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# Diversity of actinomycetes on plant rhizosphere of karst ecosystem of Gorontalo, Indonesia

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**Abstract.** *Retnowati Y, Kandowangko NY, Katili AS, Pembengo W. 2024. Diversity of actinomycetes on plant rhizosphere of karst ecosystem of Gorontalo, Indonesia. Biodiversitas 25: 907-915.* The objective of this study was to isolate and identify the actinomycetes associated with the rhizosphere of plants in the karst ecosystem. This study was descriptive-quantitative. Soil sampling was conducted at three locations of the karst ecosystem of Gorontalo, i.e., Bangga Hills at Bangga Bubaa Coastal area of Gorontalo District, Hills around Lake Limboto of Gorontalo District, and Oluhuta Beach Hills at Bone Bolango District. Rhizosphere-soil sample was collected from 15-30 cm of soil depth. Isolation of actinomycetes was performed using the plate method. The screening of phosphate-solubilizing bacteria was based on the ability to solubilize phosphates indicated by the clear zone on the Pikovskaya medium. Identification of actinomycetes bacteria based on morphology and molecular characters. The morphology character included the color of aerial and substrate mycelium. Molecular characteristics based on the sequence of 16S rRNA gene compared to sequence data on the gene bank of NCBI. The phylogenetic tree reconstruction was based on the Neighbor-joining algorithm. The results successfully found six isolates, including three actinomycetes isolates, two non-actinomycetes bacteria, and one yeast. They were isolated from the rhizosphere of *Malastoma malabathrum, Chromolaena odorata, Cycas rumphii*, *Leucaena leucocephala*, and *Jatropha curcas*. The actinomycetes isolate showed plant-growth-promoting potential indicated by solubilizing phosphate and Indole 3-Acetic Acid production activities. Based on the molecular analysis, actinomycetes were closely related to *Streptomyces aegyptia* (MT505707.1:42-1426), *Streptomyces* sp. GGCR-6 (MH718844.1:5-1391), and *Streptomyces carpaticus* strain PES-A23 (MH712039).

**Keywords:** Actinomycetes, IAA, karst ecosystem, PGPR, rhizosphere, *Streptomyces*, yeast

## INTRODUCTION

Karst ecosystems are extremely marginal lands with low soil fertility, both chemical, physical, and biological. Karst soils are characterized by high calcium (Ca) content, which can affect the availability of Phosphorus (P) (Fan et al. 2019). This element in karst soil is bound by Ca into a form that is not available to plants. Fulfillment of plant P needs in marginal soil is largely determined by the activity of phosphate solubilizing bacteria which are in symbiosis with the root system. Fulfillment of nutrient needs for plant growth in karst ecosystems is supported by the association of plants with rhizosphere microbes (Ahemed et al. 2014; Glick 2012). Karst ecosystems are found in many areas, one of them is Gorontalo. The diversity of actinomycetes of the karst ecosystem of Gorontalo, especially actinomycetes associated with the plant rhizosphere in karst ecosystems has not been studied much.

Actinomycetes are native soil bacteria that are important in the biogeochemical cycle of elements. Actinomycetes are also important microorganisms as sources for the discovery of secondary metabolites for application in medicine (Oli et al. 2022) and agriculture (Chitraselvi 2018; Das et al. 2021; Silva et al. 2020), including antibiotics (Simeis and Serra 2021), anticancer (Davies-Bolorunduro et al. 2019; Elsayed et al. 2020). Interestingly, actinomycetes exhibit biocontrol, biopesticide agents, antifungal compounds, and biocorrosion and agroactive compounds (Prathyusha and Bramhachari 2018). Actinomycetes are plant growth-promoting rhizobacteria that promote plant growth either directly or indirectly (Karnwal 2009; Glick 2012; Sreevidya et al. 2016; Sathya et al. 2017). Several types of actinomycetes have the potential to produce PGP properties (Goudjal et al. 2018; Singh et al. 2018; Franco-Correa and Chavarro-Anzola 2015; Myo 2019; Wahyudi et al. 2019), including IAA hormones (Matsukawa 2007; Sameera et al. 2018; Myo et al. 2019), phosphate solubilizing (Faried et al. 2018, Boubekri et al. 2021), and siderophores (Takehana et al. 2017; Fatmawati et al. 2019). Myo et al. (2019) reported that *Streptomyces fradiae* NKZ-259 has the potential to produce IAA.

Actinomycetes are widely found in terrestrial, freshwater aquatic marine habitats, also associated with other organisms (Ghorbani-Nasrabadi et al. 2013). Actinomycetes also can interact with plants on their stem, leaf, and root on rhizospheres or plant root systems (Anwar et al. 2016; Walida et al. 2019). Ara et al. (2013), reported that the actinomycetes population on rhizosphere was higher than non-rhizosphere area, and varied based on kind of vegetation. The rhizosphere area containing the organic matter called root exudate that support to microorganisms

activities (Elshafie and Camele 2022). Some rhizosphere microbes act as a biocontrol against various pathogenic microbes in plants. Actinomycetes can survive under extreme soil conditions such as low levels of moisture (Zviagintsev et al. 2007), hypersaline habitats (Jose and Jebakumar 2014; Hamdali et al. 2008), marine habitats (Subramani and Sipkema 2019; Sarkar and Suthindhiran (2022), and karst ecosystems (Retnowati et al. 2017). Retnowati et al. (2017) reported that actinomycetes were found on the rhizosphere of some types of mangroves in the karst ecosystem, Gorontalo. The density, distribution, and diversity of actinomycetes are significantly affected by environmental factors, such as soil type and pH, and vegetation type (Zhang et al. 2019). The objective of this study was to isolate and identify the actinomycetes associated in the rhizosphere of plants in the karst ecosystem. This research was focused on exploring endemic actinomycetes from the rhizosphere of plants in karst ecosystems in the southern coastal region of Gorontalo. This research may be very important to answer the challenge of the widespread application of synthetic pesticides and fertilizers in agricultural systems that affect the physicochemical, and biological characteristics of soil, including the increasing plant pathogen resistance.

## MATERIALS AND METHODS

### Study area of karst ecosystem, Gorontalo, Indonesia

Soil sampling of plant rhizosphere on karst ecosystem of Gorontalo was conducted on three locations, i.e. Bangga Hills at Bangga Bubaa Coastal area of Gorontalo District (0°29'47.55"N; 122°33'6.17"E), Hills around Lake Limboto of Gorontalo District (0°32'43.22"N; 122°59'51.80"E), and Oluhuta beach hills of Bone Bolango District (0°25'10.05"N; 123°5'53.44"E) (Figure 1).

### Procedures

*Sampling of rhizosphere-soil sample on karst ecosystem*

Soil sampling was conducted on three locations of the karst ecosystem, i.e., Bangga Hills, the Bangga Bubaa Coastal area, Gorontalo District, Hills around Lake Limboto, Gorontalo District, and Oluhuta beach hills, Bone Bolango District. A total of 36 soil samples were collected from 33 types of plants. Rhizosphere-soil sample was collected at a depth of 15-30 cm in soil. The physicochemical analysis of soil samples including soil acidity and soil moisture.

*Isolation and purification of actinomycetes*

The initial treatment of rhizosphere soil samples was based on the dry wet method at 60°C for 15 minutes in a sterile ringer solution (Retnowati et al. 2017). Rhizosphere-soil samples of about 5.0 g were suspended in 45 mL of sterile physiological saline. The soil suspension was carried out with serial dilution up to $10^{-3}$. As much as 200 µL of each dilution was spread on Starch Casein Agar (SCA) and Raffinose Histidine Agar supplemented by 25 mg/mL of Cyclohexamide. The plates were incubated at 30°C for 14 days. Colonies showing different morphology characteristics were purified on SCA media. The actinomycetes isolates were stored for further analysis.

*Screening of plant-growth-promoting potential*

Solubilizing-phosphate activity: Actinomycetes isolate assayed the activity of solubilizing phosphate on the Pikovskaya medium (10 g/L glucose, 5 g/L $Ca_3PO4$, 0.5 g/L $(NH_4)2SO_4$, 0.2 g/L KCl, 0.1 g/L $MgSO_4.7H_2O$, 0.1 g/L $MnSO_4.H_2O$, 0.5 g/L yeast extract, and 0.01 g/L $FeCl_3.6H_2O$ at pH 7.0). The phosphate-solubilizing activity was determined based on the formation of clear zones (Widawati and Sulasih 2006).

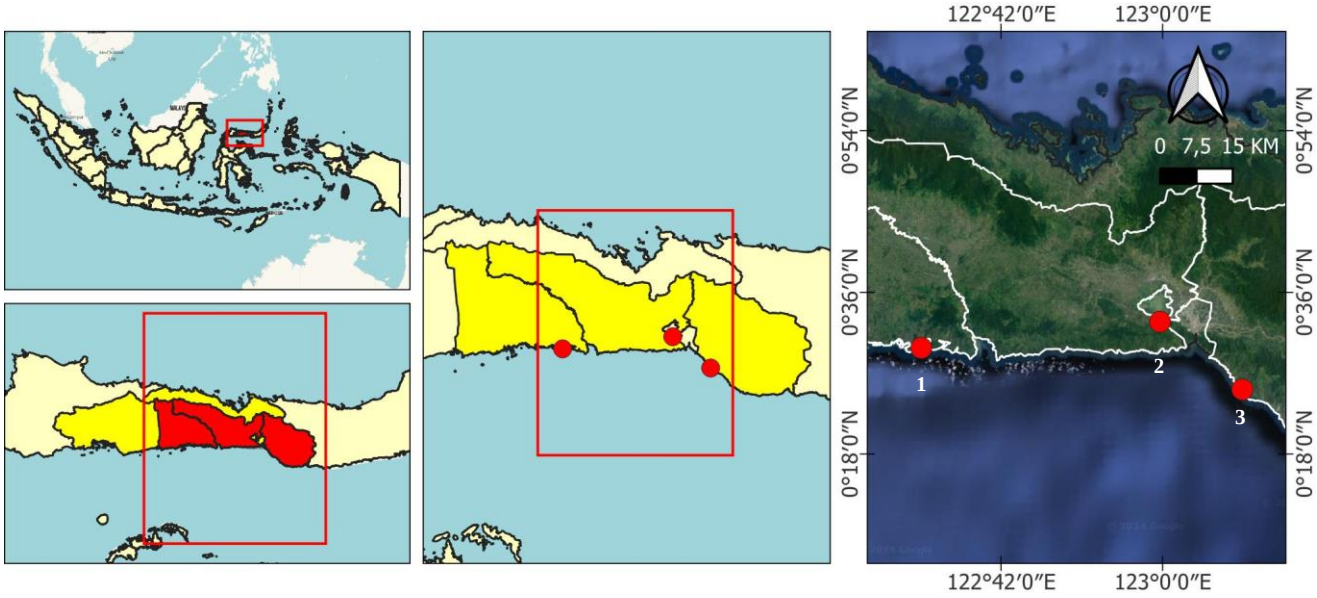


**Figure 1.** Location of karst ecosystem of Gorontalo, Indonesia. Note: 1. Bangga Hills at Bangga Bubaa Coastal area of Boalemo District (0°29'47.55"N; 122°33'6.17"E), 2. Hills around Lake Limboto of Gorontalo District (0°32'43.22"N; 122°59'51.80"E), and 3. Oluhuta beach hills of Bone Bolango District (0°25'10.05"N; 123°8'53.44"E)

IAA production activity: All pure isolates were inoculated into 250 mL conical flasks containing 50 mL LB broth media. The liquid cultures were incubated on a shaker incubator at 180 rpm for 2 days at 28 ± 1°C. Cultures were centrifuged at 12000 rpm for 10 min and 500 µL of supernatant from liquid cultures were taken into a 1.5 mL tube, and 1 mL of Salkowski reagent was added. Salkowski reagent was prepared by dissolving 2% of 0.5 M $FeCl_3$ in 35% perchloric acid. Reaction tubes were incubated for 30 min in the dark at room temperature. The development of pink color indicated the IAA production. Optical absorbance was measured at 530 nm (Baggam et al. 2017).

*Diversity analysis of actinomycetes*

The diversity of actinomycetes isolated from the rhizosphere of plants on the karst ecosystem of Gorontalo was based on molecular methods. Molecular characterization of actinomycetes followed the stages of extraction of genomic DNA, amplification of 16S rRNA gene, sequencing of 16S rRNA, and reconstruction of phylogenetic tree based on the neighbor-joining algorithm.

Extraction of genomic DNA: The extraction of genomic DNA actinomycetes and non-actinomycetes was carried out by following the protocol of the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) (Retnowati et al. 2023). Amplification of 16S rRNA gene: The amplification of the 16S rRNA gene was conducted by using kit MyTaq HS Red Mix, 2X (Bioline, BIO-25048) (B/7.2.1/IKP/002. The PCR Master Mix about 25 µL was consist of 9.5 µL ddH2O; 12.5 µL MyTaq HS Red Mix, 2x; 1 µL 10 µmol/µL 27F Primer (5'-AGAGTTTGATCMTGGCTCAG-3'); 1 µL 10 µmol/µL 1492R Primer (5'- GGTTACCTTGTTACGACTT-3'), and 1 µL DNA Template (Retnowati et al. 2017). The PCR program was operated for about 35 cycles, followed by an initial denaturation of 95°C for 3 min; denaturation at 95°C for 15 sec; annealing at 52°C for 30 sec; extension at 72°C for 45 sec; and final extension at 72°C for 3 min. The concentration of amplicon was determined using nanodrop, while the purity was determined based on spectrometry at 260 and 280 wavelengths. The qualitative detection of amplicon followed the visualizing of 0.8% of agarose gel electrophoresis in TBE Buffer (Retnowati et al. 2023).

The sequencing of 16S rRNA gene and analysis of phylogenetic: The PCR products of the 16S rRNA gene were purified by using the ZymocleanÔ Gel DNA Recovery Kit (Zymo Research). The amplicon was sequenced based on Sanger DNA Sequencing by using Capillary Electrophoresis. The 16S rRNA gene sequences were analyzed and edited by using BioEdit software (Retnowati et al. 2017). Then the 16S rRNA gene sequences were compared to the data of gene sequence on the GenBank of NCBI by using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned by using ClustalW, and a phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) XI software. The phylogenetic reconstructions are based on a neighbor-joining algorithm using the sequence of the 16S rRNA gene *Streptomyces sp*. as an outgroup (Retnowati et al. 2023).

**Data analysis**

The vegetation condition on karst ecosystem of Gorontalo, actinomycetes on plant rhizosphere, the phosphate solubilizing potential, and IAA production of actinomycetes were analyzed based on descriptive analysis. Molecular characterization data were compared with the NCBI GenBank.

## RESULTS AND DISCUSSION

**Description of karst ecosystem, Gorontalo**

Karst ecosystem in Bangga Hills at Bangga Bubaa Coastal area of Gorontalo District (0°29'47.55" N; 122°33'6.17" E), Hills around Lake Limboto of Gorontalo District (0°32'43.22" N; 122°59'51.80" E), and Oluhuta beach hills of Bone Bolango District are part of the karst ecosystem in Gorontalo. Karst ecosystem of Gorontalo showed varying physico-chemical soil conditions (pH, temperature, and moisture) and vegetation. The rhizosphere soils at the three locations tended to be acid to neutral with soil acidity values ranged from pH 5.5 to 7, while moisture ranged from 1.2 to 3.24% (Table 1).

Vegetation components in the karst ecosystem at three observation locations showed a variation of species. There were 33 species from the total number of vegetation species. In the Bangga Hills area, the vegetation consisted of 2 divisions, 9 orders, 9 families, 10 genera, and 12 species, in the around Lake Limboto Hills area the vegetation consisted of 2 divisions, 7 orders, 10 families, 11 genera 12 species, and the Oluhuta beach hills area the vegetation included 3 divisions, 6 orders, 8 families, 11 genera, 12 species. A total of 6 dominant species were found in those three locations. These dominant species, included *Cychas rumpii*, *Leucaena leucocephala, Imperata cylindrica, Malastoma malabathrum*, *Chromolaena odorata*, and *Jatropa curcas* (Table 2). The vegetation was found to be more dominated by groups of lower plants groups and tree-dwelling plants.

**Actinomycetes on plant rhizosphere of karst ecosystem, Gorontalo**

Actinomycetes are one component of the Gorontalo karst ecosystem. The observation results show that actinomycetes were found associated with several types of plants, especially in the rhizosphere of plants in the karst ecosystem of Gorontalo. A total of 3 actinomycetes isolates and 3 non-actinomycetes isolates were successfully cultured from rhizosphere soil samples using Starch Casein Agar (SCA) and Raffinose Histidine Agar (RHA) medium (Table 3). Result shows that each actinomycetes isolate was found to be associated with certain plant types. This shows that there was a specific interaction between actinomycetes and plant species in the karst ecosystem of Gorontalo. The results also showed that non-actinomycetes bacteria were also found specifically associated with specific types of plants. The actinomycetes isolated from plant rhizosphere tend to be similar in morphology character, dominated by the white color of aerial mycelium (Figure 2). The actinomycetes isolate also showed a similar spore ornamentation (Figure 3).

**Plant-growth promoting potential**

Actinomycetes and non-actinomycetes isolates were tested for their potential as growth-promoting agents based on their activity in dissolving phosphate and producing IAA. The research results showed that actinomycetes isolates showed potential as plant-growth-promoting bacteria, and vice versa for non-actinomycetes isolates (Table 4).

**Diversity microbes on the karst ecosystem of Gorontalo**

Actinomycetes and non-actinomycetes associated with the rhizosphere of plants in the karst ecosystem, Gorontalo were identified based on molecular characters based on the 16S rRNA gene sequence. Extraction of the genomic DNA using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) obtained about 1.92 to 2.02 of DNA purity. The amplification of the 16S rRNA gene using 27F and 1492R primer was successfully amplified at about 1500 bp based on visualizing based on DNA agarose gel electrophoresis.

**Table 1.** Physicochemical condition of three sites sampling site

| Locations | pH | Moisture |
|---|---|---|
| Bangga Hills | 6.5 | 1.2% |
| Hills around Lake Limboto | 5.5 | 2.5% |
| Oluhuta beach hills | 7.04 | 3.24% |

**Table 4.** The plant growth-promoting activities of actinomycetes isolated from the rhizosphere

| Isolates | Plant-growth promoting potential | |
|---|---|---|
| | Phosphate solubilizing | IAA producing |
| **Actinomycetes** | | |
| RACr isolate | + | + |
| RALl isolate | + | + |
| RAIc isolate | + | + |
| **Non-actinomycetes** | | |
| RAMm isolate | - | - |
| RACo Isolates | - | - |
| RAJc isolate | - | - |

Note: +: possess plant-growth promoting potential; -: possess no plant-growth promoting potential

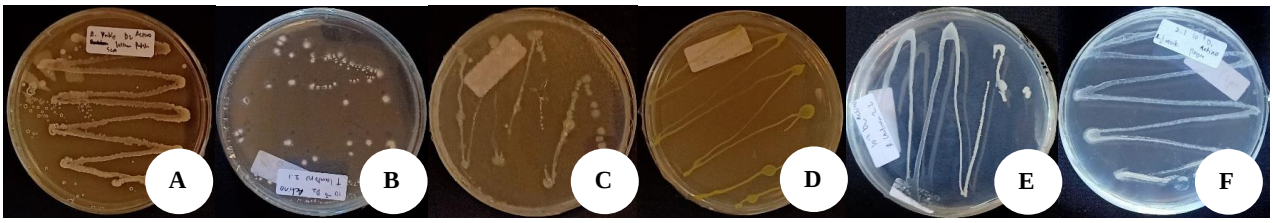


**Figure 2.** The colony of actinomycetes and non-actinomycetes isolates. A-C: actinomycetes isolates: a. RACr; b. RALl; c. RAIc; and d-f: non-actinomycetes isolates: d. RAMm; e. RACo; f. RAJc

**Table 2.** The vegetation on three sites of karst ecosystem on Gorontalo, Indonesia

| Bangga Hills | Hills around Lake Limboto | Oluhuta beach hills |
|---|---|---|
| *Leucaena leucocephala* (Lam.) de Wit | *Imperata cylindrica* (L.) Raeusch. | *Andrachne* L. |
| *Morinda citrifolia* L. | *Cascabela thevetia* (L.) Lippold | *Jatropha curcas* L. |
| *Desmostachya bipinnata* (L.) Stapf | *Jasminum* L. | *Celtis philippensis* Blanco |
| *Ficus retusa* L. | *Baleria priontis* L. | *Phyllanthus niruri* L. |
| *Lindera benzoin* (L.) Blume | *Melastoma malabathricum* L. | *Acalypha indica* L. |
| *Murraya paniculata* (L.) Jacq. | *Jatropha curcas* L. | *Persea americana* Mill. |
| *Baleria priontis* L. | *Chromolaena odorata* (L.) R.M.King & H.Rob. | *Macaranga tanarius* (L.) Müll.Arg. |
| *Justicia pinesis* S.Moore | *Porophyllum ruderale* (Jacq.) Cass. | *Origanum majorana* L. |
| *Justicia gendarussa* Burm.fil. | *Veronica cymbalaria* Bodard | *Chromolaena odorata* (L.) R.M.King & H.Rob. |
| *Ficus americana* Aubl. | *Leucaena leucocephala* (Lam.) de Wit | *Vallisneria spiralis* L. |
| *Terminalia catappa* L. | *Rivina humilis* L. | *Cycas rumphii* Miq. |
| *Jatropha curcas* L. | *Priva lappulacea* (L.) Pers. | *Lantana camara* L. |

Note: *): the vegetation found at some location of karst ecosystem, Gorontalo

**Table 3.** Actinomycetes on plant rhizosphere on karst ecosystem of Gorontalo, Indonesia

| Code of isolates | Characteristics of isolates | Associate plants |
|---|---|---|
| **Actinomycetes** | | |
| RACr isolate | The white color of aerial mycelium and black color of substrate mycelium | Pakis (*Cycas rumphii*) |
| RALl isolate | The white color of aerial and substrate mycelium | Lamtoro (*Leucaena leucocephala*) |
| RAIc isolate | The white color of aerial and substrate mycelium | Alang-alang (*Imperata cylindrica*) |
| **Non-actinomycetes** | | |
| RAMm isolate | Yellow color colony | *Malastoma malabathrum* |
| RACo isolate | White color colony (RACo Isolates) | Gulma siam (*Chromolaena odorata*) |
| RAJc isolate | The white color of aerial and substrate mycelium | Jarak pagar (*Jatropa curcas*) |

The sequencing of the 16S rRNA gene of Actinomycetes and non-actinomycetes isolates based on the bidirectional method obtained the length of sequence about 1371 to 1387 bp, even about 604 bp of RAJc isolate. The results of the analysis revealed that isolates RACr, RALl i, and RAIc showed 98.92-99.66% - similarities to the genus *Streptomyces*, including *Streptomyces aegyptia* (MT505707.1:42-1426), *Streptomyces* sp. GGCR-6 (MH718844.1:5-1391), and *Streptomyces carpaticus* strain PES-A23 (MH712039), while RAMm and RAJc isolates were closely related with *Microbacterium paraoxydans* strain SMV194#22 (KP780213.1) and *Bacillus Velezensis* strain LE51 (MN841781.1), respectively (Figure 4). RACo isolate showed similarity with the yeast of *Meyerozyma caribbica* strain AM89 (KM822611.1) (Figure 5).

## Discussion

Karst is a unique ecosystem that consists of a thin soil layer on the carbonate rocks. The uniqueness of karst ecosystem causes the type of vegetation to be more specific. The results of observations in the three locations of the karst ecosystems of Gorontalo showed that the plant can grow well in karst ecosystems. This triggers plants that live in this region to have adaptive properties to limestone content and drought. The high lime content in karst ecosystems causes some types of essential elements for plants to become unavailable forms. Some types of plants respond to these conditions through morphological and physiological adaptations of plant root systems. Morphological adaptation in the form of the ability to penetrate karst rock fracture gaps to reach the boundary of water sources (Whitten et al. 1999). While physiological adaptation through the ability to produce organic compounds (root exudate) that can dissolve phosphate (Farda et al. 2022).

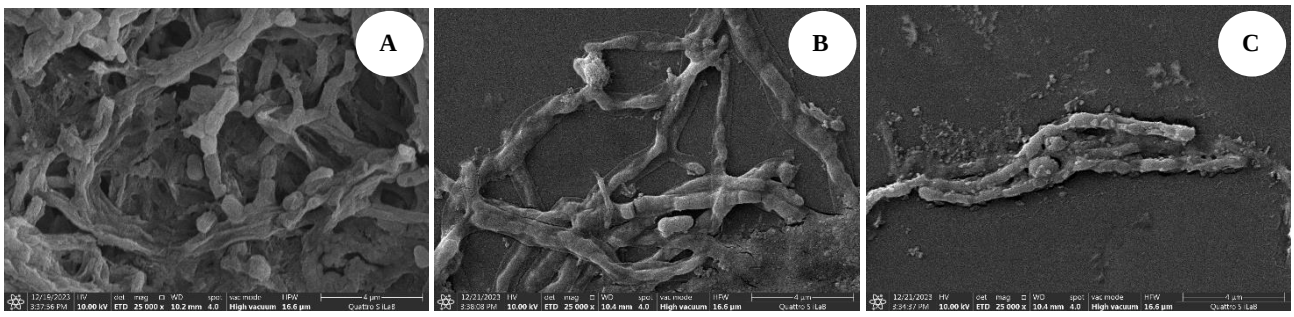


**Figure 3.** The ornamentation of spore-chain morphology and spore-surface of actinomycetes isolates at the magnification of ×25000. A. RACr isolate; B. RALl isolate; C. RAIc isolate


**Figure 4.** Neighbor-joining phylogenetic tree inferred from 16S rRNA gene sequence of actinomycetes from plant rhizosphere of karst ecosystem Gorontalo. The phylogenetic tree shows the phylogenetic relationship of isolates with related genera

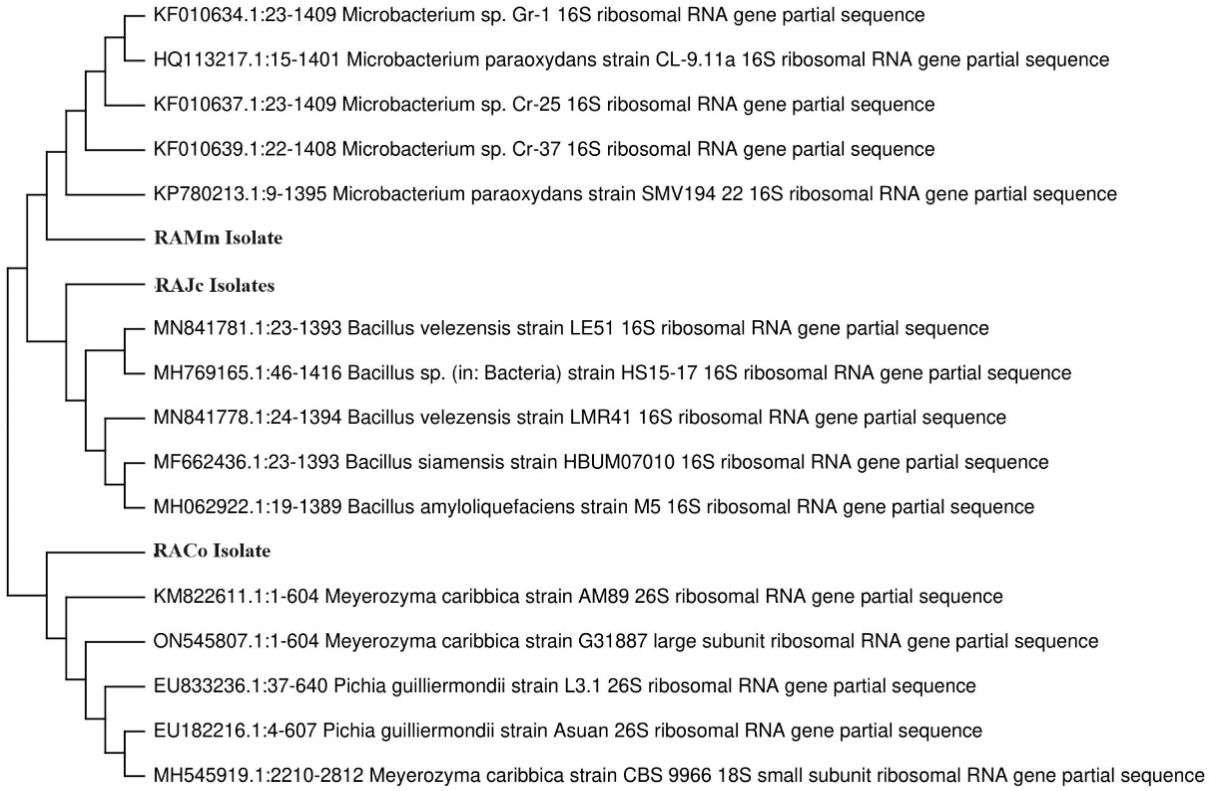


**Figure 5.** Neighbor-joining phylogenetic tree inferred from 16S rRNA and 26S rRNA gene sequence of non-actinomycetes from plant rhizosphere on karst ecosystem of Gorontalo. The phylogenetic tree shows the phylogenetic relationship of isolates with related genera

Physicochemical and biological characteristics of the root system of vegetation in the karst ecosystem at three observation sites revealed the presence of microorganisms associated with the root system. One of the microorganisms associated was actinomycetes. Actinomycetes play a role in the biogeochemical cycle of elements in the karst ecosystem at the observation site. Actinomycetes are spore-forming aerobic gram-positive bacteria that are abundant in the soil and play a major role in the organic matter cycle and decompose a complex mixture of polymers in the dead plant, animal, and fungal material (Bhatti et al. 2017). The presence of actinomycetes in the root system in the hill karst ecosystem at three observation sites shows the role of plant physiological activity. Bhatti et al. (2017) and Selim et al. (2021) suggest that actinomycetes in the plant rhizosphere play a role in decomposing a complex mixture of polymers in the dead plant, animal, and fungal material, produce extracellular enzymes that conductive for crop production, and protect plant roots by inhibiting the growth of plant pathogenic fungi in the rhizosphere.

Plants in the karst ecosystem, bacteria were found associated with bacteria in their root systems, including actinomycetes and non-actinomycetes groups. According to Putri and Sumerta (2020), there are 11 genera of actinomycetes found in the karst ecosystem of Semeulue Island. Elshafie and Camele (2022), also reported that the root system of rosemary, acacia, strawberry, and olive was associated with some kinds of actinomycetes strains. The results of the present study also showed that actinomycetes and non-actinomycetes were found to be specifically associated with certain types of plants in the karst ecosystems of Gorontalo. Pérez-Jaramillo et al. (2017) reported that the presence of microorganisms in the rhizosphere are dependent on the soil type, host species or kinds of plants, host plant genotype, and the root system architecture. Saleem et al. (2018), also reported that the rhizosphere is a narrow zone strongly affected by the roots plants activity, called by rhizosphere effect. This zone is characterized by intense biological activity owing to the release of root exudates, which stimulate or inhibit rhizosphere organisms. The root exudate, in a process known as rhizodeposition, consists of a complex mixture of carbon/nutrient sources, such as amino acids, sugars, and other nutrients provided by the plant. The exudate root is governed by the dynamic of the rhizosphere area by complex interactions between plants and organisms in close association with the root. The root exudates composition and pattern influenced the population, distributions, diversity, and microbial activity. This environment attracts a plant species-specific microbial community (Essarioui et al. 2017). The result shows that the association of actinomycetes and non-actinomycetes bacteria in the rhizosphere of plants in karst ecosystems may be influenced by rhizosphere physicochemical factors including root exudates produced by plant roots in karst ecosystems, Gorontalo.

Actinomycetes are commonly found in various types of soil, one of which is karst soil. The results showed that

actinomycetes and several non-actinomycetes bacteria showed the ability to grow in the karst ecosystem, Gorontalo. This shows the physiological adaptability of actinomycetes in karst ecosystems, namely the ability to dissolve phosphate to fulfill the need for the element phosphorus. Isolates of actinomycetes in the rhizosphere of several types of plants in the Gorontalo karst ecosystem show the ability to dissolve phosphate. According to Geekiyanage et al. (2019), karst soils tend to be low in phosphorus because lime-stone and dolomite have low phosphorus contents, and the high pH from $Ca^{2+}$ saturation further reduces the bioavailability of phosphorus. Farda et al. (2022) reported that caves or karst are rich in carbonates, sulfates, phosphates, and potassium-rich sediments, with diverse but approximately stable temperatures and humidity. These conditions shape a unique mineral solubilizing microflora. Soil mineral solubilizing microflora transforms complex and insoluble forms of minerals into simple nutrients. Farda et al. (2022) reported that the adaptation of actinomycetes to extreme environments and the associated interactions have led to the evolution of different biosynthetic potentials, instead some karst plants secrete organic acids with root exudates to increase phosphorus solubility. Widawati and Sulasih (2006) reported that actinomycetes which were isolated from the soil of Waigeo Island were able to dissolve phosphate into orthophosphate. The presence of actinomycetes and non-actinomycetes bacteria in the rhizosphere of plants in karst ecosystems and their potential to solubilize phosphate indicates an association between microbes and plants. The result showed that actinomycetes found in the rhizosphere of several types of plants in karst ecosystems show the ability to produce IAA. Marwati et al. (2017) also reported that there are 50 out of 85 isolates of *actinomycetes* capable of producing IAA hormones.

Bacteria associated with the plant root system in the Gorontalo karst ecosystem were identified as members of the genus *Streptomyces*, and several non-actinomycetes bacteria, including the genus *Microbacterium*, and *Bacillus*, and even Yeast, members of the genus *Meyerozyma*. The presence of microbes shows their involvement in the element cycles that occur in karst ecosystems. Santillán et al. (2021) found that karst soils in tropical forests have a diverse microbial component related to soil physicochemical and environmental characteristics. These microorganisms are related to critical biogeochemical processes that increase N-pool, P-availability, and C-cycling. Nurkanto (2007) reported that some kinds of actinomycetes are capable of solubilizing phosphate, including member genera *Nocardia* sp., *Streptomyces*, *Micromonospora* sp., *Actinoplanes* sp., *Microbispora* sp., *Microtetraspora* and *Streptosporagium* sp. Putri and Sumerta (2020) also found about eleven genera of actinomycetes from the karst cave soils of Simeulue island, one of which is *Streptomyces*. *Streptomyces griseorubens* BC10 and *Nocardiopsis alba* BC11 are promising candidates as phosphate solubilizing bacteria (Boubekri et al. 2021). According to Fan et al. (2019), indigenous microbial communities in soil are fundamental for healthy ecosystem function, owing to their role in mediating the circulation of various important materials, such as soil organic carbon and nitrogen. The soil bacterial community changes constantly with plant development and root growth, depending on the history of co-evolution among plants and soil microbial communities, to meet nutritional or physiological requirements. In addition, changes in soil factors can influence the composition of the microbial community. The relative abundances of rhizosphere resident microbial genera indicated that they were influenced by the geological conditions and vegetation type.

In conclusion, plants in the karst ecosystem of Gorontalo, show specific associations with various types of microbes in the rhizosphere. Actinomycetes and non-actinomycetes bacteria, even yeast groups were found to be specifically associated with certain types of plants. The physicochemical conditions which tend to be uniform in the karst ecosystem of Gorontalo were responded to by the presence of actinomycetes which were dominated by members of the genus *Streptomyces*. Actinomycetes could solubilize phosphate and produce plant growth Indole Acetic Acid. According to the results, rhizosphere-associate actinomycetes of plants in the karst ecosystem of Gorontalo potential to be developed as Plant Growth Promoting Rhizobacteria (PGPR).

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