BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY
Volume 15, Number 1, April 2026 | Pages: 1363-1371 | DOI: 10.14421/biomedich.2026.151.1363-1371
ISSN 2089-6514 (paper)
ISSN 2540-9328 (online)

Distribution and Abundance of *Actinomycetes* in the Rhizosphere Soil of The Hungayono Karst Ecosystem, Gorontalo

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Manuscript received: 23 March 2026. Revision accepted: 10 June 2026, Published: 11 July 2026.

Abstract

Karst ecosystems have unique environmental conditions that can influence the presence of soil microorganisms, including *Actinomycetes* in the rhizosphere. This study aimed to determine the distribution and abundance of *Actinomycetes* in the rhizosphere soil of plants in the Hungayono karst ecosystem, Gorontalo. This study employed a quantitative descriptive method with an exploratory approach. Rhizosphere soil samples were collected from several plant species at three observation points: the lower, middle, and upper parts of the karst area. *Actinomycetes* were isolated using Starch Casein Agar, while their abundance was determined using the Total Plate Count method and expressed in CFU/g of soil. The results showed that actinomycetes were found at all observation points, with abundances ranging from 2×10^3 to 3×10^5 CFU/g. The highest abundances were found in the rhizosphere of *Calamus* spp*.*, *Anthocephalus macrophyllus*, and *Ficus microcarpa*, while the lowest abundances were found in the rhizosphere of *Alocasia macrorrhizos*. The *Actinomycetes* isolates obtained exhibited morphological variation based on mycelium color and colony shape. These findings indicate that the distribution and abundance of *Actinomycetes* in the rhizosphere soil of the Hungayono karst ecosystem vary among plant species and observation sites. The plant rhizosphere in this karst ecosystem functions as a microhabitat that supports the presence of *Actinomycetes* and is relevant to studies of karst soil microbiology.

Keywords: *Actinomycetes*; distribution and abundance; Hungayono Karst; rhizosphere; total plate count.

Abbreviations: CFU/g: colony-forming units per gram of soil; SCA: starch casein agar; TPC: total plate count.

INTRODUCTION

Karst ecosystems are a highly distinctive type of ecosystem with significant ecological value. Karst is formed through the dissolution of carbonate rocks such as limestone and dolomite, resulting in a distinctive landscape characterized by steep hills, caves, sinkholes, and underground water systems. These geological processes, which have taken place over a very long period of time, have created a unique landscape that is also highly sensitive to environmental changes (Katili et al., 2025). Globally, the distribution of karst landscapes largely follows the distribution of carbonate rocks, covering an area of approximately 12.5 million km² or 16.7% of the total land area. Meanwhile, the distribution of karst landscapes in Southeast Asia including China, Malaysia, and Indonesia is reported to be 1.7% (Jones & White, 2019).

In Indonesia, one of the karst areas with high conservation value is the Hungayono Karst, located in the Tulabolo Resort, Bogani Nani Wartabone National Park, Gorontalo Province, specifically in Bone Bolango Regency. The karst environment which is generally dry, rocky, and has shallow soil influences the type of vegetation that grows there. Karst vegetation interacts directly with the soil through the rhizosphere, thereby playing a crucial role in the dynamics of karst ecosystems.

The rhizosphere is the zone of soil surrounding plant roots that serves as an ideal environment for microbial growth (Tambingsila, 2016). Microbial diversity in the rhizosphere plays a role in maintaining ecosystem balance, increasing nutrient availability, improving soil structure, and suppressing the growth of pathogens. The activity of rhizosphere microorganisms is influenced by physiological interactions with plant roots and surrounding environmental conditions (Nador et al., 2019).

Among the microorganisms that play an important role in the rhizosphere are *Actinomycetes*. *Actinomycetes* are a group of Gram-positive bacteria that are widely distributed in the natural environment and are known for producing bioactive compounds, including antibiotics and antimicrobial metabolites. *Actinomycetes* in the rhizosphere help regulate the growth of soil microorganisms, particularly pathogenic ones, thereby improving soil and plant health (Faulah G, 2022).

Research in the Gorontalo karst region shows that several *Actinomycetes* isolates from the rhizosphere soil have the ability to solubilize phosphate and produce plant growth-promoting compounds (Retnowati et al., 2024). Furthermore, the distribution and abundance of *Actinomycetes* in karst ecosystems have been reported to vary among host plant species, even under relatively uniform soil physicochemical conditions. These findings suggest that rhizosphere microhabitats influence the presence of these microorganisms (Pakaya et al., 2025).

However, information regarding the distribution and abundance of *Actinomycetes* in the rhizosphere of various plant species in the Hungayono Karst ecosystem remains limited and has not yet been systematically documented. Therefore, this study aims to determine the distribution and abundance of *Actinomycetes* in the rhizosphere of plants in the Hungayono Karst ecosystem.

MATERIALS AND METHODS

Study area

This study was conducted in the Hungayono Karst ecosystem, Tulabolo Resort, Bogani Nani Wartabone National Park, Gorontalo Province. This area is a karst ecosystem with distinctive environmental characteristics, such as shallow soil, rocky substrate, varied topography, and vegetation associated with the rhizosphere. These conditions make the Hungayono Karst a relevant site for studying the distribution and abundance of *Actinomycetes* in the plant rhizosphere.

The study site consisted of three observation points representing the lower, middle, and upper parts of the karst area. Point A represented the lower part of the area with coordinates 0°30'19.4“ N and 123°17'29.5” E; Point B represented the middle part of the area with coordinates 0°30'18.13" N and 123°17'29.08“ E, while point C represented the upper part of the area with coordinates 0°30'14.7” N and 123°17'32.25" E. These three points were selected to illustrate the variation in site conditions within the Hungayono Karst ecosystem. A map of the study location is presented in Figure 1 to show the boundaries of the study area and the positions of the sampling points in the Hungayono Karst ecosystem.

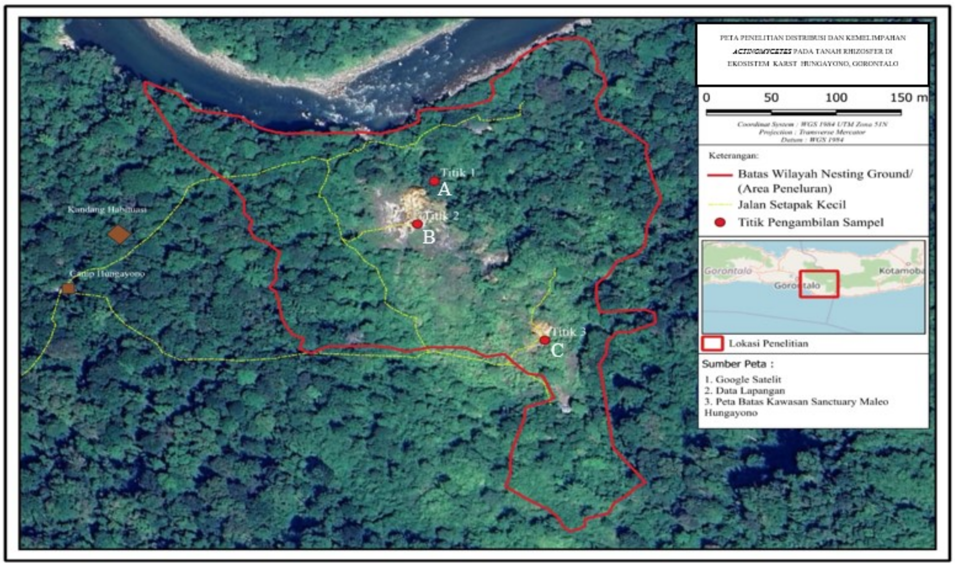


Figure 1. Sampling locations in the Hungayono Karst area, Gorontalo. (A = lower site, B = middle site, and C = upper site.).

Procedures

Soil Sampling and Parameter Measurement

Soil sampling of the rhizosphere of karst areas was conducted using the purposive sampling method, focusing on the dominant plants species at each karst site. Soil samples from the rhizosphere were collected from selected plant species at each observation site using a small shovel at several sampling points for each plant species found. According to Maulana et al. (2022), collecting rhizosphere soil samples at a depth of 10–15 cm maximizes the concentration of Actinomycetes bacteria in the soil. Soil samples were then stored in sterile plastic bags, labeled with location codes, and stored in a cool box to ensure their safety (Katili & Retnowati, 2017). The GPS Maps Camera app was used to record coordinate points. Environmental parameters, including soil pH, soil temperature, and soil moisture, were measured using a soil tester in the karst area.

Isolation, Cultivation, and Purification of Actinomycetes from karst areas.

Soil samples collected from the Hungayono karst area were ground using a mortar and pestle; 5 grams of the sample were then mixed with 45 ml of Ringer's solution and vortexed until homogeneous using a shaker incubator at 220 rpm. Next, the soil suspension was heated in a water bath at 60°C for 15 minutes (Mangamuri et al., 2012; Retnowati et al., 2017).

Next, a series of dilutions of the soil suspension was performed by transferring 1 ml of the suspension into a test tube containing 9 ml of Ringer's solution (10^{-1} tube), vortexing it, and then transferring 1 ml from the first tube into each subsequent test tube from 10^{-2} to 10^{-5}. A total of 100 µl of the soil suspension at dilution levels 10^{-3}–10^{-5} was taken and inoculated onto the surface of SCA medium using the surface/spread plate technique in duplicate. The Petri dishes were then incubated at 37°C for 7–14 days. Nystatin was added to the SCA medium at a concentration of 25 µl/ml to prevent the growth of contaminating fungal colonies during incubation (Baskaran et al., 2011).

Actinomycetes colonies were counted using the Total Plate Count (TPC) method. Microbial colonies growing on each sample plate were counted using a colony counter; the number of microbial colonies analyzed fell within the range of 30–300 colonies per plate (Sukmawati, 2018). If the number of colonies does not fall within the 30–300 range per plate, a plate with a colony count closest to 300 is selected (Waluyo, 2018). The formula for calculating the colony count is as follows:

$$Number\ of\ colonies/gram\ = \frac{1}{Dilution\ factor} x Number\ of\ colonies\ in\ the\ dish$$

Actinomycetes colonies grown on SCA medium were inoculated into new plates containing SCA medium for purification using the single-cell colony method via streak plating and incubated for 1 week at 37°C (Budhathoki et al., 2020).

Morphological Characterization of Actinomycetes Isolates and the Distribution of Actinomycetes

The morphological characterization of *Actinomycetes* isolates was conducted macroscopically and microscopically. Macroscopic observations involved examining colony characteristics, including the color of the substrate mycelium, the color of the aerial mycelium, and the colony shape. Microscopic observations were performed using the Gram staining technique as described by Kurniati et al. (2019). Isolates prepared on microscope slides were stained with crystal violet, iodine, alcohol, and safranin, then observed under a microscope to examine filamentous structures and mycelium-like forms. The distribution of *Actinomycetes* was recorded based on the presence of each isolate in the rhizosphere soil of each plant species.

Data analysis

The research data were analyzed using quantitative descriptive methods. The abundance of *Actinomycetes* was analyzed based on the number of colonies growing on isolation media using the Total Plate Count (TPC) method. The results were expressed in colony-forming units per gram of soil (CFU/g). The abundance data were then presented in tabular form to illustrate the differences in the number of *Actinomycetes* colonies in each rhizosphere soil sample. The distribution of *Actinomycetes* was analyzed based on the presence of isolates in each plant species and sampling location. This distribution data is presented in the form of a scatter plot to illustrate the distribution patterns of *Actinomycetes* isolates in the rhizosphere soil of plants in the Hungayono Karst ecosystem, Gorontalo. This analysis is used to describe the variation in abundance and distribution of *Actinomycetes.*

RESULTS AND DISCUSSION

Environmental conditions and plant characteristics at the rhizosphere sampling sites

This study aim**ed** to examine the presence of *Actinomycetes* in the rhizosphere soil of the Hungayono Karst ecosystem in Gorontalo, focusing on their distribution and abundance. The results of the study indicate that *Actinomycetes* populations vary across each observation site. Environmental conditions in karst ecosystems can influence the characteristics of rhizosphere soil, particularly through differences in soil pH, soil temperature, and soil moisture.

Table 1. Soil environmental conditions at the study site in the Hungayono Karst.

Site	Soil pH	Soil temperature (°C)	Soil moisture (%)
Lower site	6.8	29	20
Middle site	6.8	32	10
Upper site	7.0	37	10

Based on Table 1, the soil pH at the study sites ranged from 6.8 to 7. The lower and middle sites had a soil pH of 6.8, while the upper site had a soil pH of 7. These data indicate that the soil at all three observation sites was near neutral.

Soil temperature increased from the lower site to the upper site. The lower site had a soil temperature of 29 °C, the middle site 32 °C, and the upper site 37 °C. Conversely, soil moisture was highest at the lower site, at 20%, while the middle and upper sites had the same soil moisture level, at 10%.

Descriptively, the results of the environmental parameter measurements show that the three locations have relatively similar soil pH but differ in soil temperature and moisture. The lower location has moister soil conditions, while the upper location has the highest soil temperature with lower moisture. These data serve as the basis for examining variations in the abundance and distribution of *Actinomycetes* in the rhizosphere soil of plants in the Hungayono Karst area.

In addition to environmental conditions, the presence of *Actinomycetes* in the rhizosphere soil is also related to the plants from which samples were collected. Each plant has a different growth habit and root type, which can result in different rhizosphere characteristics. Therefore, the following research results present data on plant species, growth habits, and root types at each observation site in Table 2.

Table 2. Plant species at the study site in the Hungayono Karst.

Site	Plant species	Plant habit	Root type
Lower site	*Calamus spp.*	Climbing plant	Fibrous root
	Acrostichum aureum	Seedling	Fibrous root
	Alocasia macrorrhizos	Seedling	Fibrous root
Middle site	*Anthocephalus macrophyllus*	Tree	Taproot
	Acrostichum aureum	Seedling	Fibrous root
	Ficus microcarpa	Tree	Taproot
	Pluchea indica	Seedling	Fibrous root
	Piper aduncum	Sapling	Fibrous root
	Alstonia scholaris	Tree	Taproot
Upper site	*Imperata cylindrica*	Grass	Fibrous root
	Acrostichum aureum	Seedling	Fibrous root
	Anthocephalus macrophyllus	Tree	Taproot
	Pluchea indica	Seedling	Fibrous root

Table 2 shows that three plant species were found at the lower site: Calamus spp., *Acrostichum aureum*, and *Alocasia macrorrhizos*. At the middle site, six plant species were found: *Anthocephalus macrophyllus*, *Acrostichum aureum, Ficus microcarpa, Pluchea indica, Piper aduncum, and Alstonia scholaris*. Meanwhile, four plant species were found at the upper site: *Imperata cylindrica, Acrostichum aureum, Anthocephalus macrophyllus,* and *Pluchea indica.*

The vegetation found at the study sites consisted of seedlings, saplings, trees, and grasses with both fibrous and taproot systems. The middle location showed a greater number of plant species compared to the lower and upper locations. Differences in plant species, stature, and root types indicate that each rhizosphere source has distinct characteristics. These variations in rhizosphere sources form the basis for examining differences in the abundance and distribution of *actinomycetes* among the respective plants.

Abundance and morphological characteristics of *Actinomycetes* isolates

The abundance of *Actinomycetes* in plant rhizosphere soil was calculated based on the number of colonies that grew on isolation media. The number of colonies was expressed in Colony-Forming Units per gram of soil, or CFU/g. The abundance data were used to describe the size of the *Actinomycetes* populations obtained from each type of rhizosphere soil at the study site. The results of the actinomycetes abundance calculations in plant rhizosphere soil are presented in Table 3.

Table 3. *Actinomycetes* Population.

Site	Plant species	Abundance (CFU/g)
Lower site	*Calamus spp.*	3×10^5
	Acrostichum aureum	4×10^3
	Alocasia macrorrhizos	2×10^3
Middle site	*Anthocephalus macrophyllus*	3×10^5
	Acrostichum aureum	4×10^4
	Ficus microcarpa	3×10^5
	Pluchea indica	3×10^4
	Piper aduncum	2×10^4

Site	Plant species	Abundance (CFU/g)
Upper site	*Alstonia scholaris*	2×10^4
	Imperata cylindrica	2×10^4
	Acrostichum aureum	2×10^4
	Anthocephalus macrophyllus	2×10^5
	Pluchea indica	4×10^4

Based on Table 3, the abundance of *Actinomycetes in* the rhizosphere soil of the Hungayono Karst area varied by plant species and observation site. The lowest abundance was found in the rhizosphere of *Alocasia macrorrhizos* at the lower site, at 2×10^3 CFU/g, while the highest abundance was found in the rhizosphere of Calamus spp. at the lower site, as well as in the rhizospheres of *Anthocephalus macrophyllus* and *Ficus microcarpa* at the middle site, each at 3×10^5 CFU/g. At the upper site, the highest abundance was found in the rhizosphere of *Anthocephalus macrophyllus* at 2×10^5 CFU/g, while the lowest abundance was found in the rhizosphere of *Imperata cylindrica* and *Acrostichum aureum,* at 2×10^4 CFU/g each. These results indicate that the abundance of *actinomycetes* varies among the rhizospheres of the plants observed.

After the yield was calculated, the morphological characteristics of the *Actinomycetes* isolates were observed. Macroscopic observations were conducted by examining the color of the substrate mycelium, the color of the aerial mycelium, and the colony shape. The results of the macroscopic observations of the isolates are presented in Table 4.

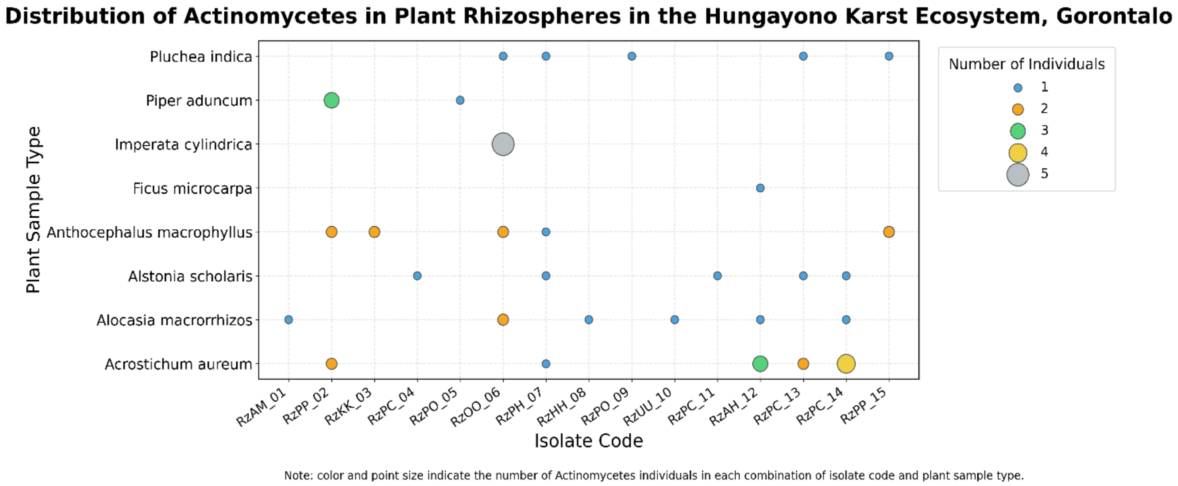
Table 4. Macroscopic characteristics of *Actinomycetes* isolates from plant rhizosphere soil.

Isolate code	Rhizosphere source	Substrate mycelium color	Aerial mycelium color	Colony shape
RzAM_01	*Alocasia macrorrhizos*	Grey	Red	Circular
RzPP_02	*Anthocephalus macrophyllus*	White	White	Irregular
	Piper aduncum			
	Acrostichum aureum			
RzKK_03	*Anthocephalus macrophyllus*	Yellow	Yellow	Circular
RzPC_04	*Alstonia scholaris*	White	Brown	Irregular
RzPO_05	*Piper aduncum*	White	Orange	Circular
RzOO_06	*Imperata cylindrica*	Orange	Orange	Irregular
	Pluchea indica,			
	Anthocephalus macrophyllus			
	Alocasia macrorrhizos			
RzPH_07	*Anthocephalus macrophyllus*	White	Black	Irregular
	Alstonia scholaris			
	Acrostichum aureum			
	Pluchea indica			
RzHH_08	*Alocasia macrorrhizos*	Black	Black	Circular
RzPO_09	*Pluchea indica*	White	Orange	Circular
RzUU_10	*Alocasia macrorrhizos*	Purple	Purple	Circular
RzPC_11	*Alstonia scholaris*	White	Cream	Circular
RzAH_12	*Ficus microcarpa*	Grey	Black	Irregular
	Acrostichum aureum			
	Alocasia macrorrhizos			
RzPC_13	*Acrostichum aureum*	White	Cream	Circular
	Pluchea indica,			
	Alstonia scholaris			
RzPC_14	*Acrostichum aureum*	White	Cream	Circular
	Alocasia macrorrhizos			
	Alstonia scholaris			
RzPP_15	*Anthocephalus macrophyllus*	White	White	Circular
	Pluchea indica			

Based on Table 4, the *Actinomycetes* isolates obtained exhibited a diversity of macroscopic characteristics. This variation was evident in the color of the substrate mycelium, the color of the aerial mycelium, and the shape of the colonies formed on the isolation media. The observed mycelium colors included white, gray, yellow, red, orange, cream, black, and purple. In addition, the colony shapes found consisted of circular and irregular forms. These characteristics indicate morphological variation among the *Actinomycetes* isolates obtained from various plant rhizospheres in the Hungayono Karst region. Microscopic observation through Gram staining showed that the observed isolates exhibited Gram-positive characteristics and filamentous, mycelium-like structures, supporting their identification as *Actinomycetes*.

Distribution of *Actinomycetes* isolates

Actinomycetes are a group of soil microorganisms commonly found in the rhizosphere of plants. The presence of *Actinomycetes* in different plant species may exhibit varying distribution patterns depending on environmental conditions and vegetation characteristics at the study site. The distribution of *Actinomycetes* isolates in the rhizosphere soil of the Hungayono Karst region was examined based on the presence of isolates in each plant species found at the study site. Data on the distribution of *Actinomycetes* isolates in plant samples are presented in Figure 2. Figure 2 showed that *Actinomycetes* are unevenly distributed in the plant rhizosphere of the Hungayono karst ecosystem. Isolates RzOO_06 and RzPH_07 were found to be associated with 4 plant species; isolates RzPP_02, RzAH_12, RzPC_13, and RzPC_14 were associated with 3 plant species while isolate RzPP_15 was associated with two plant species, namely *Anthocephalus macrophyllus* and *Pluchea indica*, whereas the other isolates were associated with only one plant species. Each *Actinomycetes* isolate was found in varying populations within the rhizosphere of each plant species. Isolate RzOO_06 was most frequently associated with the rhizosphere of *Imperata cylindrica*. Several isolates were also found on a single plant species, such as RzAM_01 on *Alocasia macrorrhizos*, RzKK_03 on *Anthocephalus macrophyllus,* RzPC_04 on *Alstonia scholaris*, RzPO_05 on *Piper aduncum*, RzHH_08 on *Alocasia macrorrhizos*, RzPO_09 on *Pluchea indica*, RzUU_10 on *Alocasia macrorrhizos,* and RzPC_11 on *Alstonia scholaris*.


Distribution of Actinomycetes in Plant Rhizospheres in the Hungayono Karst Ecosystem, Gorontalo

Note: color and point size indicate the number of Actinomycetes individuals in each combination of isolate code and plant sample type.

Figure 2. Scatter plot of the distribution of *Actinomycetes* isolates in the rhizosphere soil of plants in the Hungayono Karst ecosystem.

Discussion

The results of the study show that *Actinomycetes* were found in the rhizosphere soil of plants at all observation sites in the Hungayono Karst ecosystem, Gorontalo, but their abundance and distribution patterns were not uniform. The abundance of Actinomycetes ranged from 2 × 10^3 to 3 × 10^5 CFU/g of soil. The highest abundance was found in the rhizosphere of *Calamus* spp. at the lower site and in the rhizosphere of *Anthocephalus macrophyllus* and *Ficus microcarpa* at the middle site, each at 3 × 10^5 CFU/g. In contrast, the lowest abundance was found in the rhizosphere of *Alocasia macrorrhizos* at 2 × 10^3 CFU/g. These differences indicate that the plant rhizosphere in karst ecosystems is not homogeneous but rather constitutes a microhabitat influenced by soil environmental conditions, plant species, root type, and the adaptive capacity of the isolates. These findings support Retnowati et al. (2024), who reported the presence of *Actinomycetes* in the plant rhizosphere of the Gorontalo karst ecosystem. These results are also consistent with those of Pakaya et al. (2025), who showed that the abundance of *Actinomycetes* in the plant rhizosphere of the Gorontalo karst ecosystem varies among plant species and observation sites.

Variations in *Actinomycetes* abundance in this study were related to soil environmental conditions at each observation point. Soil pH values at the lower, middle, and upper sites ranged from 6.8 to 7.0, indicating that the soil was near neutral. These conditions still support the growth of soil microorganisms, including *Actinomycetes*. However, since pH values at the three sites were relatively similar, pH cannot be used as a single factor to explain differences in abundance. More pronounced differences were observed in soil temperature and moisture. Temperature increased from 29 °C at the lower site, to 32 °C at the middle site, and up to 37 °C at the upper site, while the highest soil moisture was recorded at the lower site at 20% and was lower at the middle and upper sites, at 10%. Dewi et al. (2024) demonstrated that *Actinomycetes* growth is influenced by variations in incubation temperature and medium pH. Thus, variations in *Actinomycetes* abundance in the Hungayono Karst are best understood as the result of interactions between pH, temperature, moisture, and plant rhizosphere characteristics, rather than as the result of any single environmental factor acting in isolation.

Differences in plant species and root types are also important factors in explaining variations in *Actinomycetes* abundance. The rhizosphere is the soil zone directly influenced by root activity, including the release of root exudates. Root exudates can serve as a source of carbon, energy, and chemical signals for soil microorganisms. Qu et al. (2024) demonstrated that metabolites in root exudates can influence the bacterial and fungal profiles in the rhizosphere. Feng et al. (2024) explained that root exudates act as a bridge between plant roots and rhizosphere microbes and influence the formation and function of the rhizobiome. Wu et al. (2024) also confirm that root exudates play a role in shaping the rhizosphere microbial community and enhancing soil microbial function. Based on this, the differences in *Actinomycetes* abundance among plant rhizospheres in this study can be interpreted as an ecological response to differences in rhizosphere characteristics, root types, and possible differences in the organic compounds released by each plant. However, since the composition of root exudates has not been chemically analyzed, the discussion of root exudates in this article should be viewed as an ecological interpretation rather than direct causal evidence.

The highest abundance in the rhizosphere of *Calamus* spp. can be attributed to its fibrous root system, which has numerous fine branches, thereby expanding the contact area between the roots and the soil. These conditions allow for the formation of a more active rhizosphere zone as a space for microbial colonization. The high abundance in *Anthocephalus macrophyllus* and *Ficus microcarpa* can be attributed to their tree-like stature and relatively stable taproot systems. Tree-like plants have the potential to provide organic matter through litter, root decomposition, and root activity that persists over a longer period. Conversely, the low abundance in *Alocasia macrorrhizos* indicates that higher soil moisture does not always result in larger *Actinomycetes* populations. These findings demonstrate that soil physical conditions must be interpreted in conjunction with plant characteristics and rhizosphere traits. Wankhade et al. (2025) emphasize that microbial interactions in the rhizosphere are influenced by nutrient availability, root chemical signals, environmental stress, and microbial adaptability. Therefore, differences in *Actinomycetes* abundance among plant species are best interpreted as the result of interactions between abiotic and biotic factors within each individual rhizosphere.

Morphological observations showed that *Actinomycetes* isolates from the rhizosphere of Karst Hungayono plants exhibited a variety of macroscopic and microscopic characteristics. The colors of the substrate and aerial mycelia ranged from white, gray, yellow, red, orange, cream, black, brown, and purple, while colony shapes were circular and irregular. Microscopically, the isolates exhibited filamentous forms and mycelium-like structures. This variation indicates that the obtained *Actinomycetes* isolates are not phenotypically uniform. Matalauni et al. (2025) also reported that *Actinomycetes* isolates from the rhizosphere of plants in the Gorontalo karst region exhibited variations in colony shape, mycelium color, and microscopic characteristics. Thus, morphological characteristics can serve as an initial step in distinguishing isolates. However, morphological characteristics alone are insufficient to confirm taxonomic identity down to the genus or species level. Further molecular-based identification, such as through 16S rRNA gene analysis, remains necessary to determine the taxonomic position of the isolates more accurately.

The distribution pattern of the isolates indicates that *Actinomycetes* are unevenly distributed across various plant species. Isolates RzOO_06 and RzPH_07 were found to be associated with four plant species. Isolates RzPP_02, RzAH_12, RzPC_13, and RzPC_14 were found on three plant species, while RzPP_15 was found on two plant species, namely *Anthocephalus macrophyllus* and *Pluchea indica.* The other isolates were found on only one plant species. This pattern indicates that some isolates have the ability to associate with several types of rhizosphere, while others have a more limited distribution. Isolates with a wide distribution, such as RzOO_06 and RzPH_07, can be interpreted as having greater ecological tolerance to variations in rhizosphere conditions. Conversely, isolates found on only one plant may indicate a more specific association with certain rhizosphere characteristics. Distinguishing between abundance and distribution is important because a high CFU/g value does not always indicate a large number of isolate codes. For example, *Ficus microcarpa* has high abundance, but according to the distribution data, it is associated only with isolate RzAH_12. Conversely, *Acrostichum aureum* has several isolate codes, but its abundance varies by location.

Theoretically, the results of this study reinforce the understanding that karst ecosystems are not only important from geological, hydrological, and vegetation perspectives, but also possess a significant microbiological dimension. The plant rhizosphere has been shown to be a microecological niche that supports the presence of *Actinomycetes* with varying levels of abundance and distribution. The novelty of this article lies in the integration of data on abundance, morphological characteristics, and distribution patterns of *Actinomycetes* isolates based on plant species in the

Hungayono karst ecosystem. Practically, the rhizosphere of *Calamus* spp., *Anthocephalus macrophyllus*, and *Ficus microcarpa* can be prioritized as initial sources for *Actinomycetes* exploration because they exhibit the highest abundance. Furthermore, widely distributed isolates such as RzOO_06 and RzPH_07 warrant further investigation as they are associated with multiple plant species. Boukhatem et al. (2022) explain that Actinobacteria have potential as plant growth-promoting microbes and bioinoculant candidates through both direct and indirect mechanisms. Therefore, further research should focus on the molecular identification of isolates, analysis of soil organic matter, analysis of root exudate metabolites, and biological potential testing so that the relationship between environmental conditions, plant species, rhizosphere characteristics, and the presence of *Actinomycetes* can be explained more scientifically and validly.

CONCLUSIONS

Actinomycetes were found at all observation sites in the Hungayono Karst ecosystem, Gorontalo, namely in the lower, middle, and upper parts of the karst area. The distribution patterns of *Actinomycetes* varied across plant rhizospheres and observation sites, indicating that their presence was not uniform across plant species. This indicates that the plant rhizosphere in the Hungayono Karst ecosystem serves as a microhabitat that supports the presence of *Actinomycetes*.

The abundance of *Actinomycetes* in the rhizosphere soil varies among plant species, ranging from 2×10^3 to 3×10^5 CFU/g of soil. The highest abundance was found in the rhizosphere of *Calamus* spp., *Anthocephalus macrophyllus*, and Ficus microcarpa, while the lowest abundance was found in the rhizosphere of *Alocasia macrorrhizos*. The *Actinomycetes* isolates obtained also exhibited morphological variation based on mycelium color and colony shape. These findings indicate that the distribution and abundance of *Actinomycetes* in the Hungayono Karst ecosystem are influenced by differences in plant rhizospheres and site conditions.

Acknowledgements: The authors would like to thank Gorontalo State University, particularly the Department of Biology, the Faculty of Mathematics and Natural Sciences, and the Microbiology Laboratory, for the facilities and technical support provided during the conduct of this research. The authors also extend gratitude to Bogani Nani Wartabone National Park (TNBNW) in Limboto for granting permission and providing support for sample collection in the study area. Thanks are also extended to all those who assisted with field sampling, laboratory analysis, and the preparation of this research manuscript.

Authors' Contributions: Apriliyanti Mohulaingo conducted laboratory work, and manuscript preparation. Yuliana Retnowati contributed to the formulation of the concept and methodology, supervision, and manuscript review. Abubakar Sidik Katili contributed to field supervision, ecological interpretation, and manuscript review. All authors have read and approved the final version of this manuscript.

Competing Interests: The authors state that there are no competing interests in this study.

Funding: This study was supported by the research team's internal funds. These funds were used to support sampling activities and laboratory analysis.

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