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Isolation and Antimicrobial Activity of Endophytic Bacteria from *Rhizophora mucronata* Roots in the Kupang Mangrove Ecotourism Area

Isolasi dan Uji Aktivitas Antimikrobia Bakteri Endofit Akar Mangrove Rhizophora mucronata di Kawasan Ekowisata Mangrove Kupang

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Abstract. Semi-arid mangrove ecosystems harbor significant potential as sources of endophytic bacteria producing adaptive antimicrobial compounds. This study aimed to isolate and evaluate the antimicrobial activity of endophytic bacteria from *Rhizophora mucronata* prop and lateral roots across three management zones (core, buffer, and utilization) in the Kupang Mangrove Ecotourism Area. Sampling was conducted in March–April 2026, followed by root surface sterilization and isolation on Nutrient Agar. Phenotypic characterization was performed macroscopically, microscopically, and biochemically, while antimicrobial activity was assessed using the Kirby-Bauer disk diffusion method against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. From 69 initial colonies, 18 pure isolates were obtained, with *Bacillus* sp. as the dominant genus (44.4%). Notably, *Bacillus* sp. isolates ZS-04 and ZI-05 exhibited the most potent bioactivity, yielding very strong (>20 mm) inhibition zones against *S. aureus* and strong (10–20 mm) inhibition against *E. coli*. One-way ANOVA revealed no significant zonal differences for *S. aureus* ($p = 0.785$), whereas a statistically significant zonal effect was observed for *E. coli* ($p = 0.045$), particularly between the utilization and buffer zones. These findings confirm that endophytic *Bacillus* sp. from *R. mucronata* roots, particularly isolates ZS-04 and ZI-05, produce bioactive compounds with strong, targeted antibacterial activity against Gram-positive pathogens, highlighting semi-arid mangrove endophytes as promising, stress-adapted reservoirs for novel natural antimicrobial agents.

Keywords*:* antimicrobial_activity; endophytic_bacteria; mangrove_zonation; *rhizophora_mucronata*

Abstrak. *Ekosistem mangrove semi-arid menyimpan potensi besar sebagai sumber bakteri endofit penghasil senyawa antimikrobia yang adaptif. Penelitian ini bertujuan mengisolasi dan mengevaluasi aktivitas antimikrobia bakteri endofit dari akar tunjang dan lateral Rhizophora mucronata pada tiga zona pengelolaan (inti, penyangga, dan pemanfaatan) di Kawasan Ekowisata Mangrove Kupang. Pengambilan sampel dilakukan pada Maret–April 2026, diikuti sterilisasi permukaan akar dan isolasi pada media Nutrient Agar. Karakterisasi fenotipik dilakukan secara makroskopik, mikroskopik, dan biokimia, sedangkan uji aktivitas antimikrobia menggunakan metode difusi cakram Kirby-Bauer terhadap Staphylococcus aureus ATCC 25923 dan Escherichia coli ATCC 25922. Dari 69 koloni awal, diperoleh 18 isolat murni dengan Bacillus sp. sebagai genus dominan (44,4%). Secara khusus, isolat Bacillus sp. ZS-04 dan ZI-05 menunjukkan bioaktivitas paling poten, menghasilkan zona hambat yang sangat kuat (>20 mm) terhadap S. aureus dan kuat (10–20 mm) terhadap E. coli. Analisis ANOVA menunjukkan tidak ada perbedaan zonasi yang signifikan terhadap S. aureus (p = 0,785), namun terdapat pengaruh zonasi yang signifikan terhadap E. coli (p = 0,045), khususnya antara zona pemanfaatan dan penyangga. Hasil ini mengonfirmasi bahwa endofit Bacillus sp. dari akar R. mucronata, khususnya isolat ZS-04 dan ZI-05, menghasilkan senyawa bioaktif dengan aktivitas antibakteri yang kuat dan terarget terhadap patogen Gram-positif, yang menyoroti endofit mangrove*

semi-arid sebagai reservoir yang adaptif terhadap stres dan menjanjikan untuk pengembangan agen antimikrobia alami yang baru

***Kata Kunci**: aktivitas_antimikrobia; bakteri_endofit; rhizophora_mucronata; zona_mangrove*

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1. Introduction

Mangrove ecosystems are highly productive coastal habitats that play a crucial role in shoreline protection and blue carbon sequestration (1). As naturally functioning ecosystems, mangrove forests harbor high biodiversity and deliver essential ecological services that sustain broader coastal and terrestrial forest ecosystems (2). Indonesia encompasses approximately 3,455,628 hectares of mangrove forests distributed along its extensive archipelagic coastline, including East Nusa Tenggara Province (NTT) (3). This region, characterized by semi-arid climatic conditions and extreme salinity fluctuations, supports an estimated 23,016 hectares of mangrove ecosystems. In this region, *Rhizophora mucronata* (locally known as *bakau* or red mangrove) is the dominant species within the family Rhizophoraceae, distinguished by its characteristic prop root system. These roots interact directly with anoxic sediments and brackish waters exhibiting fluctuating salinity, creating an extreme microenvironment that drives unique adaptations in the symbiotic microorganisms associated with them (4).

R. mucronata has long been utilized by coastal communities in Southeast Asia as traditional medicine for treating skin infections, wounds, and fever. Modern phytochemical studies have confirmed that extracts of *R. mucronata* bark, leaves, and roots contain bioactive secondary metabolites, including tannins, flavonoids, saponins, and terpenoids, that exhibit significant antimicrobial activity against both Gram-positive and Gram-negative pathogens (5,6). Given that endophytic bacteria are known to synthesize bioactive compounds structurally similar to or identical with those produced by their host plants, the documented antimicrobial properties of *R. mucronata* provide a strong rationale for bioprospecting its root-associated endophytes as alternative sources of novel antibacterial agents.

Endophytic bacteria that colonize plant tissues in a mutualistic association have been demonstrated to produce secondary metabolites with high biological activity in response to environmental stressors (7). Extreme environmental pressures, including sediment anoxia, elevated salinity, and pronounced diurnal temperature fluctuations in semi-arid mangrove habitats, are hypothesized to selectively drive the biosynthesis of unique bioactive compounds rarely identified in wet tropical mangrove ecosystems (8). Amid the global antimicrobial resistance (AMR) crisis, which threatens the clinical efficacy of conventional antibiotics, the bioprospecting of bioactive compounds from mangrove endophytes has emerged as a highly promising alternative strategy (9). This imperative is particularly pressing in Indonesia, where escalating cross-sectoral AMR challenges underscore the critical need to discover novel antibiotics from local biodiversity.

The Kupang Mangrove Ecotourism Area is structured into three functional management zones (core, buffer, and utilization), which collectively represent a gradient of anthropogenic disturbance and distinct ecological parameters. The core zone receives the highest level of protection with virtually no human access, the buffer zone functions as an ecological transition area with restricted entry, and the utilization zone experiences the highest tourist visitation and the most intensive human disturbance. These contrasting environmental conditions are hypothesized to shape endophytic microbial communities with distinct diversity profiles and antimicrobial potential.

While recent studies have documented the antimicrobial activity of endophytic bacteria isolated from various

mangrove species (5,6), the root-associated endophytic communities of *R. mucronata* across distinct management zones in semi-arid eastern Indonesia remain unexplored. Roots were specifically selected as the target organ in this study because they interact directly with the most extreme microenvironment within the mangrove ecosystem—namely, anoxic and highly saline sediments. Root-associated plant growth-promoting bacteria (PGPB) have evolved under these harsh conditions to produce a diverse array of beneficial compounds, including exopolysaccharides and secondary metabolites, which not only aid in host stress tolerance but also enhance resistance against soil-borne pathogens (10). Because the root system serves as the primary interface and first line of defense against environmental pathogens, endophytes colonizing the roots are theoretically and empirically expected to exhibit higher antimicrobial activity compared to those isolated from above-ground organs such as leaves or stems. Furthermore, endophytic bacteria are known to synthesize secondary metabolites that are identical or comparable to the bioactive compounds produced by their host plants (11). Given the documented antimicrobial properties of *R. mucronata*, its root-associated endophytes represent a highly rational and promising target for antibacterial bioprospecting. This knowledge gap underpins the hypothesis that ecological zoning shapes both the community composition and the antimicrobial potential of *R. mucronata* root endophytes. Accordingly, this study aims to isolate, identify, and comparatively evaluate the antimicrobial potential of root-associated endophytic bacteria across the three functional zones. Preliminary findings demonstrate that these root endophytes, predominantly *Bacillus* sp., possess notable antibacterial bioactivity. Specifically, isolates ZS-04 and ZI-05 exhibited strong inhibitory activity against *Staphylococcus aureus* (inhibition zones >20 mm), while activity against *Escherichia coli* showed significant zonal variation, highlighting the influence of ecological gradients on metabolite production.

2. Methods

This laboratory-based experimental study employed an exploratory comparative design to assess how ecological zoning influences endophytic diversity and antimicrobial potential.

Sample Collection and Environmental Profiling

The research was conducted from March to April 2026. Healthy *R. mucronata* roots, specifically a combination of prop (support) roots and their associated fine lateral roots, were collected from a depth of 0–20 cm below the sediment surface from three individual trees per zone (core, buffer, utilization) in the Kupang Mangrove Ecotourism Area (Figure 1). Prop roots were targeted as they represent the primary interface with the anoxic, saline sediment environment, while the inclusion of fine lateral roots ensured the capture of the most active zones of microbial colonization and nutrient exchange. *In situ* measurements of air/water temperature, salinity, and water/soil pH were recorded concurrently. Samples were transported at 4–8°C to the UNWIRA Laboratory for processing.

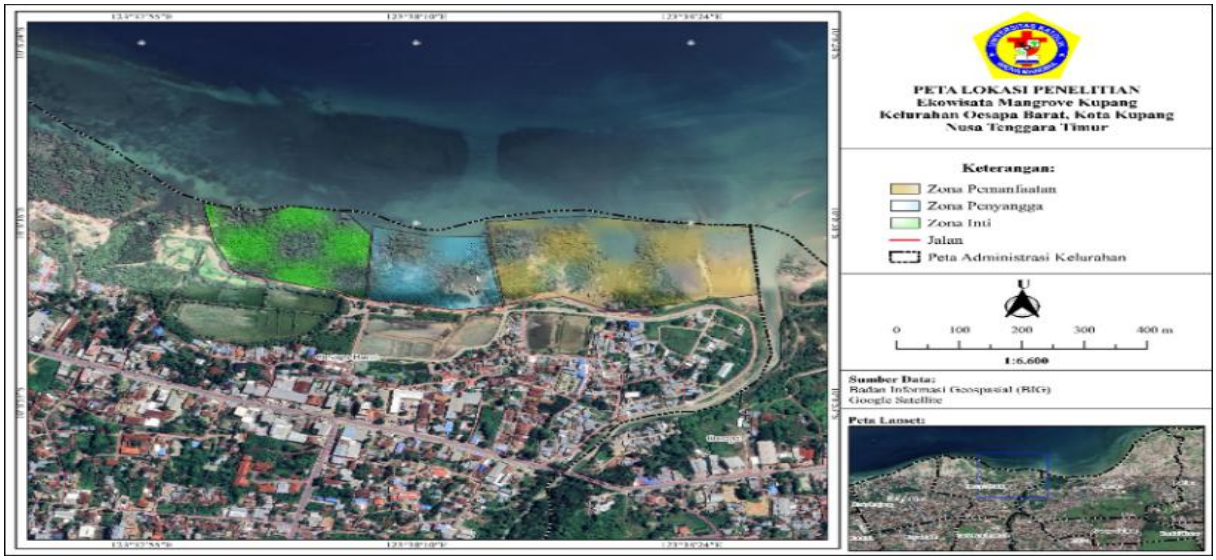

Figure 1. Research location map of the Kupang Mangrove Ecotourism Area. The green, blue, and yellow shaded areas represent the core, buffer, and utilization zones, respectively. Sampling points are marked with symbols indicating the zone: ZI (Core), ZP (Utilization), and ZS (Buffer)

Isolation & Characterization

Surface sterilization followed the protocol described by Pakaya et al. (2024) (12) with specific modifications tailored for mangrove root tissues. While the original protocol utilized 1% NaOCl for 5 minutes on sponge tissues, this study adjusted the concentration and exposure time to 3% NaOCl for 3 minutes to effectively penetrate the thicker, tannin-rich cuticle of *R. mucronata* roots. Furthermore, the rinsing step was increased to three cycles with sterile distilled water to ensure the complete removal of residual sterilizing agents. Finally, instead of Marine Agar supplemented with ketoconazole, sterilized root fragments were plated on standard Nutrient Agar (NA) and incubated at 28°C for 48 h, optimizing conditions for mesophilic endophytic bacteria. To verify sterilization efficacy, 100 µL of the final rinse water was plated onto NA and incubated; the absence of microbial growth confirmed successful surface sterilization. Macroscopic traits (colony morphology, diameter) and microscopic/biochemical profiles (Gram stain, catalase, oxidase, starch hydrolysis) were recorded following Bergey's Manual (13)

Antimicrobial Activity Assay

Antimicrobial activity was evaluated using the Kirby-Bauer disk diffusion method against two reference pathogen strains: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The diameter of the zone of inhibition was measured using a vernier caliper (accuracy: ±0.01 mm) and categorized according to the Davis-Stout criteria: very strong (>20 mm), strong (10–20 mm), moderate (5–10 mm), weak (<5 mm), and no activity (0 mm).

Data Analysis

Data are reported as mean ± SD (n=3). Zonal differences were analyzed via one-way ANOVA (α = 0.05) with Tukey's HSD post hoc test using IBM SPSS v25.

3. Result and Discussion

In situ measurements of environmental parameters were conducted concurrently with root sample collection at all sampling points across the three functional management zones. The parameters assessed included air temperature, water temperature, water pH, soil pH, and salinity. The recorded environmental conditions for each zone are presented in Table 1. Results indicate a clear ecological gradient across the core, buffer, and utilization zones, as reflected by

variation in temperature, pH, and salinity values at each sampling location. These physicochemical differences are hypothesized to play a significant role in shaping the diversity of endophytic microbial communities associated with *R. mucronata* roots across the respective zones.

Table 1. Environmental conditions were recorded during root sample collection across the three functional management zones

Zone	Sampling Point	Air Temp (°C)	Water Temp (°C)	Water (pH)	Soil (pH)	Salinity (‰)	Weather
Core	ZI-T1	31.4	29.7	7.2	6.8	28.2	Clear
Core	ZI-T2	31.7	30.1	7.1	6.7	28.6	Clear
Core	ZI-T3	32.1	30.4	7.3	6.9	29.1	Clear
Buffer	ZP-T1	32.8	30.8	7.0	6.6	26.4	Clear
Buffer	ZP-T2	33.2	31.2	7.1	6.5	25.9	Clear
Buffer	ZP-T3	33.5	31.6	7.0	6.4	25.3	Clear
Utilization	ZS-T1	34.1	32.0	6.9	6.2	23.7	Clear
Utilization	ZS-T2	34.4	32.3	6.8	6.1	23.1	Clear
Utilization	ZS-T3	34.7	32.7	6.7	6.0	22.8	Clear

Legend: temperature and salinity values exhibit a gradient between the core zone (higher humidity, elevated salinity) and the utilization zone (higher temperature, reduced salinity attributable to surface freshwater influence). Data represent mean values from single measurements per sampling point; weather conditions were recorded visually at the time of collection.

Environmental Conditions

Measurements of environmental parameters across the three functional zones revealed a distinct ecological gradient (Table 1). Air and water temperatures gradually increased from the core zone (31.4–32.1°C and 29.7–30.4°C) to the utilization zone (34.1–34.7°C and 32.0–32.7°C), while both water and soil pH showed a slight decline. In contrast, salinity decreased progressively from the core zone, which exhibited relatively high values (28.2–29.1‰), to the buffer zone (25.3–26.4‰), reaching its lowest values in the utilization zone (22.8–23.7‰). These physicochemical differences are primarily driven by the core zone's direct marine influence and the utilization zone's exposure to higher solar radiation, tourist activity, and potential surface freshwater runoff.

This environmental gradient directly correlates with both the diversity of the isolated endophytes and their antimicrobial potential, validating the zoning approach proposed in this study. The core zone, characterized by the highest salinity and the most stable conditions, yielded the highest number of isolates (7 isolates), suggesting that the undisturbed, high-salinity environment supports a richer endophytic community. Conversely, the utilization zone, subjected to the highest temperatures, lowest salinity, and lowest pH, produced the fewest isolates (5 isolates), likely reflecting the combined negative impacts of anthropogenic disturbance and environmental stress on microbial colonization. Interestingly, while the core zone had the highest isolate yield, the buffer zone—which represents a transitional environment with moderate salinity and intermediate disturbance—harbored the most potent antimicrobial producers, specifically isolate ZS-04 with an inhibition zone of 22.15 mm against *S. aureus*. This pattern aligns with the ecological theory that moderate environmental stress can selectively drive the biosynthesis of potent secondary metabolites as a competitive strategy. Thus, the distinct physicochemical profiles of each management zone not only shape the quantitative diversity of the root-associated microbiome but also significantly influence the qualitative bioactivity of the endophytic bacteria.

Isolation and Phenotypic Characterization

Following the environmental profiling, a total of 69 bacterial colonies were initially observed from the root samples of nine *R. mucronata* across the

three management zones. After purification on Nutrient Agar (NA), 18 morphologically distinct pure isolates were successfully obtained: 7 from the core zone, 6 from the buffer zone, and 5 from the utilization zone in Figure 2 and Figure 3. The macroscopic, microscopic, and biochemical characteristics of these 18 purified isolates are summarized in Table 2 and Table 3.

Figure 2. Initial isolation of endophytic bacteria from *R. mucronata* root samples on Nutrient Agar (NA) plates

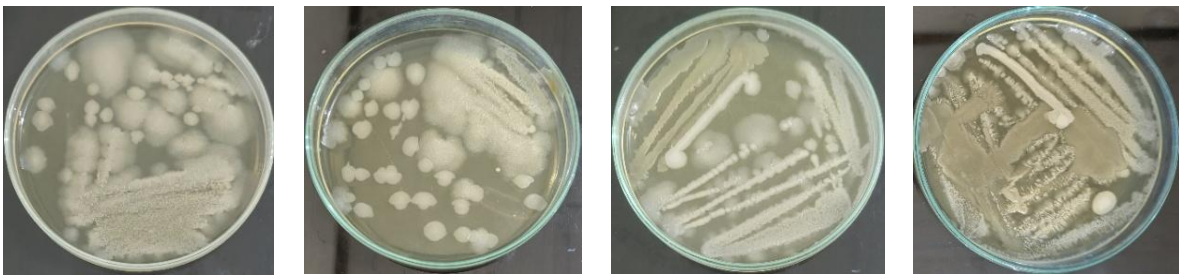

Figure 3. Morphologically distinct pure cultures obtained following quadrant-streak purification of endophytic bacteria isolated from *R. mucronata* roots

Table 2. Macroscopic colony morphology of the 18 purified endophytic bacterial isolates from *R. mucronata* roots.

Isolate Code	Zone	Colony Shape	Color	Elevation	Margin	Texture	Diameter (mm)
ZI-01	Core	Circular	Creamy white	Convex	Entire	Mucoid	3.2
ZI-02	Core	Irregular	White	Flat	Undulate	Dry	5.1
ZI-03	Core	Circular	Yellow	Convex	Entire	Shiny	2.8
ZI-04	Core	Circular	Cream	Flat	Entire	Dry	4.3
ZI-05	Core	Irregular	Creamy white	Umbonate	Lobate	Mucoid	6.2
ZI-06	Core	Circular	Orange	Convex	Entire	Shiny	2.1
ZI-07	Core	Circular	White	Flat	Entire	Dry	3.7
ZP-01	Utilization	Circular	Cream	Convex	Entire	Mucoid	3.5
ZP-02	Utilization	Irregular	White	Flat	Undulate	Dry	4.8
ZP-03	Utilization	Circular	Yellowish cream	Convex	Entire	Shiny	2.6
ZP-04	Utilization	Circular	White	Umbonate	Entire	Dry	3.9
ZP-05	Utilization	Irregular	Cream	Flat	Lobate	Mucoid	5.4
ZS-01	Buffer	Circular	Creamy white	Convex	Entire	Mucoid	4.1
ZS-02	Buffer	Circular	Yellow	Convex	Undulate	Shiny	2.9
ZS-03	Buffer	Irregular	White	Flat	Undulate	Dry	6.8
ZS-04	Buffer	Circular	Cream	Umbonate	Entire	Mucoid	3.6
ZS-05	Buffer	Circular	Orange	Convex	Entire	Shiny	2.4
ZS-06	Buffer	Irregular	White	Flat	Entire	Dry	5.5

Legend: isolate code prefixes: ZI = core zone, ZP = utilization zone, ZS = buffer zone. Colony diameters were measured using a digital vernier caliper (accuracy: ±0.01 mm). Morphological observations were recorded from pure cultures after 48 h of incubation at 28 °C on Nutrient Agar.

Table 3. Microscopic characteristics (Gram staining) and biochemical assay results for the 18 purified endophytic bacterial isolates from *R. mucronata* roots.

Isolate Code	Zone	Gram	Morphology	Arrangement	Catalase	Oxidase	Starch Hydrolysis	Putative Genus
ZI-01	Core	+	Rod	Single	+	−	+	*Bacillus* sp.
ZI-02	Core	+	Rod	Chain	+	−	+	*Bacillus* sp.
ZI-03	Core	−	Rod	Single	+	+	−	*Pseudomonas* sp.
ZI-04	Core	+	Coccus	Cluster	+	−	−	*Staphylococcus* sp.
ZI-05	Core	+	Rod	Single	+	−	+	*Bacillus* sp.
ZI-06	Core	+	Coccus	Cluster	+	+	−	*Micrococcus* sp.
ZI-07	Core	−	Rod	Single	+	−	−	*Serratia* sp.
ZP-01	Utilization	+	Rod	Single	+	−	+	*Bacillus* sp.
ZP-02	Utilization	−	Rod	Single	+	+	−	*Pseudomonas* sp.
ZP-03	Utilization	+	Coccus	Cluster	+	−	−	*Staphylococcus* sp.
ZP-04	Utilization	+	Rod	Chain	+	−	+	*Bacillus* sp.
ZP-05	Utilization	−	Rod	Single	+	−	−	*Enterobacter* sp.
ZS-01	Buffer	+	Rod	Single	+	−	+	*Bacillus* sp.
ZS-02	Buffer	−	Rod	Single	+	+	−	*Pseudomonas* sp.
ZS-03	Buffer	+	Rod	Chain	+	−	+	*Bacillus* sp.
ZS-04	Buffer	+	Rod	Single	+	−	+	*Bacillus* sp.
ZS-05	Buffer	+	Coccus	Cluster	+	+	−	*Micrococcus* sp.
ZS-06	Buffer	−	Rod	Single	+	−	−	*Serratia* sp.

Legend: isolate code prefixes: ZI = core zone, ZP = utilization zone, ZS = buffer zone. Gram reaction: + = Gram-positive, − = Gram-negative. Cell morphology: rod = Bacillus-shaped, coccus = spherical. Cell arrangement: single = solitary cells, chain = strepto-arrangement, cluster = staphylo-arrangement. Biochemical assays: + = positive reaction, − = negative reaction. Putative genus-level identification is based solely on phenotypic characteristics following Bergey's Manual of Determinative Bacteriology (13). Definitive genus and species identification requires confirmatory molecular analysis (e.g., 16S rRNA gene sequencing).

Bacillus sp. was the most dominant genus, accounting for 8 isolates (44.4%) distributed across all three zones. This dominance is likely driven by the genus's exceptional adaptability to the extreme microenvironment of the Kupang mangrove, particularly its capacity to form endospores that ensure survival under the high salinity (up to 29.1‰) and anoxic conditions recorded in the core zone (Table 1). Endophytic Bacillus isolates from *R. mucronata* are also known to exhibit cellulolytic activity, enabling the degradation of organic matter and nutrient acquisition from host tissues (14,15). Furthermore, Bacillus strains from mangrove rhizospheres are well-documented for plant growth-promoting (PGP) traits, such as nitrogen fixation and phosphate solubilization, which foster mutualistic symbioses with the mangrove host (16). While Bacillus dominated the overall isolate collection, the presence of other functionally distinct genera further highlights the metabolic diversity of this root-associated microbiome.

The detection of three *Pseudomonas* sp. isolates (ZI-03, ZP-02, ZS-02) can be attributed to the genus's established ecological role in nutrient cycling and its adaptation to partially anaerobic conditions within the mangrove rhizosphere. Pseudomonas is among the most prevalent endophytic genera in Indonesian mangrove ecosystems, largely due to its production of diverse extracellular enzymes and bioactive metabolites that enhance host plant resilience to environmental stress (17). Research indicates that Pseudomonas efficiently colonizes Rhizophora root zones through chemotactic responses to organic-rich root exudates and exhibits notable heavy metal bioremediation capabilities (18,19), a trait particularly advantageous given the frequent metal accumulation in mangrove sediments. Despite its lower relative abundance compared to Bacillus, this genus remains consistently distributed across multiple habitat zones. Beyond these Gram-negative rods, the endophytic community also included Gram-positive

cocci, reflecting a broader spectrum of microbial adaptation to the saline environment.

The isolation of *Staphylococcus* sp. and *Micrococcus* sp. (2 isolates each) from *R. mucronata* roots highlights the diversity of endophytic bacteria capable of thriving under high-salinity conditions. *Staphylococcus* sp. have been documented in mangrove habitats globally, suggesting they possess effective osmotic stress tolerance mechanisms that facilitate tissue colonization (20). Studies position Micrococcus and Staphylococcus as key members of heterotrophic communities, contributing to organic matter decomposition in environments enriched with complex aromatic compounds from Rhizophora leaf litter (21). Their presence here underscores a remarkable adaptive capacity to the unique rhizospheric microenvironment, which is dynamically influenced by tidal fluctuations (22). In addition to these osmotolerant cocci, a smaller fraction of the isolates belonged to genera traditionally recognized for their specific plant growth-promoting capabilities in nutrient-limited soils.

The detection of two *Serratia* sp. isolates and one *Enterobacter* sp. isolate can be explained by the functional roles of these genera as plant growth-promoting bacteria (PGPB). Serratia strains isolated from plant rhizospheres are well-documented for their phosphate solubilization capabilities and phytohormone production, supporting host development under stress-prone conditions (23,24). Studies on mangrove endophytes indicate that Enterobacter and Serratia effectively colonize root tissues in response to chemical signaling compounds exuded by Rhizophora roots, while their capacity for non-symbiotic nitrogen fixation contributes to overall nitrogen availability (25). Their presence highlights the functional complexity of microbial interactions in the *R. mucronata* rhizosphere.

It is important to acknowledge a key limitation of this study regarding the taxonomic identification of the isolates. The genus-level assignments presented in Table 3 are tentative and based solely on basic phenotypic and biochemical characteristics (Gram staining, catalase, oxidase, and starch hydrolysis). While these tests provide a useful preliminary classification following Bergey's Manual (13), they are insufficient for definitive taxonomic resolution, as biochemical profiles can frequently overlap between different bacterial genera. Without confirmatory molecular techniques, such as 16S rRNA gene sequencing or automated identification systems (e.g., VITEK), the exact genus and species of these isolates cannot be conclusively determined. Therefore, these phenotypic assignments should be interpreted as presumptive, and future studies should prioritize molecular identification to validate these findings and enable precise taxonomic classification of the bioactive endophytes.

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Antimicrobial Activity

Antimicrobial activity was evaluated for all 18 purified isolates using the Kirby-Bauer disk diffusion method against two reference pathogen strains: *Staphylococcus aureus* and *Escherichia coli*. Inhibition zone diameters were measured and categorized strictly according to the Davis-Stout criteria: very strong (VS, >20 mm), strong (S, 10–20 mm), moderate (M, 5–10 mm), weak (W, <5 mm), and no activity (0 mm). Chloramphenicol (30 µg) and sterile Nutrient Broth served as positive and negative controls, respectively. The comprehensive results are presented in Table 4.

Table 4. Mean inhibition zones of antimicrobial activity exhibited by endophytic bacterial isolates against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922

Zone	Isolate Code	Putative Genus	Inhibition Zone (mm)	
			S. aureus	***E. coli***
Core	ZI-01	*Bacillus* sp.	18.20 ± 0.33 (S)	12.25 ± 0.40 (S)
Core	ZI-02	*Bacillus* sp.	15.31 ± 0.42 (S)	0 (NA)

Core	ZI-03	*Pseudomonas* sp.	11.19 ± 0.34 (S)	14.55 ± 0.35 (S)
Core	ZI-04	*Staphylococcus* sp.	0 (NA)	0 (NA)
Core	ZI-05	*Bacillus* sp.	21.29 ± 0.45 (VS)	16.39 ± 0.45 (S)
Core	ZI-06	*Micrococcus* sp.	8.72 ± 0.39 (M)	0 (NA)
Core	ZI-07	*Serratia* sp.	0 (NA)	0 (NA)
Utilization	ZP-01	*Bacillus* sp.	13.62 ± 0.40 (S)	10.19 ± 0.36 (S)
Utilization	ZP-02	*Pseudomonas* sp.	0 (NA)	0 (NA)
Utilization	ZP-03	*Staphylococcus* sp.	7.86 ± 0.35 (M)	0 (NA)
Utilization	ZP-04	*Bacillus* sp.	16.79 ± 0.46 (S)	9.32 ± 0.40 (M)
Utilization	ZP-05	*Enterobacter* sp.	0 (NA)	0 (NA)
Buffer	ZS-01	*Bacillus* sp.	19.49 ± 0.60 (S)	13.82 ± 0.41 (S)
Buffer	ZS-02	*Pseudomonas* sp.	11.72 ± 0.39 (S)	15.62 ± 0.40 (S)
Buffer	ZS-03	*Bacillus* sp.	0 (NA)	0 (NA)
Buffer	ZS-04	*Bacillus* sp.	22.15 ± 0.65 (VS)	18.32 ± 0.40 (S)
Buffer	ZS-05	*Micrococcus* sp.	8.92 ± 0.39 (M)	0 (NA)
Buffer	ZS-06	*Serratia* sp.	0 (NA)	0 (NA)
Active isolates (n)			**12**	**8**
-	Positive control	Chloramphenicol (30 µg)	31.19 ± 0.36 (S)	26.39 ± 0.45 (S)
-	Negative control	Sterile Nutrient Broth (NB)	0	0

Legend: values represent mean inhibition zone diameter ± standard deviation (SD) from three independent replicates. Activity categories: VS = very strong (>20 mm), S = strong (10–20 mm), M = moderate (5–10 mm), W = weak (<5 mm), NA = no activity (0). Positive control: chloramphenicol disk (30 µg); negative control: sterile nutrient broth. Isolate code prefixes: ZI = core zone, ZP = utilization zone, ZS = buffer zone.

Against the Gram-positive pathogen *S. aureus*, antimicrobial activity varied considerably among the isolates. Notably, isolates ZS-04 and ZI-05 (both *Bacillus* sp.) demonstrated the most potent inhibitory effects, yielding mean inhibition zones of 22.15 ± 0.65 mm and 21.29 ± 0.45 mm, respectively. Based on the Davis-Stout criteria, these are categorized as very strong (VS) activity (Figure 4). Several other isolates exhibited strong (10–20 mm) or moderate (5–10 mm) inhibition, while a subset showed no detectable activity. These findings align with prior studies by Prihanto (26) and Fatimah (27), who reported that endophytic bacteria isolated from mangroves possess the capacity to synthesize bioactive secondary metabolites, such as alkaloids, flavonoids, and terpenoids, which are highly effective against Gram-positive pathogens. While the activity against *S. aureus* was broadly distributed, the response of the Gram-negative pathogen *E. coli* revealed a distinctly different and more selective pattern.

Consistent with this selective pattern, antimicrobial testing against *E. coli* showed markedly lower overall efficacy, with 11 of the 18 isolates (61.1%) exhibiting no detectable inhibition. However, the same highly active isolates, ZI-05 and ZS-04, also produced the largest inhibition zones against this Gram-negative bacterium, measuring 16.39 ± 0.45 mm and 18.32 ± 0.40 mm, respectively. Under the corrected criteria, these values fall into the strong (S) category (10–20 mm) (Figure 5). This differential susceptibility is primarily attributed to the structural defenses of Gram-negative bacteria, specifically the outer lipopolysaccharide (LPS) membrane that acts as a formidable permeability barrier, coupled with intrinsic resistance mechanisms such as multidrug efflux pumps (28).

The markedly lower antimicrobial activity against *E. coli* compared to *S. aureus* reflects the inherent selectivity of bioactive metabolites produced by *R. mucronata* endophytes. This pattern strongly aligns with findings by Putri (29), who noted that narrow-spectrum compounds from Indonesian endophytes often exhibit greater efficacy against Gram-positive pathogens. From a therapeutic perspective, such selectivity is advantageous, as targeted antimicrobials can eradicate specific pathogens while preserving the host's commensal

microbiota. Nevertheless, isolates ZI-05, ZS-04, and ZI-03 demonstrated strong inhibition of *E. coli* and merit further chemical and mechanistic characterization to unlock their potential against Gram-negative pathogens. This inherent resistance in *E. coli* naturally leads to greater variability in inhibition data, which is further reflected in the statistical analysis of zonal effects.

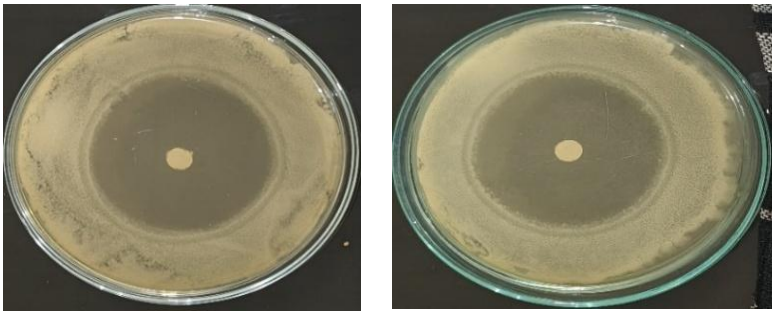

Figure 4. Representative inhibition zones exhibited by endophytic bacterial isolates ZS-04 (*Bacillus* sp.) and ZI-05 (*Bacillus* sp.) against *S.aureus*

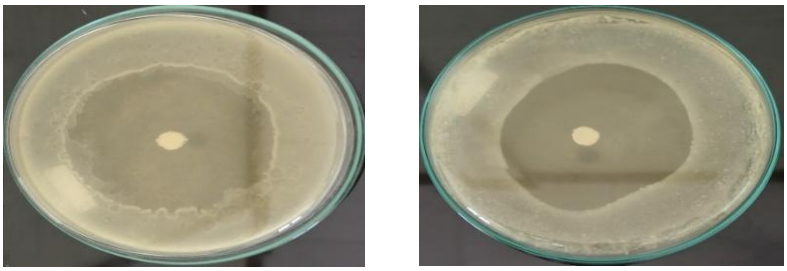

Figure 5. Representative inhibition zones exhibited by endophytic bacterial isolates ZS-04 (*Bacillus* sp.) and ZI-05 (*Bacillus* sp.) against *E. coli*

Comparative Statistical Analysis

To evaluate the influence of ecological zoning on antimicrobial potential, the inhibition zone data from active isolates were subjected to one-way ANOVA (α = 0.05), preceded by Shapiro-Wilk normality and Levene's homogeneity of variance tests. Where significant differences were detected, Tukey's HSD post hoc test was applied to identify specific zonal variations. The summary of these statistical evaluations is detailed in Table 6.

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Table 6. Summary of assumption testing, one-way ANOVA, and pairwise comparisons of antimicrobial activity across functional management zones.

Pathogen	n	Normality (Shapiro-Wilk)	Homogeneity (Levene's Test)	ANOVA (*p*)	Post hoc (*p*)		
					ZI vs ZP	ZI vs ZS	ZP vs ZS
S. aureus	12	Normal	Homogeneous	*0.785* (ns)	*0.847* (ns)	*0.984* (ns)	*0.779* (ns)
E. coli	8	Normal	Homogeneous	*0.045* (s)	*0.104* (ns)	*0.635* (ns)	*0.041* (s)

Legend: ZI = Core Zone; ZP = Utilization Zone; ZS = Buffer Zone. (s) = significant; (ns) = not significant. The value (n) = 18 for all isolates (value 0 was not included in the analysis). Post hoc Tukey HSD test for *E. coli*. The number of isolates (n) selected is those that have bacterial inhibition zone values.

Descriptive analysis for *S. aureus* revealed a gradient in mean activity, highest in the buffer zone (15.57 mm), followed by the core (14.94 mm) and utilization zones (12.76 mm). However, one-way ANOVA indicated no statistically significant differences among the zones ($p = 0.785$). This lack of statistical significance is largely attributed to high within-group variance, driven by the inclusion of non-active isolates (zero inhibition) and the limited sample size per zone. Such intra-zone heterogeneity reflects the inherent variability in biosynthetic capacity among endophytic isolates, influenced by genetic diversity and microenvironmental

conditions, as observed by Annisa et al. (2024). Furthermore, this pattern is corroborated by coastal bacterial exploration studies, such as those by Astrid (30), wherein a significant portion of isolates exhibited weak or no activity, thereby increasing data dispersion. Despite this statistical dispersion, it is important to note that the mean inhibition zones of the active isolates across all zones consistently fall within the strong activity category (10–20 mm), with peak values exceeding 20 mm classified as very strong, confirming their biologically meaningful inhibitory potential. In contrast to the uniform response of *S. aureus*, the statistical analysis for *E. coli* revealed a significant ecological gradient.

Indeed, antimicrobial activity against *E. coli* demonstrated a statistically significant effect of ecological zoning (ANOVA, $p = 0.045$). Tukey's HSD post hoc testing specifically identified a significant difference between the utilization and buffer zones ($p = 0.041$). This differential statistical significance between the two pathogens underscores the impact of bacterial cell wall architecture: while the exposed peptidoglycan layer of Gram-positive *S. aureus* makes it generally susceptible to a wider range of compounds (masking zonal differences), the complex outer membrane of Gram-negative *E. coli* confers greater intrinsic resistance, resulting in more variable and zone-sensitive responses, as highlighted by Sarmira (31). Consequently, only the most potent, zone-specific metabolites can overcome this barrier, making Gram-negative pathogens more discriminative indicators of ecological gradients in endophyte bioprospecting studies.

4. Conclusion

This study successfully isolated and characterized 18 endophytic bacterial isolates from the prop and lateral roots of *Rhizophora mucronata* across three distinct management zones (core, buffer, and utilization) in a semi-arid mangrove ecosystem. *Bacillus* sp. emerged as the dominant genus (44.4%), demonstrating exceptional adaptability to extreme rhizospheric conditions. Notably, *Bacillus* sp. isolates ZS-04 and ZI-05 exhibited the most potent bioactivity, yielding very strong (>20 mm) inhibition against *Staphylococcus aureus* and strong (10–20 mm) inhibition against *Escherichia coli*. Statistically, while intra-zone variability masked significant zonal differences for *S. aureus* ($p = 0.785$), a significant ecological gradient was observed for *E. coli* ($p = 0.045$), indicating that Gram-negative pathogens serve as more sensitive indicators of zonal variations in endophytic metabolite production. These findings validate semi-arid mangrove root endophytes as promising, stress-adapted reservoirs of targeted natural antibacterial agents.

However, as the current taxonomic assignments are based solely on phenotypic characteristics, future research must prioritize 16S rRNA gene sequencing for definitive taxonomic validation, coupled with LC-MS/MS-based metabolite profiling to elucidate the specific bioactive compounds responsible for this selective antimicrobial activity.

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