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Rhizobacteria profiling of endemic aromatic rice *Pulu Mandoti* from Enrekang, South Sulawesi, Indonesia through shotgun metagenomic

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Abstract. *Masniawati A, Manguntungi B, Umar MR, Nurhikmah, Sari AP, Salsabila AS. 2025. Rhizobacteria profiling of endemic aromatic rice* Pulu Mandoti *from Enrekang, South Sulawesi, Indonesia through shotgun metagenomic. Biodiversitas 26: 3183-3192. Pulu Mandoti* is an indigenous rice with a unique aroma, texture, and cultural significance in local communities. The rhizosphere microbial community plays a pivotal role in supporting its growth, nutrient cycling, and stress resilience. Understanding the microbial diversity associated with *Pulu Mandoti* rice is essential for developing strategies to improve its cultivation and conservation. Therefore, this research aimed to conduct a comprehensive analysis of rhizobacteria profile of *Pulu Mandoti* through shotgun metagenomic. The research methods include soil analysis, DNA extraction, and shotgun metagenomic analysis. Shotgun metagenomic sequencing reported a diverse microbial community dominated by bacteria, with Proteobacteria (42%) as the most abundant phyla, and Alphaproteobacteria (15%) in the class. In the family level, Anaeromyxobacteraceae and Bradyrhisobiaceae were particularly abundant. The genus *Bradyrhizobium* within Bradyrhisobiaceae showed the highest richness, with 97 species. *Anaeromyxobacter*, such as *Singulisphaera* and *Rhodoplanes* dominated the species at 66% and 83%, respectively. This microbial profiling showed the intricate relationship between *Pulu Mandoti* rice and the rhizospheric microbiome, emphasizing the role of microbial diversity in supporting growth and resilience. It's essential for offering potential applications for sustainable agriculture and crop optimization.

Keywords: Aromatic rice, Enrekang, *Pulu Mandoti*, rhizobacteria, shotgun metagenomic

INTRODUCTION

Rhizosphere is a soil region directly influenced by plant roots and serves as a critical interface for plant-microbe interactions, facilitating communication through chemical signaling mediated by root exudates and microbial metabolites (Checcucci and Marchetti 2020; Berg et al. 2021). Rhizosphere is a biological hotspot where plant-microbe communication primarily occurs at the root level, where plants release organic compounds, such as sugars, amino acids, and secondary metabolites, collectively known as root exudates. These exudates act as a food source for microbial communities, shaping the composition and activity of the rhizospheric microbiome (Berg et al. 2021; Jamil et al. 2022). The microbial community influences plant growth and health through nutrient cycling, hormone production, and pathogen suppression (Malviya et al. 2020; Hakim et al. 2021).

Rhizospheric bacteria, particularly Plant Growth-Promoting Rhizobacteria (PGPR), directly stimulate plant growth by secreting hormones, such as auxin (Indole Acetic Acid-IAA), cytokinins, gibberellins (GA3), and ethylene (Saeed et al. 2021; Kumar et al. 2022; Vocciante et al. 2022). Additionally, PGPR enhances nutrient availability by fixing atmospheric nitrogen, solubilizing phosphate, and producing siderophores that chelate iron. It also contributes to plant tolerance against biotic and abiotic stresses. Some induce systemic resistance, making plants more resistant to pathogens, while others produce osmoprotectants to help plants withstand drought or salinity (Khan et al. 2021; Zia et al. 2021).

The analysis of rhizospheric microbial interactions holds the potential for improving food crop productivity. Understanding the complex interactions between plants and microbial communities can help develop strategies to harness beneficial rhizobacteria for enhanced crop yields and resilience (Koza et al. 2022; Kumar et al. 2022). Indonesia is one of the rice-producing country in the world and has a significant consumer (Setyaningsih et al. 2019). Among the various varieties, glutinous rice holds a unique position due to its cultural and economic significance. An important variety is *Pulu Mandoti*, a red-hued, fragrant, and high-value glutinous rice native to Salukanan Village, Enrekang District, South Sulawesi (Sukmawaty et al. 2022). This rice is valued for its nutritional benefits since aromatic and pigmented varieties are rich in phytochemicals, including antioxidants and anti-diabetic compounds (Masniawati et al. 2024; Nurjannah et al. 2024).

Despite attempts to cultivate *Pulu Mandoti* in other regions, the unique qualities, including the distinct aroma, have not been replicated. In some cases, rice fails to grow entirely, showing that the characteristics are tied to the

local environment, including soil conditions and microbial communities. The rhizospheric microbial community significantly influences the quality of *Pulu Mandoti* rice by enhancing nutrient uptake, stress resilience, and the production of secondary metabolites that contribute to its unique aroma, flavor, and nutritional profile. Specific microbes may play a role in synthesizing volatile organic compounds or improving the accumulation of essential nutrients, which define the rice's distinctive characteristics. Understanding these microbial interactions is critical for preserving the endemic rice's unique properties, emphasizing the relevance of studying rhizospheric communities to maintain its cultural and economic value (Sukmawaty et al. 2022).

The soil characteristics of Salukanan Village have been analyzed extensively, however, the rhizobacterial profile of *Pulu Mandoti* remains poorly understood. The investigation of the microbial communities associated with the endemic rice variety could provide insights into the factors contributing to the uniqueness and inability to thrive outside the native environment. A promising method for analyzing the rhizobacterial profile of *Pulu Mandoti* is shotgun metagenomic (Lu et al. 2018). This high-throughput method enables comprehensive analysis of the entire microbial community in the rhizosphere, providing insights into taxonomic and functional diversity (Lan et al. 2019). Shotgun metagenomic analysis allows an unbiased exploration of microbial diversity and identifies key microbial species to improve understanding and conservation of *Pulu Mandoti* unique properties. Therefore, this research aimed to analyze the rhizosphere microbial community associated with *Pulu Mandoti* rice using shotgun metagenomics, to uncover the taxonomic and functional diversity of the microbial populations.

MATERIALS AND METHODS

Research site

This research was conducted in the villages of Salukanan and Kendenan Villages, located in the Baraka Sub-district, approximately 60 km from Enrekang District, South Sulawesi, Indonesia (Figure 1). The villages are situated at an altitude of 700 m above sea level (asl), with temperatures ranging from 17 to 22°C. *Pulu Mandoti* is a rare local rice variety found in mountainous regions at an altitude of approximately 700 m asl.

Procedures

Sampling and sample preparation

Rhizosphere soil samples were meticulously collected to capture the diverse microbial communities associated with plant roots. There are three soil samples taken at three different points. Plants were gently uprooted to minimize disruption to the rhizosphere environment, ensuring the integrity of the root-soil interface. Furthermore, extraneous soil was carefully removed, and the remaining tightly adhering to the root surface was collected through gentle shaking and brushing. Root fragments were also included in the samples to maintain the association with the microbial community. Plant litter was incorporated into the collected rhizosphere soil to replicate natural conditions and enhance microbial diversity. In addition, sampling was conducted with precision to preserve the spatial heterogeneity inherent in the rhizosphere, ensuring a representative and accurate reflection of the microbial ecosystem. This method guarantees the reliability of the data for subsequent analysis and interpretation (Babalola et al. 2020; Akinola et al. 2021; Shami et al. 2022).

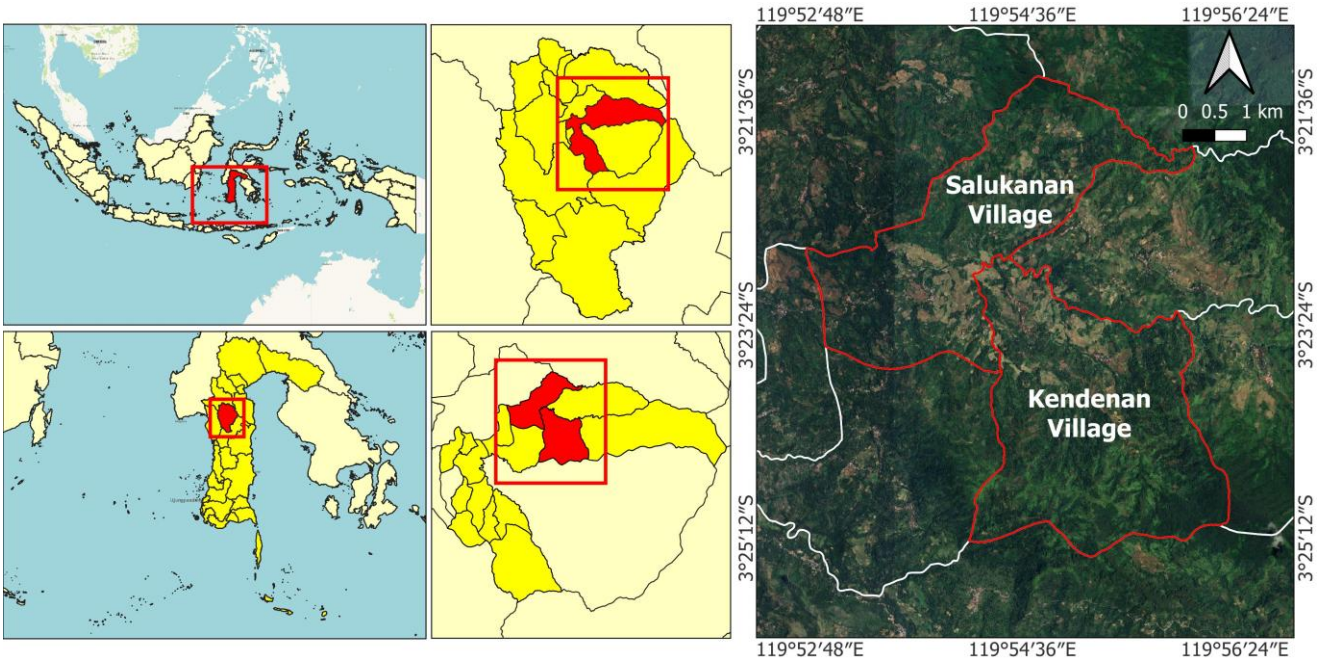

Figure 1. Location profile of *Pulu Mandoti* rice field in Salukanan and Kendenan Villages, Baraka Sub-district, Enrekang District, South Sulawesi, Indonesia

Soil analysis

Soil analysis was conducted to determine the micro and macronutrient content of the soil used as a sample for metagenomic shotgun analysis. The samples used were the same soil samples for shotgun metagenomic analysis. Soil analysis includes organic matter, i.e C, N (spectrophotometer), and P_2O_5(Olsen method), cation exchange capacity, i.e Ca, Mg, and K), and other compounds, such as Fe, Mo, Mn, Zn, Cu, Cl, and So_4 (AAS, flamephotometer, pectrophotometer). The analysis was conducted in the testing laboratory of the Faculty of Agriculture and Forestry, Universitas Sulawesi Barat, Majene, Indonesia.

Metagenomic DNA isolation and shotgun sequencing

Microbial community was extracted from 0.25 g of soil samples using Powersoil Community DNA Isolation Kit (Mo Bio Laboratories, Inc.). The Illumina Nextera DNA Flex Kit was used for library preparation, adhering to the protocols provided by the manufacturer. Initial DNA concentrations were quantified using the Qubit® dsDNA HS Assay Kit from Life Technologies. During library preparation, 50 ng of extracted DNA was used, showing simultaneous fragmentation and adapter addition. Adapters and unique indices were incorporated through 6 cycles of PCR. After library preparation, the concentration of the libraries was measured using the Qubit® dsDNA HS Assay Kit, and library sizes were determined using the Agilent 2100 Bioanalyzer. The libraries, pooled in an equal-molar ratio of 0.7 nM, were sequenced using paired-end sequencing for 300 cycles on the NovaSeq 6000 system (Illumina). This sequencing process was conducted by Mr. DNA at the Molecular Research Laboratory in the USA (Rong et al. 2020; Shami et al. 2022).

Data analysis

The MG-RAST online server processed the shotgun raw sequences obtained from microbial community DNA (Meyer et al. 2008). Using the standard protocol, this online annotation system pipeline performed multiple quality control steps, including dereplication, removal of host-specific sequences, elimination of ambiguous bases, and read length filtering to exclude artificial sequences introduced during the process. For sequence annotation, the BLAT (BLAST-like alignment tool) algorithm was used to compare sequences against the M5NR database (Kent 2002). Taxonomic profiling was conducted using the Best Hit method with an E-value cutoff of 1×10^{-5}, as well as minimum balance length and percentage identity cutoff of 15 bp and 60%, respectively. This is based on the RefSeq annotation database through MG-RAST (Wilkie et al. 2015. The taxonomic analysis included the distribution of domains, phyla, classes, and genera, with a particular focus on bacteria due to their dominance in the samples. A taxonomic abundance heatmap, using relative abundance data and a z-score range of −1 to 1, was generated with the Heatmapper online tool (www1.heatmapper.ca/expression/). The evenness, Simpson, and Shannon diversity indices were calculated for the samples, while diversity comparisons across treatments were conducted using the Kruskal-Wallis test in PAST statistical software (Rong et al. 2020; Navgire et al. 2022; Jensen et al. 2024).

RESULTS AND DISCUSSION

Soil condition

The chemical properties of the soil require careful consideration to ensure the successful cultivation of *Pulu Mandoti* rice. According to the composition analysis, soil pH is classified as slightly acidic (5.5-6.5), with a low carbon-to-nitrogen (C/N) ratio ranging from 5% to 10%. Additionally, the Cation Exchange Capacity (CEC) is low, measuring less than 30 cmol/kg (Table 1).

Metagenomic DNA isolation and shotgun sequencing

Shotgun metagenomic DNA sequencing of *Pulu Mandoti* rice soil rhizosphere was performed**.** The resulting sequences were mapped and classified at the phylum, class, order, family, and genus levels (Figure 2). The most abundant bacterial phyla were Proteobacteria (42%), Acidobacteria (11%), and Actinobacteria (11%). At the class level, the most abundant groups were Alphaproteobacteria (15%), Deltaproteobacteria (14%), Actinobacteria (9%), and Betaproteobacteria (8%) (Figure 3). Bacteria were the dominant organisms, but other groups were also detected, including archaea (0.3%), eukaryotes (0.02%), viruses (0.08%), and unclassified organisms (0.3%). Bacterial diversity at the family level is represented by four predominant families, namely Anaeromyxobacteraceae (8%), Bradyrhizobiaceae (4%), Isosphaeraceae (2%), and Acidobacteriaceae (2%) (Figure 4). Anaeromyxobacteraceae and Bradyrhizobiaceae with 11 genera have a higher number of genera than other bacterial families. The percentage of unassigned genera within Anaeromyxobacteraceae, Bradyrhizobiaceae, Isosphaeraceae, and Acidobacteriaceae is 4%, 18%, 1%, and 12%, respectively.

Table 1. Soil rhizosphere compound analysis of *Pulu Mandoti* rice

Analysis	Element	Quantity	Categorization (Sulaeman et al. 2009)
Organic matter	C (%)	0.72	Very low
	N (%)	0.08	Very low
	C/N	9	Low
	P_2O_5 (ppm)	7.08	Low
Cation exchange capacity	Ca (cmol/kg)	3.83	Low
	Mg (cmol/kg)	1.08	Moderate
	K (cmol/kg)	0.27	Low
Other compounds	Fe (ppm)	5.36	Sufficient
	Mo (ppm)	1.74	High
	Mn (ppm)	26.35	High
	Zn (ppm)	1.05	Sufficient
	Cu (ppm)	1.12	Sufficient
	SO_4 (ppm)	42.15	Low
	Cl (ppm)	14.63	Very low
pH	5.53		Slightly acidic

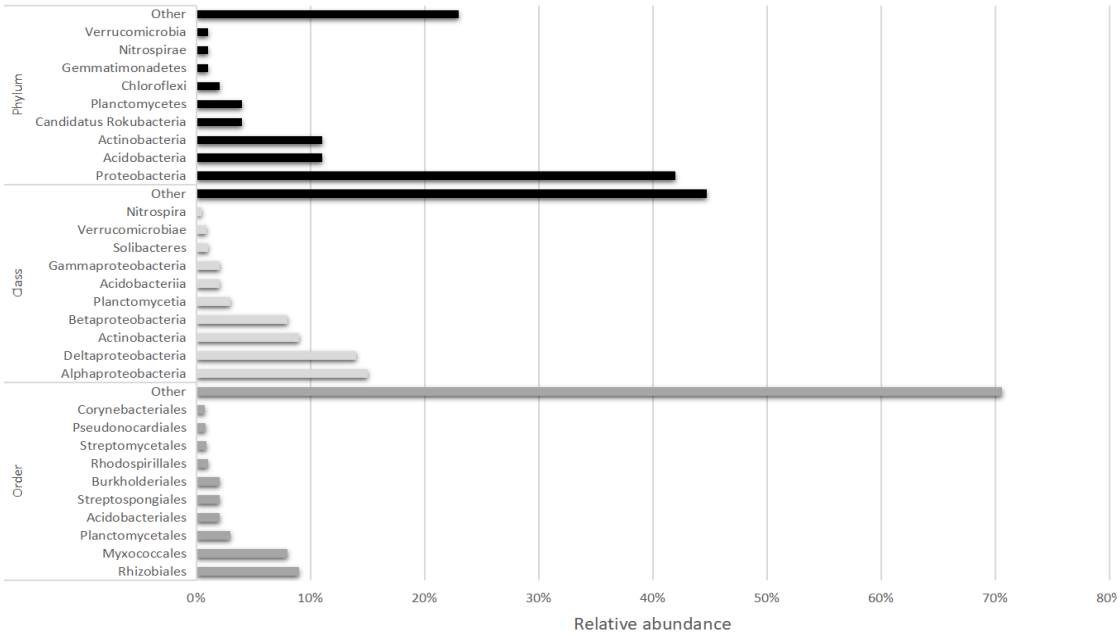

Figure 2. Relative abundance of bacteria phyla, class, and order from *Pulu Mandoti* rice rhizosphere

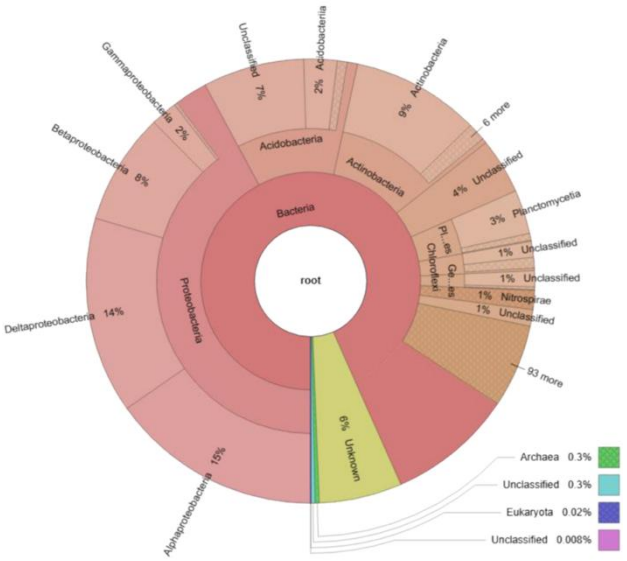

Figure 3. Krona charts showing the phylum-level and class-level diversity of microbial populations in *Pulu Mandoti* rice soil rhizosphere

Among the bacterial genera identified, *Bradyrhizobium* shows the highest richness, comprising 97 species. Meanwhile, *Bradyrhizobium erythrophlei* is the most abundant species within the genus, accounting for 17% of the total population (Figure 5). *Anaeromyxobacter* consists of three predominant species, with *Anaeromyxobacter* sp. showing the highest abundance at 29%, In contrast, the genera *Singulisphaera* and *Rhodoplanes* contain two species each, with *Singulisphaera* sp. and *Rhodoplanes* sp. being the most abundant, representing 66% and 83%, respectively.

The microbial diversity of *Pulu Mandoti* rice rhizosphere soil, as revealed by shotgun metagenomic sequencing, exhibited high species richness and balanced microbial composition. The Shannon Diversity Index indicated a diverse community, dominated by bacterial phyla, such as Proteobacteria (42%), Acidobacteria (11%), and Actinobacteria (11%), reflecting the presence of multiple taxa contributing to the community structure. The Simpson diversity index showed a low dominance value, with several bacterial families contributing significantly to the population, particularly Anaeromyxobacteraceae (8%), Bradyrhizobiaceae (4%), Isosphaeraceae (2%), and Acidobacteriaceae (2%), suggesting that no single taxon dominated the entire community. The evenness index demonstrated a moderate level of species distribution, where abundant genera, such as *Bradyrhizobium* (97 species), *Anaeromyxobacter* (29%), *Singulisphaera* (66%), and *Rhodoplanes* (83%) were consistently represented across samples. Despite the predominance of certain genera, the relatively balanced distribution across microbial taxa highlights the importance of microbial interactions in the rhizosphere. The diverse and evenly distributed bacterial community may contribute to key ecological functions, such as nutrient cycling, nitrogen fixation, and stress tolerance, which are essential for the adaptation and growth of Pulu Mandoti rice in its native environment.

Discussion

According to Amrullah et al. (2020) and Sukmawaty et al. (2022), altitude and climate are critical factors shaping microbial diversity, with higher elevations often fostering distinct microbial communities adapted to cooler temperatures and lower atmospheric pressure. In this context, beneficial microbial taxa, such as *Pseudomonas* and *Bacillus*, which are known for their roles in nitrogen fixation, phosphate solubilization, and disease suppression are likely to dominate

the rhizosphere, enhancing nutrient availability and stress tolerance in *Pulu Mandoti* rice (Mendes et al. 2013). These microbial interactions not only support the rice's growth in mountainous terrain, but also contribute to its unique fragrant and glutinous qualities, which are highly valued by local communities (Nurjannah et al. 2024). By elucidating the relationship between environmental factors, microbial community structure, and rice quality, this research underscores the importance of preserving these specific agroecological conditions to sustain the ecological and economic value of *Pulu Mandoti*, while also providing insights into optimizing traditional agricultural practices in the face of climate change and global agricultural homogenization.

The chemical properties of the soil in Salukanan and Kendenan villages play a critical role in the successful cultivation of *Pulu Mandoti* rice. Soil pH is acidic, which significantly influences nutrient availability and microbial activity. Acidic soil conditions (pH<6.5) can limit the solubility of essential nutrients, such as P_2O_5, Ca, and Mg, while increasing the availability of Al and Mn, which may be toxic to plants at high concentrations (Deng et al. 2020). This acidic environment also shapes the rhizosphere microbial community, favoring acid-tolerant bacterial taxa, such as *Acidobacteria* and *Proteobacteria*, which play vital roles in nutrient cycling and organic matter decomposition (van der Sloot et al. 2022).

Additionally, the soil reports a low carbon-to-nitrogen (C/N) ratio, ranging from 5% to 10%, indicating limited organic matter decomposition and slower nutrient cycling. A low C/N ratio often results in nitrogen immobilization, where microorganisms compete with plants for available nitrogen, thereby constraining its availability for rice growth and yield (Geng et al. 2024). This condition may also influence the dominance of microbial groups specialized in breaking down complex organic compounds, such as *Actinobacteria* and *Firmicutes*, which are efficient in decomposing recalcitrant organic matter under nutrient-limited conditions.

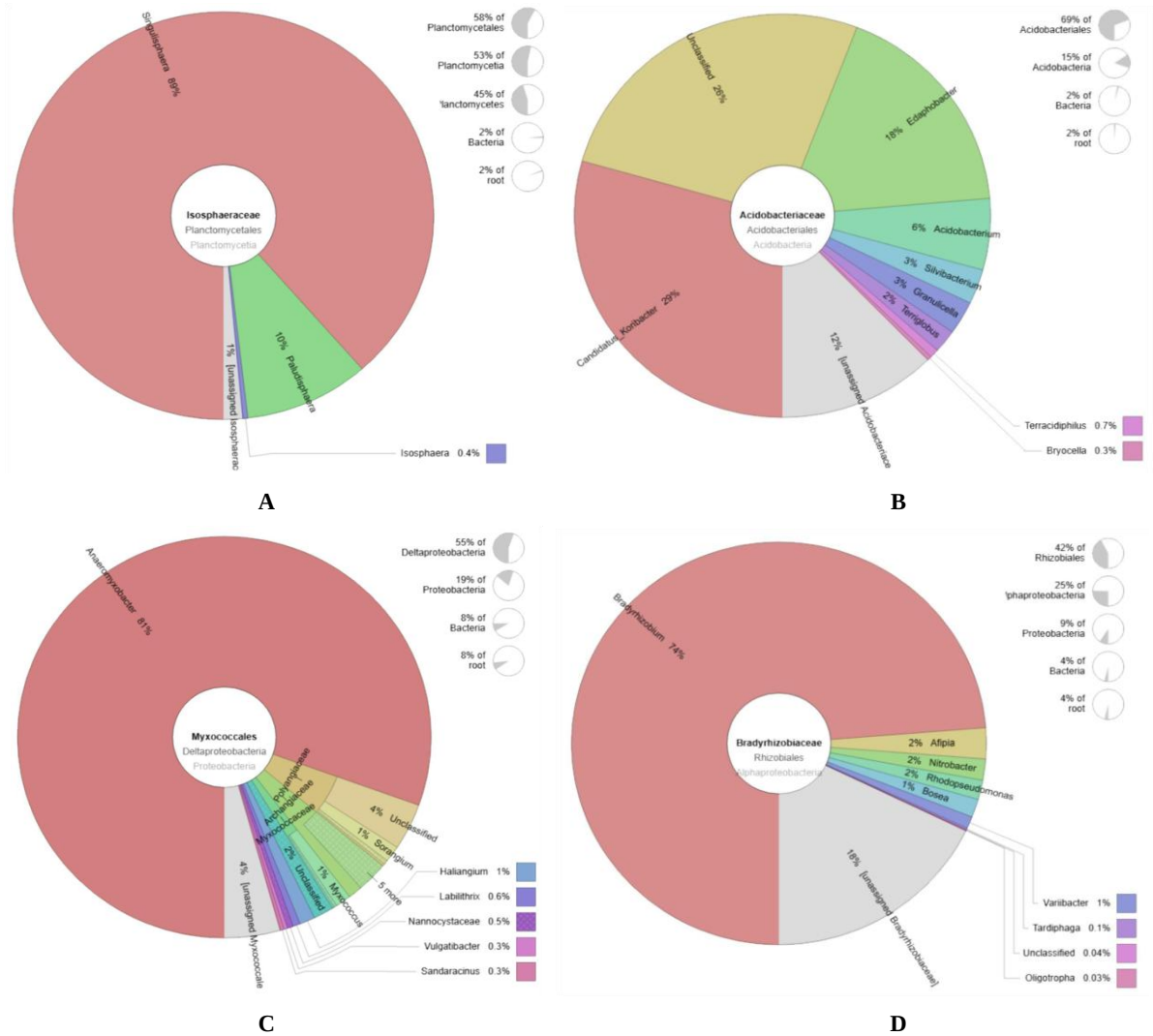

Figure 4. Krona charts showing the four most abundant family-level bacterial diversity from *Pulu Mandoti* rice soil rhizosphere: A. Anaeromyxobacteraceae, B. Bradyrhizobiaceae, C. Isosphaeraceae, D. Acidobacteriaceae

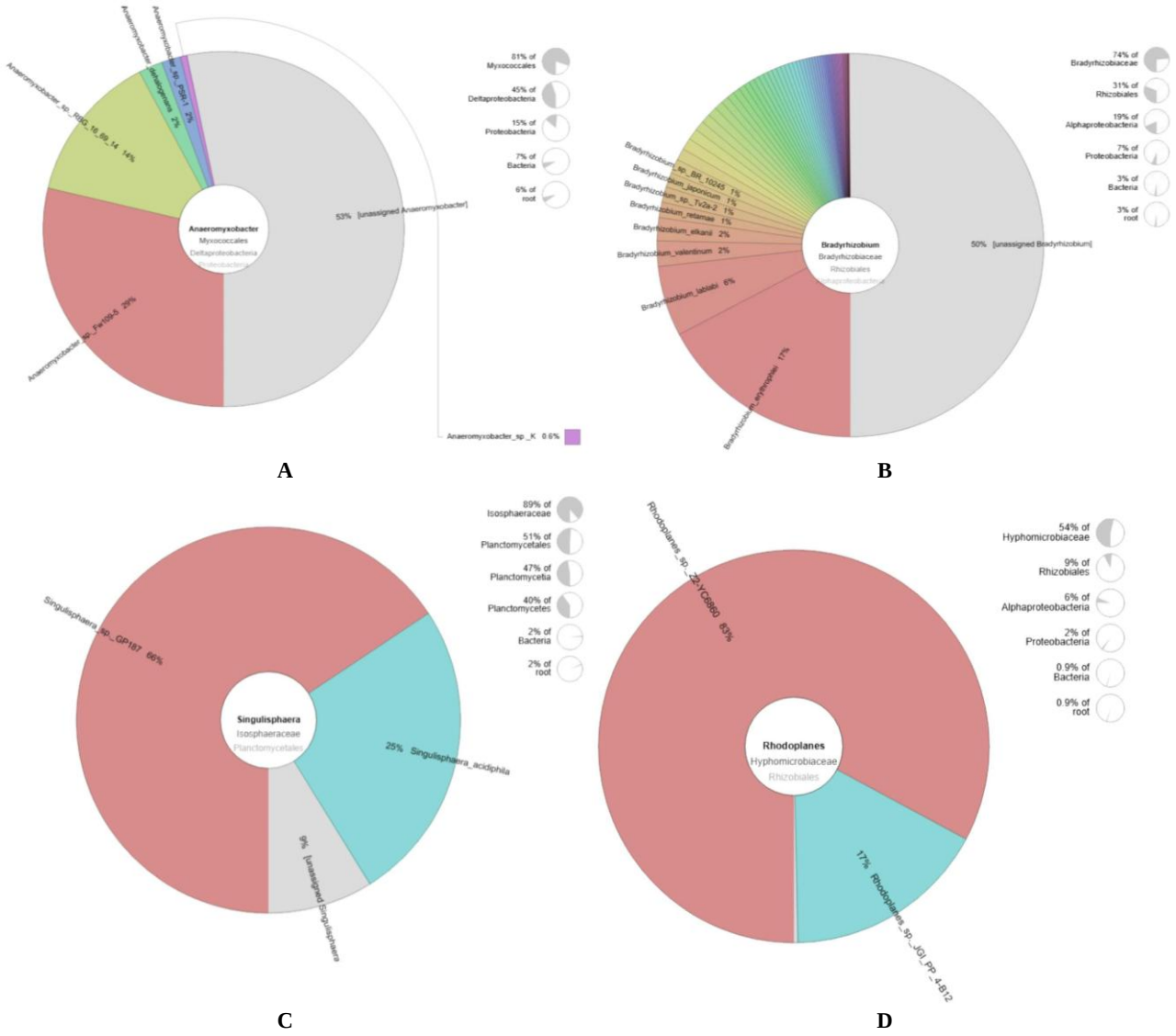

Figure 5. Krona charts showing the four most abundant genus-level bacterial diversity from *Pulu Mandoti* rice soil rhizosphere: A. *Anaeromyxobacter*, B. *Bradyrhizobium*, C. *Singulisphaera*, D. *Rhodoplanes*

The soil's low Cation Exchange Capacity (CEC), measuring less than 30 cmol/kg, suggests a reduced ability to retain and supply essential cations like Ca, Mg, and K, further exacerbating nutrient limitations. These soil characteristics highlight the challenges faced by farmers in maintaining soil fertility and productivity. Addressing these limitations through targeted soil management practices, such as organic amendments to improve C/N ratios or liming to adjust pH, can enhance microbial diversity, nutrient availability, and overall soil health, thereby supporting the sustainability of *Pulu Mandoti* cultivation (Deng et al. 2020; Tong et al. 2023). The integration of microbial ecology with agronomic practices offers significant potential for optimizing soil health, nutrient availability, and crop productivity in high-altitude rice cultivation systems such as *Pulu Mandoti*. By leveraging insights from shotgun metagenomic DNA sequencing, specific microbial consortia with beneficial traits such as nitrogen fixation, phosphate solubilization, and production of plant growth-promoting hormones can be identified and developed into biofertilizers. These microbial consortia-based biofertilizers could be tailored to the unique agroecological conditions of Salukanan and Kendenan villages, enhancing nutrient cycling and stress tolerance while reducing reliance on chemical inputs. This approach not only aligns with sustainable agricultural practices, but also provides a practical solution for improving the productivity and resilience of endemic rice varieties like *Pulu Mandoti*, particularly in the face of climate change and soil fertility challenges (van der Sloot et al. 2022; Tong et al. 2023; Geng et al. 2024).

Shotgun metagenomic DNA sequencing allows for the identification of both culturable and unculturable microorganisms, offering insights into the taxonomic diversity and functional genes involved in nutrient cycling, stress tolerance, and plant growth promotion. By analyzing the entire genetic material present in the rhizosphere,

shotgun metagenomics reveals the metabolic pathways and interactions between microbes and *Pulu Mandoti* rice, such as nitrogen fixation, phosphate solubilization, and production of secondary metabolites. The sequencing data were processed using high-throughput bioinformatics pipelines, allowing for precise taxonomic classification at multiple hierarchical levels, including phylum, class, order, family, and genus (Figure 2). The analysis showed that bacteria were the dominant microorganisms, with Proteobacteria (42%), Acidobacteria (11%), and Actinobacteria (11%) reported as the most abundant phyla. The dominance of Proteobacteria, particularly members of Alphaproteobacteria (15%) and Betaproteobacteria (8%) suggests a microbial community highly adapted to nutrient cycling, symbiotic interactions, and plant growth promotion. The presence of Deltaproteobacteria (14%) shows a potential role in anaerobic metabolism and sulfur cycling to maintain soil biogeochemical stability (Enebe and Babalola 2021; Toole et al. 2021). Additionally, Actinobacteria (9%), known for the ability to produce secondary metabolites and degrade complex organic matter report the functional diversity within the soil microbiome. The coexistence of microbial groups also suggests a highly interactive and dynamic microbial network, influenced by soil acidity, nutrient availability, and plant root exudates. Since *Pulu Mandoti* rice is cultivated in acidic soils with low CEC, the microbial consortia play critical roles in soil fertility enhancement, organic matter decomposition, and stress tolerance to support plant health in high-altitude rice agroecosystem (Abraham et al. 2020; Omotayo et al. 2022).

Shotgun sequencing identified other microbial domains, including archaea (0.3%), eukaryotes (0.02%), viruses (0.08%), and unclassified organisms (0.3%). These microbial groups may contribute significantly to soil ecosystem functions, such as methanogenesis, organic matter decomposition, and plant-microbe interactions. The detection of archaea suggests the presence of methanogenic and ammonia-oxidizing communities with the capacity to influence nitrogen cycling and greenhouse gas emissions (Maeda et al. 2020; Wahid et al. 2020; Wani et al. 2024). The presence of eukaryotic microorganisms, including fungi and protists contributes to nutrient turnover, organic matter degradation, and potential symbiotic relationships with plant roots. Meanwhile, the detection of viral sequences shows the potential role of bacteriophages in microbial community dynamics, including the regulation of bacterial populations and horizontal gene transfer. The relatively high percentage of unclassified organisms suggests that *Pulu Mandoti* rhizosphere harbors novel microbial lineages, emphasizing the need for functional metagenomic and genome-resolved analyses. Since microbial diversity and community composition are strongly influenced by environmental factors such as soil pH, temperature, and organic matter content, future research should explore the functional traits of microbial communities, particularly the roles in nutrient mobilization, plant defense mechanisms, and adaptation to acidic soil conditions (Jagadesh et al. 2024; Wani et al. 2024). These insights are critical for developing microbiome-based strategies to enhance soil health and sustain *Pulu Mandoti* rice cultivation in the native highland environment.

The bacterial diversity at the family level within *Pulu Mandoti* rice rhizosphere reported four predominant families, namely Anaeromyxobacteraceae (8%), Bradyrhizobiaceae (4%), Isosphaeraceae (2%), and Acidobacteriaceae (2%) (Figure 4). These microbial families play important ecological roles, particularly in nutrient cycling, biogeochemical transformations, and plant-microbe interactions essential for maintaining soil fertility in high-altitude agricultural systems. Anaeromyxobacteraceae and Bradyrhizobiaceae are the most abundant families, collectively including 11 bacterial genera. The higher abundance of Anaeromyxobacteraceae suggests a potential inclusion in anaerobic metabolism and bioremediation, particularly in redox-driven nutrient transformations to enhance detoxification and metal bioavailability. In contrast, members of Bradyrhizobiaceae are well known for their Symbiotic Nitrogen Fixation (SNF) capabilities for sustaining plant growth in nitrogen-limited environments (Mantri et al. 2021; Kifle et al. 2024). The presence suggests a potential mutualistic relationship with *Pulu Mandoti* rice, contributing to natural soil enrichment by reducing the dependency on synthetic fertilizers. Additionally, the presence of Isosphaeraceae and Acidobacteriaceae, despite the lower relative abundances, shows the role in carbon cycling and acid tolerance mechanisms. This is because the families are often found in oligotrophic, low-pH environments and contribute to organic matter degradation and soil stabilization. The proportion of unassigned genera within Anaeromyxobacteraceae (4%), Bradyrhizobiaceae (18%), Isosphaeraceae (1%), and Acidobacteriaceae (12%) suggests that novel or under-characterized taxa may be present, warranting functional and taxonomic characterization using metagenomic and whole-genome sequencing to uncover ecological significance (Priya et al. 2021; Wang et al. 2021; Jagadesh et al. 2024).

At the genus level, *Bradyrhizobium* reported the highest richness, including 97 species, reinforcing the critical role in nitrogen cycling and rhizosphere colonization. The high species diversity within the genus shows the adaptive potential in fluctuating environmental conditions in low-nitrogen and acidic soils. *B. erythrophlei* was reported as the most dominant, constituting 17% of the total population, suggesting the significant inclusion in Biological Nitrogen Fixation (BNF), phosphate solubilization, and plant growth promotion (Wekesa et al. 2022; Lafay et al. 2024). This dominance is particularly relevant for *Pulu Mandoti* rice ecosystem in enhancing soil fertility and improving plant resilience against abiotic stressors. Similarly, the genus *Anaeromyxobacter*, known for anaerobic respiration and metal-reducing capabilities, was represented by three predominant species, with *Anaeromyxobacter* sp. being the most abundant at 29%. This result was consistent with previous research where Anaeromyxobacteraceae played an essential role in Fe and Mn cycling to impact soil redox potential and nutrient availability. The characteristics are particularly beneficial in paddy soils, where periodic flooding and oxygen-limiting conditions can alter microbial community dynamics. The high abundance of

Anaeromyxobacter suggests that redox-sensitive metabolic pathways are key factors in structuring microbial interactions in the rhizosphere (Wang et al. 2020; Li et al. 2024; Sarkodie et al. 2024). Furthermore, genomic and transcriptomic analyses of the dominant species show adaptive mechanisms, metabolic pathways, and contributions to soil health, providing valuable insights for biofertilization and bioremediation applications.

Even though not a dominant genus, *Singulisphaera* sp. and *Rhodoplanes* sp. have quite high abundance of 66% and 83%, respectively. *Singulisphaera*, a member of Planctomycetes, is typically associated with acidophilic and oligotrophic environments. These genera plays a role in polysaccharide degradation, biofilm formation, and secondary metabolite production to influence soil microbial interactions and organic matter turnover. The genus *Rhodoplanes* belonging to Alphaproteobacteria is known for photoheterotrophic metabolism, nitrogen fixation, and hydrogen oxidation. The predominance of *Rhodoplanes* sp. (83%) suggests the potential inclusion in organic matter decomposition and symbiotic interactions with rice roots. The results collectively emphasize that the microbial community in *Pulu Mandoti* rice rhizosphere is highly structured and functionally diverse, with taxa contributing to nutrient mobilization, organic matter decomposition, and plant health promotion (Kosunen et al. 2020; Yang et al. 2021; Ignateva et al. 2024). The presence of novel taxa within the microbial groups shows the need for investigations using metagenomic functional profiling, Stable Isotope Probing (SIP), and genome-resolved metagenomic to elucidate precise biogeochemical roles and potential applications in sustainable agriculture. Additionally, the integration of microbial ecology with agronomic practices facilitates the development of microbial consortia-based biofertilizers, optimizing soil health and crop productivity in high-altitude rice cultivation systems.

In conclusion, the rhizobacteria profile within *Pulu Mandoti* rice rhizosphere was highly diverse, with Proteobacteria (42%), Acidobacteria (11%), and Actinobacteria (11%) as the dominant phyla. At the class level, Alphaproteobacteria (15%), Deltaproteobacteria (14%), and Actinobacteria (9%) showed the highest relative abundances. Meanwhile, archaea, eukaryotes, viruses, and unclassified organisms were detected in lower proportions. Among the four predominant bacterial families, Anaeromyxobacteraceae (8%) and Bradyrhizobiaceae (4%) were the most abundant, including 11 genera. *Bradyrhizobium* reported the highest richness of 97 species, with *B. erythrophlei* as the dominant (17%). The genus *Anaeromyxobacter* comprised three predominant species, with *Anaeromyxobacter* sp. (29%) being the most abundant. Further characterization of microbial groups was essential to explore the potential bioactivity and functional applications in sustainable agriculture.

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