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Exploring the bacteriome of *Etlingera balikpapanensis* for potential biofertilizer development and conservation strategies

ALMANDO GERALDI[1,2,3,♥], FATIMAH[1,2], RICO RAMADHAN[1,4], NI'MATUZAHROH[1,2], HERY SUWITO[1,4], NABILLA HAPSARI WIJAYA[1,2], YANA ZAINADA VIRDAUS[5], RICA HANIM FADHILAH[2], ARRIZAL RAHMAN[6], LUKMAN RIYADI[6]

[1]University CoE-Research Center for Bio-Molecule Engineering, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia.
Tel.: +62-823-5938-6346, ♥email: almando.geraldi@fst.unair.ac.id
[2]Department of Biology, Faculty of Science and Technology, Universitas Airlangga. Jl. Teknik Mesin No.173, Surabaya 60115, East Java, Indonesia
[3]Institute of Life Science, Technology and Engineering (LIHTR), Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia
[4]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia
[5]School of Life Sciences and Technology, Institut Teknologi Bandung. Jl. Ganeca No.10, Bandung 40132, West Java, Indonesia
[6]Environmental Office of Balikpapan. Jl. Ruhui Rahayu 1 No. 8, Balikpapan 76115, East Kalimantan, Indonesia

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Abstract. *Geraldi A, Fatimah, Ramadhan R, Ni'matuzahroh, Suwito H, Wijaya NH, Virdaus YZ, Fadhilah RH, Rahman A, Riyadi L. 2025. Exploring the bacteriome of* Etlingera balikpapanensis *for potential biofertilizer development and conservation strategies. Biodiversitas 26: 2548-2555. Etlingera balikpapanensis*, an endangered and endemic plant species from the rainforests of East Kalimantan, Indonesia, faces significant threats due to habitat loss and environmental changes. Conservation strategies for this species are limited, and there is an urgent need for innovative approaches to enhance its survival, particularly in ex-situ environments. One promising avenue is the development of biofertilizers derived from beneficial microbial communities. These Plant Growth-Promoting Bacteria (PGPB), which exist in both endophytic and rhizospheric environments, can improve nutrient uptake, enhance stress tolerance, and protect against pathogens. We used high-throughput 16S rRNA gene sequencing to characterize the endophytic and rhizospheric bacterial communities associated with *E. balikpapanensis*. We identified 1,183 Operational Taxonomic Units (OTUs) from plant tissues and soil samples. Among these, we found bacterial species known for their plant growth-promoting properties, including *Pantoea dispersa* and *Kluyvera intermedia* in plant tissues, as well as *Burkholderia pyrrocinia*, and *Bradyrhizobium elkanii* in soil samples. These potential microbes, with their proven plant growth-promoting properties, offer hope for the conservation of *E. balikpapanensis*. They can be used in biofertilizer formulation that could support the species' survival by improving its resilience to environmental stresses. The identification of such microbial communities offers a foundation for developing sustainable conservation practices that leverage natural symbiotic relationships. Future efforts should focus on isolating and characterizing these bacterial strains for experimental biofertilizer applications, aiming not only to enhance the growth of *E. balikpapanensis*, but also to aid the conservation of other endangered species within the Zingiberaceae family.

Keywords: Bacteriome, biodiversity, endangered species, endophytic bacteria, *Etlingera balikpapanensis*, rhizosphere

INTRODUCTION

Etlingera balikpapanensis A.D. Poulsen, commonly known as Balikpapan ginger, is an endangered and endemic species of the Zingiberaceae family, native to the rainforests of East Kalimantan, Indonesia (Robiansyah et al. 2021). Like other members of the *Etlingera* genus, this plant holds significant ecological, medicinal, and cultural value. It is particularly known for its unique aromatic properties and has been utilized by local communities for traditional culinary and medicinal purposes (Manurung et al. 2019). This genus is rich in bioactive compounds, including flavonoids, phenolics, and terpenoids, which contribute to their anti-inflammatory, antioxidant, and antimicrobial activities (Al-Mansoub et al. 2021; Tan et al. 2023; Koch et al. 2024). For instance, extracts from several *Etlingera* species have shown strong antimicrobial effects against common pathogens, highlighting their potential in pharmaceutical applications (Sahidin et al. 2022; Ferreira et al. 2025). Despite its ecological and medicinal importance, the distribution of *E. balikpapanensis* is limited to only two locations near Balikpapan, East Kalimantan, Indonesia (Robiansyah et al. 2021), making it prone to habitat destruction, deforestation, and climate change. This species is categorized as endangered; thus, conservation efforts are considered critical.

The role of endophytic and rhizospheric bacteria in the conservation of endangered plants has increasingly gained recognition since it has shown the potential to increase plant growth, stress tolerance, and resilience (Rigg et al. 2017; Dutta et al. 2021). Microbial communities contribute to plant health by promoting nutrient acquisition, producing phytohormones, and protecting against pathogens (Compant et al. 2019). For instance, endophytic bacteria increase plant growth by affecting crucial factors such as improvement in nitrogen fixation, hormone production, and phosphate solubilization (Santoyo et al. 2016). These microbial interactions are not only beneficial for plant growth but also contribute to the plant's ability to withstand environmental stresses such as drought, salinity, and heavy metal contamination. Research on the endophytic bacterial communities of *E. balikpapanensis* remains limited. The only study conducted to date successfully isolated bacterial strains, including *Micrococcus luteus* and *Bacillus cereus*, which exhibited antibacterial activity against human pathogenic bacteria (Fatimah et al. 2024). However, other members of the family, such as *Alpinia officinarum* Hance, *Amomum compactum* Roem. & Schult., and *Curcuma longa* L., host diverse microbial communities, including Plant Growth-Promoting Bacteria (PGPB) like *Bacillus*, *Methylobacterium*, and *Priestia*. These bacteria enhance plant growth through mechanisms such as phosphate solubilization, nitrogen fixation, and production of phytohormones like Indole-3-Acetic Acid (IAA) (Gamalero and Glick 2019). For example, *A. officinarum* endophytes produce chitinase and β-glucanase, which aid in biocontrol, while *A. compactum* bacteria exhibit anti-phytopathogenic activity (Li et al. 2015; Geraldi et al. 2024). Co-inoculation of *C. longa* with *Bacillus megaterium* and arbuscular mycorrhizal fungi improves soil health and bacterial diversity, further supporting plant growth (Sarathambal et al. 2022). These findings highlight the potential of PGPB in Zingiberaceae species to enhance plant resilience and productivity, which is essential for endangered plants like *E. balikpapanensis*.

A case study by Boanares et al. (2024) involving the threatened plant species from Amazon forest, *Carajasia cangae* R.M.Salas, E.L.Cabral & Dessein, demonstrated the potential of microbial communities in plant conservation. The research indicated that endophytic and rhizospheric bacteria, including *Bacillus*, *Planococcus*, and *Lysinibacillus*, contributed to a reduction in the germination time of this species, ultimately enhancing seedling yield. Similarly, the endangered *Solanum fernandezianum* Phil. showed increased seed germination rate through the symbiotic relationships with *Serratia*, *Raoultella*, and *Pseudomonas* (Carrasco-Fernández et al. 2020). These findings align with other studies, such as those on *Abies hickelii* Flous & Gaussen and *Abies religiosa* (Kunth) Schltdl. & Cham., where biopriming with PGPB like *Pseudomonas fluorescens* and *Bacillus subtilis* improved germination rates up to 91% and 68%, respectively, without compromising growth (Zulueta-Rodríguez et al. 2015). Additionally, research on *Pinus squamata* X.W.Li emphasized the importance of soil properties and native microbial communities, such as *Bradyrhizobium* and *Burkholderia-Caballeronia Paraburkholderia*, in enhancing plant health and growth in both wild and conservation settings (Li et al. 2024). These examples collectively underscore the critical role of PGPB and microbial interactions in improving germination, growth, and resilience of threatened plant species, offering valuable strategies for conservation and sustainable propagation efforts.

Given the critical conservation status of *E. balikpapanensis* and the lack of prior research on its associated bacterial communities, it is plausible that this plant hosts beneficial endophytic and rhizospheric bacteria that contribute to growth promotion and environmental adaptability. Understanding these bacterial communities is essential for developing innovative biofertilizer formulations that can support ex-situ conservation and improve the resilience of this endangered species. To explore this possibility, we employed high-throughput 16S rRNA gene sequencing to comprehensively characterize the bacterial communities associated with *E. balikpapanensis*. This approach enables the identification of Plant Growth-Promoting Bacteria (PGPB) that may facilitate nutrient acquisition, stress tolerance, and pathogen resistance. By elucidating the composition and potential functions of these bacteriomes, our study aims to provide foundational knowledge that can inform sustainable conservation strategies, including the formulation of biofertilizers tailored to *E. balikpapanensis*. Ultimately, these efforts will not only contribute to the preservation of this endemic species but also enhance our broader understanding of plant-microbe interactions within the Zingiberaceae family, paving the way for novel biofertilizer developments and sustainable agricultural practices (Fadiji and Babalola 2020).

MATERIALS AND METHODS

Genomic DNA extraction

Plant samples (whole leaf, aerial stem, and rhizome) of *E. balikpapanensis*, as well as the rhizospheric soil from a depth of 30 cm around the root zone of *E. balikpapanensis* were collected from 17 locations within the Balikpapan Botanical Garden, East Kalimantan, Indonesia (01°07.823'S to 01°07.960'S and 116°51.321'E to 116°51.515'E) in October 2019. Leaf, stem, and rhizome samples were subjected to surface sterilization using ethanol, followed by a mercury chloride (HgCl) solution (Boanares et al. 2024) to eliminate surface contaminants. Genomic DNA was then isolated from the sterilized plant tissues and the rhizospheric soil with the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Cambridge, UK). The concentration of the extracted DNA was quantified using a NanoDrop spectrophotometer and a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA integrity and purity were confirmed through agarose gel electrophoresis, with the gel images captured using the Gel-Doc EZ imaging system (Bio-Rad, California, USA).

16S rRNA gene amplicon sequencing

The extracted genomic DNA was processed with dilution using sterile deionized water until it reached 1 ng/µL concentration. The samples were sent to Novogene AIT Genomics, Singapore, for high-throughput amplicon sequencing targeting the V3-V4 hypervariable regions of the 16S rRNA gene. The V3-V4 region was amplified with PCR by utilizing the 341F forward primer (5’-CCTAYGGGRBGCASCAG-3’) and the 806R reverse primer (5’-GGACTACNNGGGTATCTAAT-3’), along with Phusion® High-Fidelity PCR Master Mix (New England Biolabs) (15). The obtained PCR products were utilized to generate sequencing libraries according to the protocol set by the manufacturing company (Ion Plus Fragment Library Kit, Thermo Fisher Scientific). Next, the quality of the libraries was inspected using a Qubit® 2.0

Fluorometer (Thermo Fisher Scientific), and sequencing was done on an IonS5TM XL platform (Thermo Fisher Scientific).

Bioinformatics analysis

After sequencing, the paired-end reads were processed with demultiplexing according to sample-specific barcodes. The sequences containing the primer and barcode were trimmed. The resulting reads were merged using the FLASH software (v1.2.7) (Fadiji and Babalola 2020). The raw sequences were subjected to quality filtering using QIIME (v1.7.0) (Yang et al. 2023) to ensure high-quality data. Any potential chimeric sequences were identified and removed by comparing the data against the Gold reference database by the UCHIME algorithm (Singh et al. 2021). Further processing of clean sequences was done using the UPARSE pipeline (Singh et al. 2020), where Operational Taxonomic Units (OTUs) were clustered with a sequence similarity threshold of 97%.

Taxonomic classification of representative sequences of each OTU was performed using the MOTHUR software package against the SILVA ribosomal RNA gene database (Lv et al. 2022; Ansari et al. 2024), with a confidence threshold of 0.8-1. Alignments were generated with the MUSCLE software (v3.8.31) to analyze phylogenetic relationships among OTUs (Çelik and Sevim 2022). Next, several alpha diversity metrics were calculated, i.e., Chao1, observed species, Abundance-Based Coverage Estimator (ACE), Shannon and Simpson index, using QIIME (v1.7.0) and visualized through R software.

RESULTS AND DISCUSSION

The identification results showed a total of 1,171 Operational Taxonomic Units (OTUs) found in the soil samples, which was significantly higher compared to the OTUs found in plant tissue samples (396 in rhizomes, 371 in stems, and 299 in leaves). All rarefaction curves showed a gradual saturation with increasing sequencing depth, covering the full diversity of the microbial community in all four samples. The rarefaction analysis indicated distinct OTU abundance patterns across the plant tissues and soil samples (Figure 1.A). The plant tissues shared 193 species out of a total of 466 species, while 89, 81, and 51 species were unique to rhizomes, stems, and leaves, respectively (Figure 1.B). Of the total bacterial species observed in the plant sample, 84.98% were present in rhizomes, 79.61% in stems, and 89.6% in leaves.

The bacterial community diversity was expressed in Shannon and Simpson diversity indices that combine the number of species present with their relative abundance (Fang et al. 2015). Other indices (observed species, Chao1, and ACE) account for species richness. The bacterial community in rhizomes was the most diverse among the other samples, while leaves displayed the lowest values (Table 1).

Table 1. Five alpha diversity indices of bacterial community found in rhizospheric soil and plant tissues (rhizome, stem, and leaves) of *Etlingera balikpapanensis*

Sample	**Observed species**	**Shannon**	**Simpson**	**Chao1**	**ACE**
Rhizospheric soil	1171	7.803	0.988	1.184.016	1.182.959
Rhizome	396	3.376	0.819	1.331.200	643.188
Stem	371	3.489	0.845	437.750	444.982
Leaves	299	2.541	0.675	358.304	374.302

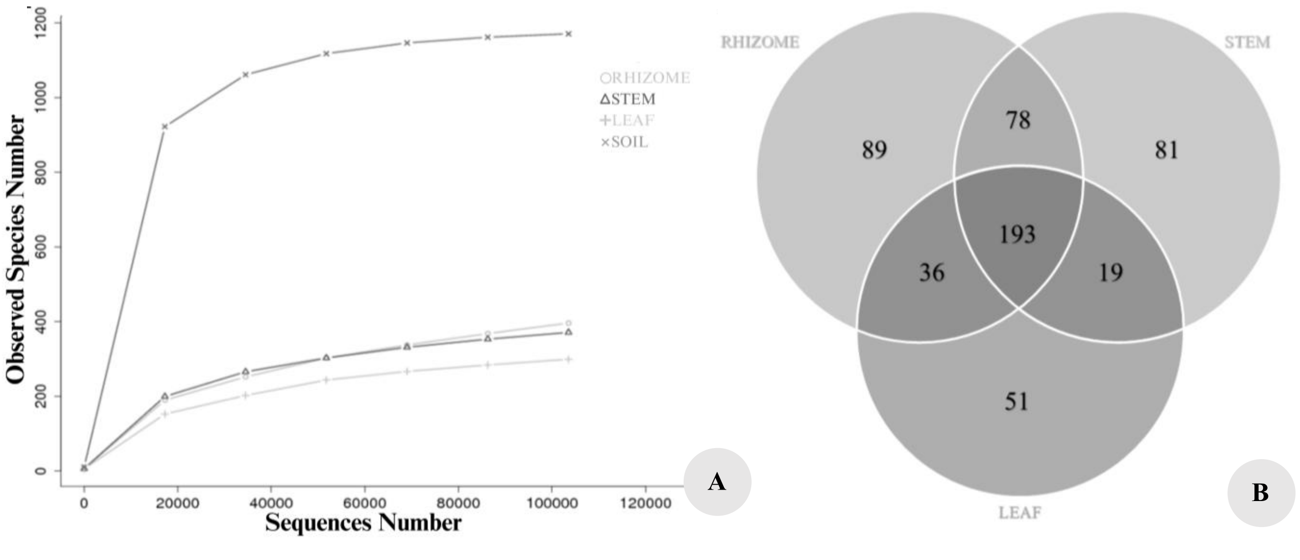

Figure 1. The result of operational taxonomic units analysis. A. Rarefaction curves of operational taxonomic units of rhizospheric soil and plant tissues (rhizome, stem, and leaves) of *Etlingera balikpapanensis*; B. Shared species among plant tissues in *E. balikpapanensis*

The most abundant bacterial phyla found in plant and soil samples are shown in Figure 2.A. Proteobacteria, a Gram-negative phylum, were the most dominant in all plant tissue (rhizome 96.46%, stem 93.37%, and leaf 95.46%) and soil samples (47.51%). In addition to Proteobacteria, the soil samples contained relatively high proportions of Acidobacteria (29.72%) and Actinobacteria (9.91%). In the plant tissues, Firmicutes was the second most abundant phylum (2-3%), while other phyla were present in proportions below 1%. At the family level, the endophytic bacterial communities in plant tissues were dominated by Enterobacteriaceae, followed by Pseudomonadaceae (Figure 2.B). As expected, the soil samples exhibited higher bacterial family diversity, with Acidobacteriaceae and Burkholderiaceae being the most dominant.

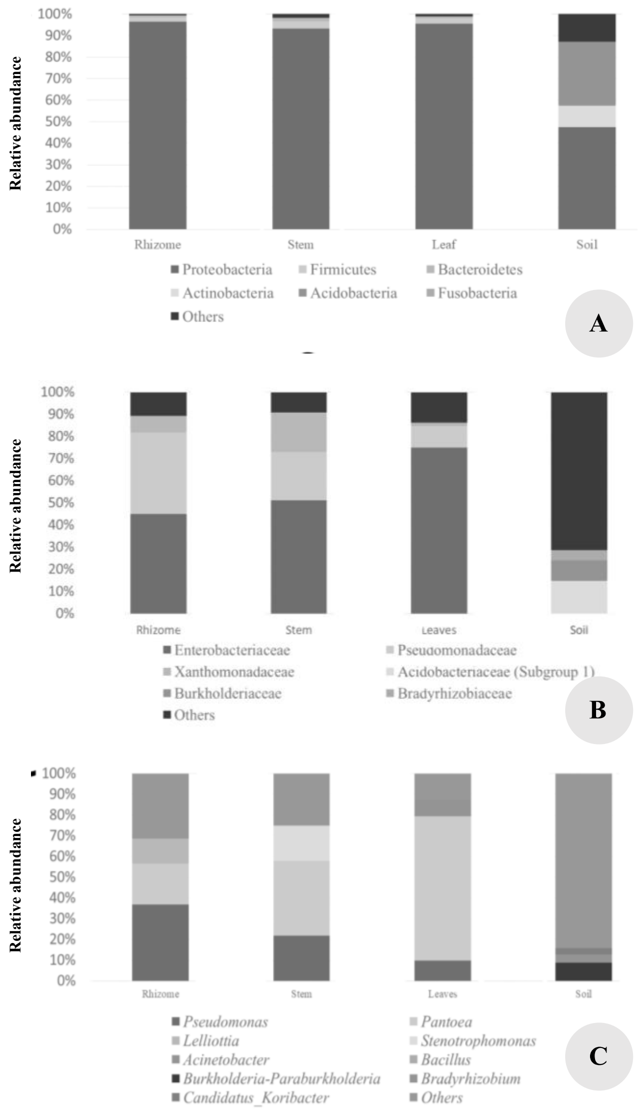

Figure 2. The relative abundance of bacterial: A. Phylum; B. Family; C. Genus in plant tissues (rhizome, stem, leaves) and rhizospheric soil samples of *Etlingera balikpapanensis*

The dominant genera found in all plant tissue samples were relatively similar, although their relative abundance differed between plant tissues. The five most dominant genera in plant tissue samples were *Pseudomonas*, *Pantoea*, *Lelliottia*, *Raoultella*, and *Stenotrophomonas* (Figure 2.C). However, some specific genera were only found in specific tissue types, i.e*.,* *Comamonas* and *Paenibacillus* were unique to rhizomes, *Serratia* and *Lactococcus* were dominant in stems, and *Acinetobacter* and *Bacillus* were specific to leaves (Figure 2.C). In soil samples, the most dominant genus was *Burkholderia-Paraburkholderia* (Figure 2.C). Besides that, *Pseudomonas* and *Pantoea* were also present in soil and plant tissues (Figure 2.C).

Pantoea dispersa and *Pantoea* sp. At-9b were among the most dominant species in plant tissues and rhizosphere soil (Table 2). Unique dominant species in the rhizome included *Comamonas testosteroni* and *Paenibacillus amylolyticus*; in the stem, *Serratia marcescens* and *Lactococcus lactis* were dominant; *Kluyvera intermedia* was dominant in rhizome and stem samples; while in the leaf sample, *S. marcescens*, *Acinetobacter* sp., and *Bacillus aerophilus* were prevalent (Table 2). Dominant species in the soil included *Bradyrhizobium elkanii*, *Burkholderia pyrrocinia*, and *Escherichia coli.* These findings are consistent with previous culture-based studies that isolated endophytic bacteria from *E. balikpapanensis*, including *Bacillus* species from leaves (Fatimah et al. 2024). Moreover, some of the genera identified here have been reported as endophytes in other plants of the Zingiberaceae family. For instance, *Pseudomonas* species with anti-phytopathogenic activity have been isolated from *C. longa*, *Zingiber officinale* Roscoe, and *Etlingera* species (Rosli et al. 2024). Similarly, *Pantoea*, *Kluyvera*, and *Stenotrophomonas* have been reported as endophytes in *Amomum* species and *Alpinia melichroa* (Sulistiyani et al. 2021).

Table 2. The five most dominant bacterial species in plant tissues (rhizome, stem, leaves) and rhizospheric soil samples of *Etlingera balikpapanensis*

Sample	Species	Relative abundance (%)
Rhizome	*Pantoea sp,* At-9b	15.14
	Pantoea dispersa	4.53
	Comamonas testosteroni	2.99
	Kluyvera intermedia	2.16
	Paenibacillus amylolyticus	1.29
Stem	*Pantoea* sp, At-9b	26.69
	Pantoea dispersa	9.02
	Serratia marcescens	2.31
	Lactococcus lactis	1.67
	Kluyvera intermedia	1.64
Leaves	*Pantoea dispersa*	52.35
	Acinetobacter sp, NIPH_2171	6.03
	Pantoea sp, At-9b	3.11
	Bacillus aerophilus	0.90
	Serratia marcescens	0.72
Soil	*Bradyrhizobium elkanii*	4.12
	Burkholderia pyrrocinia	2.59
	Pantoea sp, At-9b	0.76
	Pantoea dispersa	0.69
	Escherichia coli	0.57

Discussion

The bacterial communities identified within the tissue and rhizosphere of *E. balikpapanensis* exhibit multiple functional traits characteristic of Plant Growth-Promoting Bacteria (PGPB), highlighting their potential applications as biofertilizers. These bacteria contribute to plant health and growth through various mechanisms, including nitrogen fixation, phosphate solubilization, production of phytohormones such as Indole-3-Acetic Acid (IAA), and biocontrol activities against phytopathogens. Among the predominant taxa identified, *Pseudomonas*, *Pantoea*, *Burkholderia*, and *Paenibacillus* are renowned for their beneficial interactions with plants.

Pseudomonas species are known for their ability to produce siderophores and antimicrobial compounds, enhancing nutrient availability and plant resistance to pathogens (Hariprasad et al. 2014; Sulochana et al. 2014). *Pantoea* spp. have been recognized for their role in biological nitrogen fixation and phytohormone synthesis (Singh et al. 2021; Lorenzi et al. 2022). *Burkholderia* spp., particularly those from the plant-associated beneficial clades, contribute to nutrient mobilization and plant defense responses (Mannaa et al. 2019; Elshafie and Camele 2021). *Paenibacillus* spp. are noted for their biocontrol properties and their ability to facilitate phosphorus solubilization, making them valuable candidates for biofertilizer development (Daud et al. 2019; Din et al. 2020).

Among the identified bacterial communities, *Pantoea* species, particularly *P. dispersa*, stand out as a dominant endophytic and rhizospheric bacteria associated with *E. balikpapanensis*. This prevalence is noteworthy given the well-established roles of *Pantoea* in plant growth promotion through multiple mechanisms. Specifically, *Pantoea* is recognized for its capacity to fix atmospheric nitrogen, solubilize phosphate, and synthesize phytohormones, such as Indole-3-Acetic Acid (IAA), which collectively enhance root growth and overall plant development (Singh et al. 2021; Yang et al. 2023). These bacteria are often found in plant-associated environments, where they contribute to improved nutrient acquisition and trigger plant defense responses against phytopathogens via the production of antimicrobial compounds (Singh et al. 2020). A *Pantoea* species, *Pantoea agglomerans*, a closely related species, has been successfully employed as a biofertilizer in diverse agricultural systems, demonstrating its ability to increase plant biomass and crop yield (Lv et al. 2022; Ansari et al. 2024). The prominence of *P. dispersa* in the *E. balikpapanensis* bacteriome, coupled with the proven biofertilizer potential of other *Pantoea* species, underscores the potential for harnessing this bacterium to enhance the sustainable cultivation of *E. balikpapanensis*.

Kluyvera intermedia, a dominant species identified in the rhizome and stem of *E. balikpapanensis*, plays a significant role in plant-microbe interactions, particularly in promoting plant health and enhancing soil fertility. This bacterial species is widely recognized for its ability to suppress fungal plant pathogens through the production of antimicrobial compounds, and exhibits insecticidal properties, further contributing to plant protection, making it a valuable biocontrol agent in natural and agricultural ecosystems (Çelik and Sevim 2022; Shrestha et al. 2023). Beyond its biocontrol potential, *K. intermedia* also plays a crucial role in plant growth promotion by facilitating nutrient acquisition. Its ability to fix atmospheric nitrogen and solubilize phosphate enhances soil nutrient availability, which is particularly beneficial in nutrient-deficient or degraded environments (Li et al. 2024).

Serratia marcescens, a prevalent endophytic bacterium colonizing the stems and leaves of *E. balikpapanensis*. While it is known to act as an opportunistic pathogen in humans, particularly in immunocompromised individuals, it also exhibits significant Plant Growth-Promoting (PGP) properties that make it a valuable candidate for agricultural applications. Notably, *S. marcescens* actively solubilizes both phosphorus (P) and zinc (Zn) from insoluble forms in the plant tissues and surrounding environment, thereby significantly enhancing the bioavailability of these crucial nutrients, which are often limiting factors for plant growth (Matteoli et al. 2018). Furthermore, it synthesizes Indole-3-Acetic Acid (IAA), a key phytohormone that plays a pivotal role in stimulating root development, cell elongation, and overall plant growth and architecture (Zhang et al. 2022). Iron (Fe) acquisition, essential for various plant metabolic processes, is facilitated by *S. marcescens* via the production of siderophores, high-affinity iron-chelating compounds. In addition, this bacterium produces ACC deaminase, which reduces ethylene levels in plants under stress, thereby promoting stress tolerance (Selvakumar et al. 2008). These multifunctional PGP traits collectively highlight the potential of specific *S. marcescens* strains as promising bioinoculants for sustainable agricultural practices. However, before widespread application, rigorous research is imperative to fully characterize the specific strains inhabiting *E. balikpapanensis*, to assess their pathogenic risks to humans and other organisms comprehensively, and to develop appropriate mitigation strategies to ensure safe and effective utilization.

Burkholderia pyrrocinia and *B. elkanii,* which are dominant species in the rhizosphere of *E. balikpapanensis,* also exhibit plant growth-promoting activities. *B. pyrrocinia* can enhance soil fertility by fixing atmospheric nitrogen into a form usable by plants, thus improving the nitrogen content of the soil (Madhaiyan et al. 2010). This bacterium also produces various growth-promoting compounds and siderophores, which chelate iron and make it available to plants, ensuring the iron needs in poor soil are fulfilled (Han et al. 2021). It also shows antifungal activity, helping build plant resistance against soil-borne pathogen attacks (Yu et al. 2023). *B. elkanii* is also a rhizobial species known for its beneficial effects on plant growth, such as facilitating nitrogen fixation (Lu et al. 2017; Leng et al. 2023) producing exopolysaccharides that improve soil aggregation and moisture retention in arid and semi-arid environments (Bharti et al. 2023), also producing plant growth regulators (Nakei et al. 2022). Although this species is primarily associated with legumes, its ability to enhance nutrient uptake and improve soil structure can benefit various plants, including Zingiberaceae.

The bacterial communities identified through NGS analysis in *E. balikpapanensis* and its rhizosphere offer significant potential for biofertilizer development. The insights provided by the NGS data allow for a targeted approach to isolate and utilize PGPB. Genera such as *Pantoea*, *Pseudomonas*, *Burkholderia*, *Paenibacillus*, and *Bradyrhizobium*, which were dominant in the rhizosphere and plant tissues, are well-known for their beneficial effects on plant growth and resilience. The information provided by the NGS data allows for more efficient isolation of these bacteria and their use as biofertilizers, which will aid in the conservation of *E. balikpapanensis*.

By leveraging the NGS data, selective isolation methods can be applied to obtain key PGPB. For example, *B. elkanii* and *B. pyrrocinia* identified in the bacterial community are important nitrogen fixers, and their isolation can be carried out using nitrogen-free culture media to select bacteria with nitrogen fixation capabilities (Geraldi et al. 2024). These targeted approaches increase the chances of isolating effective PGPB for biofertilizer applications.

Functional screening of isolated bacteria can further validate their plant growth-promoting abilities. Isolation can be followed by screening of selected key traits such as phosphate solubilization, siderophore production, or phytohormone synthesis. For instance, *P. dispersa*, one of the dominant genera found in this study, is known to solubilize phosphate and produce IAA (Cui et al. 2023; Tariq et al. 2023). Similarly, *K. intermedia*, another species identified in the plant tissues, has been reported to improve nutrient uptake and enhance root growth (Saha et al. 2013). Screening these isolates for such traits will help identify the most effective PGPB candidates for biofertilizer formulation.

Once key strains have been identified and screened, they can be tested for their efficacy as biofertilizers. Trials involving isolated strains like *Pseudomonas*, *Pantoea*, and *Paenibacillus* have demonstrated positive effects on plant growth in other species, including their ability to increase biomass and nutrient uptake (Glick 2012). In a similar approach, PGPB strains isolated through NGS data have been tested as biofertilizers for various plant species, showing significant improvements in plant yield and soil nutrient levels (Mokabel et al. 2022; Anbarasan et al. 2024). For example, in sugar beet (*Beta vulgaris*), 16S rRNA metabarcoding guided the isolation of bacterial strains such as *Acinetobacter calcoaceticus* and *Bacillus australimaris*, which exhibited multiple plant-beneficial traits, including phosphate solubilization and biocontrol activity against pathogens like *Fusarium oxysporum* (Tomić et al. 2023). Studies on the model grass *Brachypodium distachyon* further illustrated how metabarcoding identifies core root microbiome members, such as Bacillus and Rhodococcus strains, which enhanced shoot biomass by up to 40% in gnotobiotic systems (Pille et al. 2024).

Additionally, biofertilizers developed from indigenous bacterial communities hold significant promise for the conservation of *E. balikpapanensis*. Indigenous PGPBs, such as those identified in this study, are well adapted to the local environment and are more likely to thrive under the specific soil and climatic conditions of East Kalimantan. This local adaptation ensures better soil health and plant resilience, thereby supporting the long-term conservation of this endangered species (Kumar et al. 2022).

This study represents the first comprehensive characterization of the endophytic and rhizospheric bacterial communities associated with *E. balikpapanensis* using high-throughput 16S rRNA gene sequencing. We identified a diverse array of bacterial taxa, including key plant growth-promoting genera such as *Pseudomonas*, *Pantoea*, *Burkholderia*, and *Paenibacillus*, which have been widely reported to enhance nutrient uptake, stress tolerance, and pathogen resistance in various plant systems. The detection of these beneficial microbes in both plant tissues and surrounding soils underscores their potential for development into biofertilizer formulations specifically tailored to support the growth and conservation of this endangered species. Compared to related studies on other Zingiberaceae members, the microbial diversity observed here is notable, with over 1,100 Operational Taxonomic Units (OTUs) detected, highlighting the rich bacteriome that could be harnessed for sustainable conservation efforts. Importantly, leveraging these microbial communities in ex-situ conservation programs could improve the resilience of *E. balikpapanensis* to environmental stresses, thereby enhancing survival rates outside its natural habitat. However, this study is limited by its reliance on amplicon sequencing, which provides taxonomic but limited functional resolution; thus, future work should prioritize isolating and experimentally characterizing the most promising bacterial strains to validate their plant growth-promoting activities and biofertilizer efficacy. Additionally, environmental variability and seasonal dynamics were not addressed here, which may influence microbial community composition and function. Overall, our findings provide a foundational framework for integrating microbiome-based strategies into conservation and agricultural practices, not only for *E. balikpapanensis* but also for other endangered plant species, thereby contributing to biodiversity preservation and sustainable ecosystem management.

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