alpha diversity analysis included the number of bacterial species found, Chao1, Shannon, Simpson, ACE (Abundance-based Coverage Estimator), and good coverage. All sample indices were calculated using Qiime program version 1.7.0 and displayed using R software version 2.15.3. The beta diversity analysis with Unifrac weighting and without weighting was calculated using the Qiime program version 1.7.0. The unweighted Pair-group Method with Arithmetic Means (UPGMA) was used as a hierarchical cluster method to interpret the distance matrix using average relationships and was calculated using the Qiime program.

RESULTS AND DISCUSSION

Alpha and beta diversity

The exploration was conducted in 2 resorts within TNGMb, namely Wonolelo and Ampel. In total, 20 terrestrial and epiphytic wild orchids were found in the 2 resorts. However, based on the abundance of orchids and their conservation status, only 7 orchid species were sampled for root bacterial community analysis. The 7 orchids were identified as *Spathoglottis plicata*, *Dendrobium sagittatum*, *Malaxis kobi*, *Apendicula alba*, *Pholidota carnea*, *Dendrobium tenellum*, and *Bulbophyllum compressa*.

All orchid root samples from TNGMb had a high bacterial diversity (Table 1). However, bacterial communities on orchid roots with a higher Shannon index also had a higher Simpson index, indicating that several bacterial species were dominant in these bacterial communities. The Goods coverage value of all orchid root samples is above 97%, indicating that sequencing has successfully captured most bacterial community members and describes the community's composition and structure.

Based on bacterial richness in the root bacterial community, the *B. compressa* had the highest values in all indices compared to the other orchid species, while the *M. kobi* was the lowest. Bacterial diversity in orchid roots originating from Ampel Resort tends to be higher than in orchids from Wonolelo Resort. During exploration, the forests in the Ampel Resort area had high air humidity levels, more than 88% during the day, with temperatures around 20°C. The extensive forest cover in the Ampel area causes high humidity levels.

The composition of the endophytic community might also be influenced by canopy structure since it affects temperature, humidity, and light (Augusto et al. 2002). Results also showed that, in general, epiphytic orchids had higher root bacterial diversity than terrestrial ones. A study on bacteria associated with epiphytic orchids' roots showed that the bacterial population on epiphytic orchid aerial roots was 30% higher than that of terrestrial roots (Tsavkelova et al. 2004).

A beta diversity analysis was performed to determine the bacterial communities in different samples of orchid roots; Figure 1A shows the heatmap generated using the Weighted Unifrac and Unweighted Unifrac distances. Based on clustering analysis, microbial communities from the same resort had higher similarity and clustered together, suggesting that they were affected by their origins (Figure 1B and 1C). This finding supported that the different environmental conditions in the two resorts influenced the diversity of bacterial populations associated with orchids.

Phylum and genus levels were evaluated to determine their composition and the taxa associated with the orchid species. Proteobacteria, Firmicutes, Bacteriodota, and Actinobacteriota were the four most abundant phyla, with Proteobacteria predominant (37 to 90%) in all samples (Figure 2). This result was consistent with previous research that these four dominant bacterial phyla are associated with several orchid species (Wang et al. 2019; Alibrandi et al. 2020; Zhu et al. 2022). Firmicutes were also prominent in *B. compressa*, *P. carnea*, and *D. tenellum* roots (33%, 18%, and 10%, respectively). Meanwhile, Actinobacteriota were abundant in the *P. carnea* (30%) and less abundant in *D. tenellum* (9%). Bacteriodota was also found in significant amounts in *B. compressa*, *P. carnea*, and *D. tenellum* but less abundant in other orchid species.

Figure 3 shows the top ten bacterial genera in all orchid species. The genus that dominates in each orchid root differs; most are bacterial genera known as plant endophytes with plant growth-promoting ability. *Pseudomonas* was the most abundant genus in *S. plicata* and *A. alba* (59 and 63%) and in significant amounts in *D. sagittatum* (30%). *Pseudomonas* was also detected in all other orchid species but with less than 5% abundance. Other species in root orchids included *P. alcaligenes*, *P. oleovorans*, and *P. psychrotolerans*.

Genera that dominate in other orchid species were *Serratia* in *D. sagittatum* (46%), *Rhodanobacter* in *M. kobi* (71%), and *Acinetobacter* in *D. tenellum* (38%). In *P. carnea* and *D. sagittatum* orchids, two genera are relatively dominant compared to other genera. *Escherichia* and *Bifidobacterium* were found in almost equal numbers in *P. carnea* (31 and 26%). Similar results were also found in *D. sagittatum*, where *Serratia* and *Pseudomonas* were 46% and 30%, respectively. Unique phenomena were observed in *B. compressa*, where the bacterial community was diverse with no predominant genera. In the *B. compressa* root bacterial community, three groups of bacteria had the highest numbers, namely *Clostridium*, *Escherichia*, and Muribaculaceae family, that could not be identified at the genus level. This OTU was suspected to be a novel genus that has never been published. Another bacterial genus found in significant amounts was *Faecalibacterium*, which reached 2.9% in all orchid root samples.

The Venn diagram in Figure 4 shows the number of unique and shared OTUs detected in orchid species. Overall, 61, 42, 838, 98, 78, 973, and 1,383 OTUs were unique to *S. plicata*, *D. sagittatum*, *M. kobi*, *A. alba*, *P. carnea*, *D. tenellum*, and *B. compressa*, respectively, whereas all seven species shared 335 OTUs. Proteobacteria, especially the genus *Pseudomonas*, dominate shared OTUs. However, their abundance varied among orchid species.

Table 1. Alpha diversity indices of the bacterial community in orchid root

Sample name	Observed species	Shannon	Simpson	Goods coverage	Origin
Sp	919	4.397	0.849	0.989	Wonolelo, terrestrial
Ds	811	3.392	0.743	0.992	Wonolelo, epiphyte
Mk	1840	3.271	0.527	0.972	Wonolelo, terrestrial
Aa	1110	4.447	0.856	0.989	Wonolelo, terrestrial
Pc	1111	4.372	0.832	0.987	Wonolelo, epiphyte
Dt	2472	6.737	0.870	0.973	Ampel, epiphyte
Bc	2870	8.853	0.989	0.996	Ampel, epiphyte

Note: Host orchid Sp: *Spathoglottis plicata*; Ds: *Dendrobium sagittatum*, Mk: *Malaxis kobi*, Aa: *Apendicula alba*, Pc: *Pholidota carnea*; Dt: *Dendrobium tenellum*; Bc: *Bulbophyllum compressa*

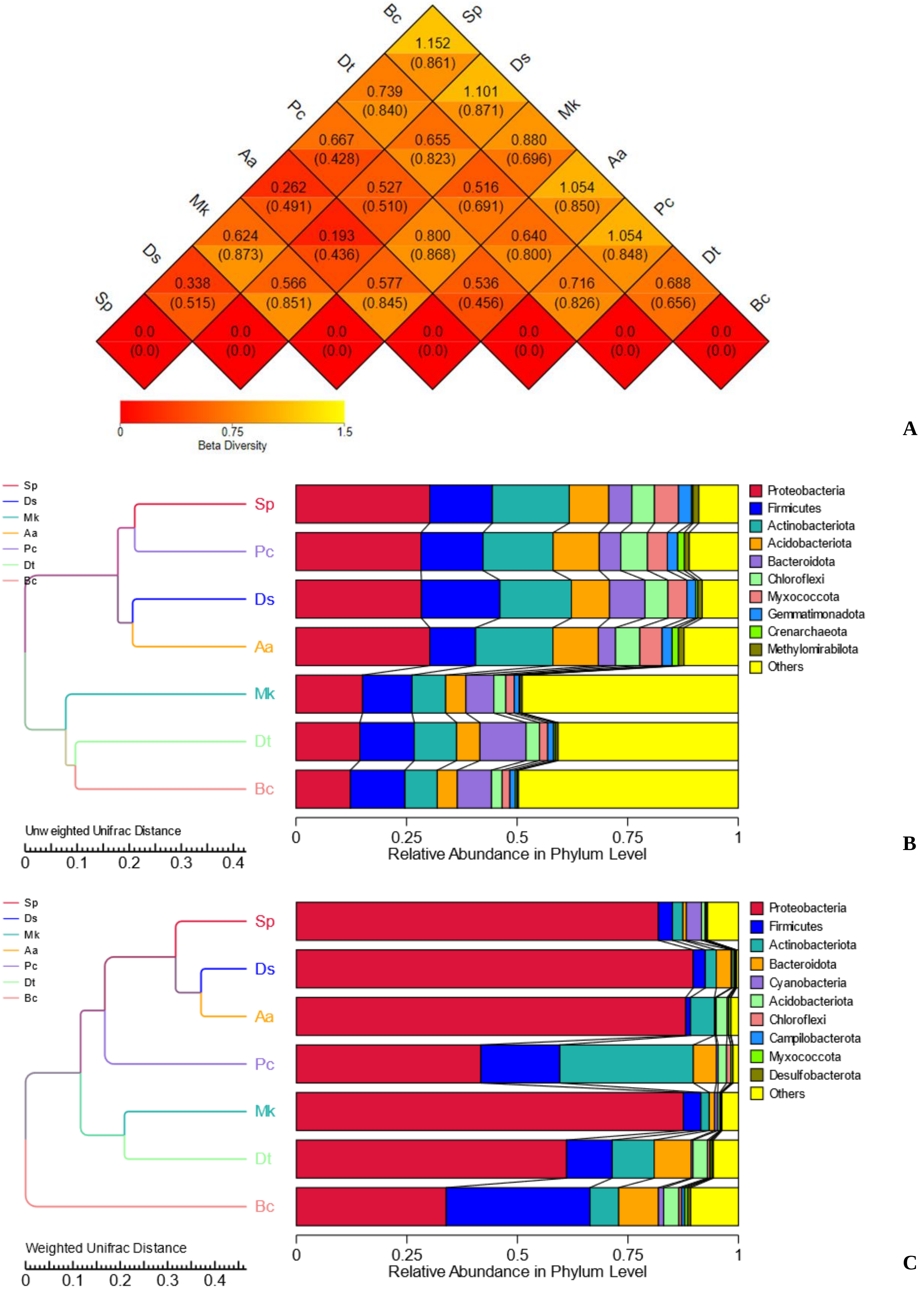

Figure 1. Beta diversity of the bacterial community in the orchid root in Mount Merbabu National Park (TNGMb), Central Java, Indonesia. A. Beta diversity heatmap, each grid illustrates the dissimilarity coefficient between pairs of samples. The first number is the weighted Unifrac distance, while the number in brackets is the unweighted Unifrac distance. B. Unweighted UniFrac distance analysis. C. Weighted UniFrac distance analysis. Note: Orchid species as host plant: Sp: *Spathoglottis plicata*; Ds: *Dendrobium sagittatum*, Mk: *Malaxis kobi*, Aa: *Apendicula alba*, Pc: *Pholidota carnea*; Dt: *Dendrobium tenellum*; Bc:*Bulbophyllum compressa*

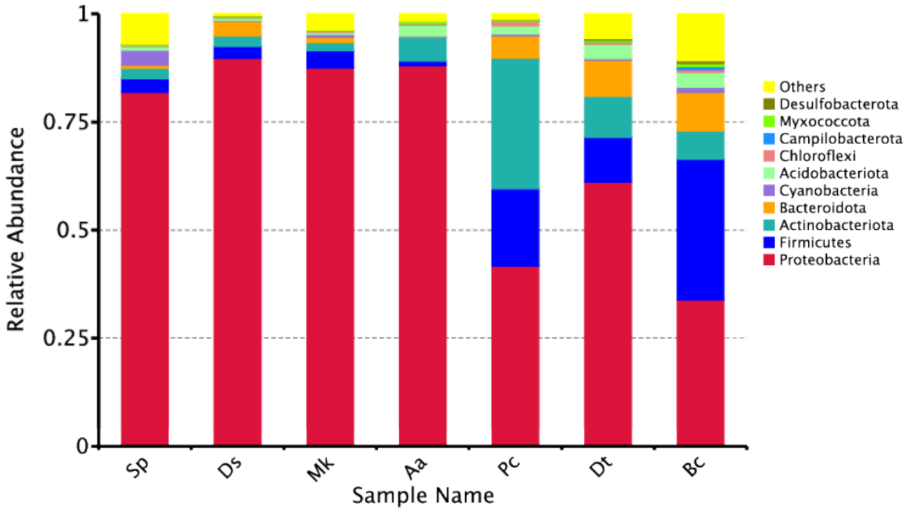

Figure 2. Relative abundance of bacterial phylum in wild orchid roots from Mount Merbabu National Park (TNGMb), Central Java, Indonesia. Note: Orchid species as host plant: Sp: *Spathoglottis plicata*; Ds: *Dendrobium sagittatum*, Mk: *Malaxis kobi*, Aa: *Apendicula alba*, Pc: *Pholidota carnea*; Dt: *Dendrobium tenellum*; Bc: *Bulbophyllum compressa*

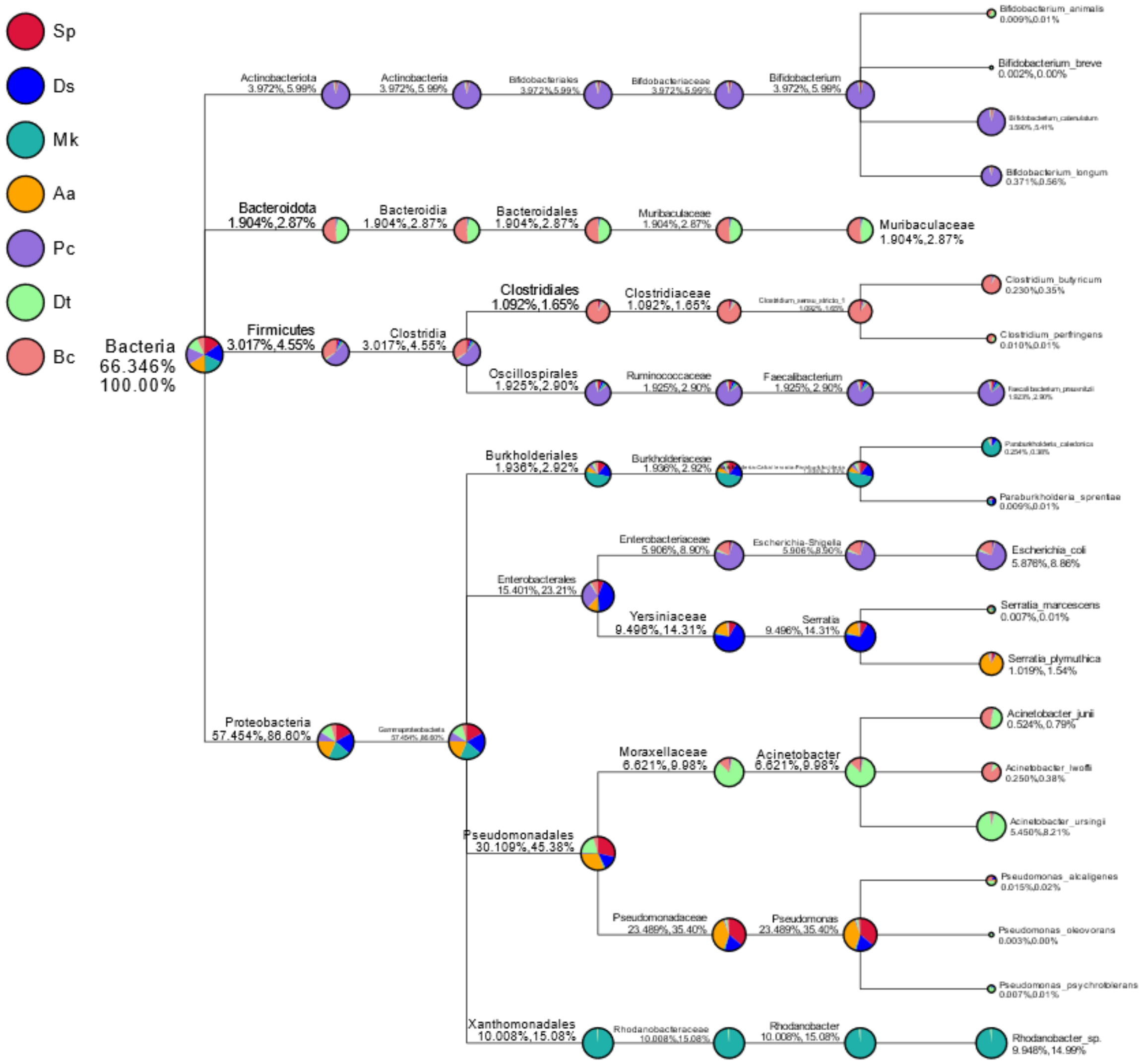

Figure 3. Taxonomy tree of bacteria in all orchid root samples from Mount Merbabu National Park (TNGMb), Central Java, Indonesia. Note: Orchid species as host plant: Sp: *Spathoglottis plicata*; Ds: *Dendrobium sagittatum*, Mk: *Malaxis kobi*, Aa: *Apendicula alba*, Pc: *Pholidota carnea*; Dt: *Dendrobium tenellum*; Bc: *Bulbophyllum compressa*

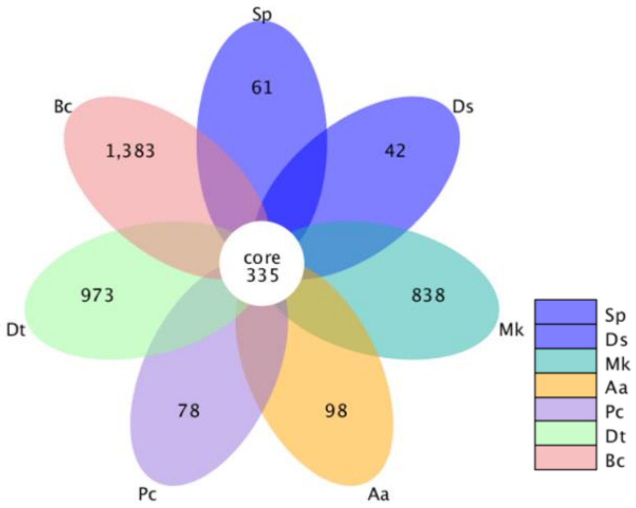

Figure 4. Venn diagram of OTUs of microbial community in all orchid species. Note: Orchid species as host plant: Sp: *Spathoglottis plicata*; Ds: *Dendrobium sagittatum*, Mk: *Malaxis kobi*, Aa: *Apendicula alba*, Pc: *Pholidota carnea*; Dt: *Dendrobium tenellum*; Bc: *Bulbophyllum compresa*

Discussion

In this study, *Pseudomonas* was associated with all orchid roots sampled. *Pseudomonas* is a well-known plant-associated bacteria that can promote plant growth by suppressing pathogenic microorganisms, synthesizing growth-stimulating plant hormones, and promoting nutrient uptake (Sah et al. 2021). Several studies have reported root-associated bacteria from the genus *Pseudomonas* in orchids, such as *Paphiopedilum appletonianum* and *Pholidota articulata*, that produced auxin (Tsavkelova et al. 2007; Kaur and Sharma 2021). Pavlova et al. (2017) reported colonization of *Pseudomonas fluorescens* in *D. nobile* Lindl roots and could enhance plant development by synthesizing substantial quantities of auxin. The auxin synthesis by root-associated bacteria can benefit orchid seed germination and tissue growth (Yadav et al. 2022). A study by Gontijo et al. (2018) showed that some bacterial strains from *Cymbidium* sp., including *Pseudomonas*, had the potential to promote orchid growth. Our study showed the presence of *Pseudomonas* in all orchid species with significant abundance, suggesting that this genus was a group of bacteria that might have an essential role in the orchid.

Pseudomonas is commonly referred to as mycorrhiza helper bacteria (MHB). MHB are microorganisms that facilitate the establishment and efficacy of mycorrhizal symbiosis, a mutualistic relationship between plant roots and fungi. According to Sangwan and Prasana (2022), *Pseudomonas* can facilitate mycorrhizal colonization and activity by producing signaling molecules, enzymes, hormones, and antibiotics that influence the growth and behavior of plants and fungi. *Pseudomonas* can detect and react to chemical signals sent by mycorrhizal fungi, including sugars and volatile compounds, leading to the establishment of mutually advantageous relationships between the two organisms (Nasslahsen et al. 2022). *Pseudomonas* enhances plant nutrition, health, and productivity across diverse agricultural settings as a microbial inoculant.

The results of this study detected the presence of *P. psychrotolerans*, a psychrophile often found associated with plants in low-temperature areas, which can produce antifreeze proteins and cold-adapted enzymes (Subramanian et al. 2011). *Pseudomonas psychrotolerans* was mainly detected in the roots of *D. tenellum* from Ampel Resort, where the temperature was relatively low. It was suggested that this bacterium helps adapt the orchid to low temperatures. *Pseudomonas psychrotolerans* had also been reported to have plant growth-promoting activity. A previous study by Kang et al. (2020) showed that inoculation with *P. psychrotolerans* CS51 in cucumber induced endogenous indole-3-acetic acid (IAA) and gibberellins (GAs), which significantly enhanced cucumber growth (root shoot length) and increased the heavy metal tolerance of cucumber plants.

Rhodanobacter, found mainly in *M. kobi* root, is a genus of bacteria with various roles in different environments, such as nitrogen fixation (Wang et al. 2020; Lv et al. 2021). *Rhodanobacter* produced IAA that increased nitrogen uptake from the soil and used both N2 and fixed nitrogen as nitrogen sources, reducing the energetic costs of nitrogen fixation (Inomura et al. 2018). *Rhodanobacter* also solubilized phosphate in association with plant roots by producing organic acids that lower the pH of the soil to dissolve the phosphate compounds or produce enzymes that hydrolyze the phosphate esters in plant residues or organic matter (Rawat et al. 2021). Furthermore, this bacterial genus is also used as a biocontrol agent against plant pathogens by producing antibiotics and siderophores (Prakash et al. 2021). A study by Zhu et al. (2022) reported the presence of *Rhodanobacter* in *D. catenatum*, which positively correlated with stem polysaccharide, leaf phenol, and flavonoid content. This genus was also found in *Platanthera cooperi* and *P. praeclara*, endemic orchids from America (Kaur et al. 2023).

Another dominant genus, *Serratia*, is also a well-known plant growth-promoting bacterium. *Serratia* acted as plant growth-promoting rhizobacteria (PGPR) by producing phytohormones, siderophores, and enzymes that enhance nutrient uptake, root development, and plant health (Herrera et al. 2022). *Serratia* could be a biocontrol agent by producing antibiotics, lytic enzymes, and volatile organic compounds that inhibit the growth of plant pathogens (Kaur and Sharma 2021). However, there needs to be more studies into the role of this genus in orchid growth.

Some isolated bacteria are known as the gut commensals in humans and animals, such as *Bifidobacterium*, *Acinetobacter*, *Escherichia*, and *Faecalibacterium*. There is no or little evidence that they are orchid-associated bacteria, and the role of these genera in orchids still needs further investigation. *Bifidobacterium* had been identified as an endophyte in *Robinia pseudoacacia*, a well-known plant species for heavy metal phytoremediation, and positively associated with Cd, Cu, Pb, and Zn bioaccumulation and translocation. *Bifidobacterium* also may have some interactions with plants. For example, *Bifidobacterium and Faecalibacterium* could metabolize some plant glycans carbohydrates found in plant cell walls and produce short-chain fatty acids that might affect the host physiology (Kelly et al. 2021). Some strains of *Acinetobacter* have been reported to have plant growth-promoting activities. For example, *Acinetobacter* sp. strain YSD YN2 was isolated from the leaves of *Cyperus esculentus* L. var. sativus and showed the ability to produce IAA, which is a phytohormone that stimulates root growth (Wang et al. 2022). *Acinetobacter* sp. CS9, which was isolated from the rhizosphere of *Catharanthus longifolius*, produced IAA and siderophores, also positive for phosphate solubilization (Yasin et al. 2018). *Escherichia* is commonly found in the environment due to fecal contamination from animals or humans. However, some studies reported *Escherichia* as a plant endophyte with growth-promoting ability. *Escherichia coli* USML2, an endophyte from oil palm, genome sequence revealed genes involved in bacterial motility towards endophytic colonization and plant growth promotion (Tharek et al. 2021).

Muribaculaceae is an incompletely understood bacterial family that was first identified from the mouse gut microbiota and later found in the intestines of other mammals (Lagkouvardos et al. 2019). Muribaculaceae have never been reported as orchid root bacteria. However, Muribaculaceae Incertae Sedis (OTU 15) was found to be the dominant OTU of rice root bacterial community in the Sundarbans region of West Bengal, India (Das and Dhal 2022), where it may contribute to plant nutrition by releasing organic acids and solubilizing phosphate. Muribaculaceae has been reported to be abundant in the rhizosphere of *Arabidopsis thaliana* under drought stress, suggesting that it may play a role in plant adaptation to water scarcity (Lagkouvardos et al. 2019). In our findings, Muribaculaceae were found in significant numbers in two orchids from Ampel Resort (6-7%). The presence of this family is likely related to the adaptation of the host plant in that location.

Clostridium is a genus of bacteria found in various environments, such as soil and the gastrointestinal tract of animals, including humans. Some species of *Clostridium* might have beneficial effects on plant growth. *Clostridium* produces GAs, phytohormones that stimulate plant growth, mainly stem elongation and seed germination (Doni et al. 2014). *Clostridium* also produces acetate, butyrate, and hydrogen, which other bacteria or plants use as substrates or energy sources (Du et al. 2020). A study on orchid-associated bacteria colonizing *Epipactis* spp. identified aerobic and anaerobic bacteria, including *Clostridium* spp., as orchid-associated bacteria (Jakubska-Busse et al. 2021).

Paraburkholderia is a genus of bacteria that can colonize plant tissues and benefit the host plant. It has been known that *Paraburkholderia* promoted plant growth and stress tolerance. It produces volatile organic compounds (VOCs) that influence plant physiology and metabolism. VOCs induce a systemic response in plants, enhancing their resistance against many pathogens and pests (Esmaeel et al. 2018). *Paraburkholderia caffeinilytica* had been reported as an endophyte from *Cattleya walkeriana* that showed an ability to promote the growth of the host plant, such as the production of IAA, N fixation, and solubilization of P, Ca, and Zn (Andrade et al. 2023). Findings in this study showed that only the terrestrial orchid *M. kobi* had a substantial abundance of *Paraburkholderia* (7%). *Paraburkholderia* is expected to reside in soil, including forest soil, and migrates to the roots of terrestrial orchids.

Although the microbiota associated with a particular host species is unique, several orchids living in the same area may share similarities in their composition, referred to as the core microbiota. The core microbiota is the microbial communities consistently present and shared among these different plant species within a specific geographic location or ecosystem. The intake of nutrients, growth, and resistance to external stressors are all influenced by these microbial communities, which are crucial for the upkeep of the health and functionality of plants. The composition of the core microbiota results from intricate interactions between plants, microbes, and the surrounding environment. The core microbiota in different plant species is influenced by the environment in which they grow. Abiotic factors, such as temperature, moisture, soil composition, nutrient availability, and host genotype, affect plant colonization by microorganisms (Agler et al. 2016). Our finding suggested that environmental conditions and the host plant's genetic makeup influence the plant microbiome's composition and diversity. Understanding these factors is crucial for ecosystem function and conservation.

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