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Molecular identification of rhizospheric Actinomycetes from karst ecosystems of Gorontalo, Indonesia, and its seed germination induction capability of *Zea mays* var. *doti*

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Abstract. *Retnowati Y, Katili AS, Kandowangko NY, Pembengo W. 2024. Molecular identification of rhizospheric Actinomycetes from karst ecosystems of Gorontalo, Indonesia, and its seed germination induction capability of* Zea mays *var.* doti. *Biodiversitas 25: 4763-4771.* Karst, as an extreme ecosystem, was a source of diverse Actinomycetes with varied biological activities. This study explored the plant-growth-promoting potential of rhizospheric Actinomycetes from the karst ecosystem of Gorontalo, with a focus on inducing seed germination in *Zea mays* var. *doti*. Four locations in Gorontalo were selected to explore Actinomycetes, targeting approximately 20 different rhizospheric plant species to isolate these microorganisms. Among the 25 isolates obtained, representing diverse morphological types from 12 rhizospheric plants, eight actinomycete isolates exhibited phosphate-solubilizing activity and produced indole-3-acetic acid (IAA). The 16S rRNA gene sequence analysis showed that approximately 75% of the isolates belonged to the *Streptomyces* genus, including *Streptomyces cavourensis* strain KRZm-02, *Streptomyces* sp. strain KRZm-03, *Streptomyces pratensis* strain KRLl-01, *Streptomyces carpaticus* strain KRIt-01, *Streptomyces* sp. strain KRIt-02, and *Streptomyces aquilus* strain KRPa-01. Additionally, 12.5% of the isolates were identified as *Nocardiopsis alba* strain KRZm-01 and *Micromonospora* sp. strain KRPt-01, respectively. The two isolates with the highest plant-growth-promoting potential, *Streptomyces pratensis* strain KRLl-01 and *Streptomyces carpaticus* strain KRIt-01, were further tested for their ability to promote germination of *Zea mays* var. *doti* seeds over 7 days. Among the two, *Streptomyces carpaticus* strain KRIt-01 exhibited the highest germination-inducing potential. Overall, the karst ecosystem of Gorontalo offers a valuable reservoir of biological resources with the potential for Plant-Growth-Promoting Rhizobacteria (PGPR). Further studies on the application of these actinomycete isolates as biofertilizers in agricultural and plantation crops could significantly contribute to improving crop growth and productivity, thereby revolutionizing agricultural practices.

Keywords: Actinomycetes, karst ecosystem, rhizosphere, seed germination, *Zea mays* var. *doti*

INTRODUCTION

Karst areas are landscapes formed by dissolving carbonate rocks, such as limestone. In Indonesia, most karst areas are composed of carbonate rocks, with only a small number formed from other materials, such as gypsum, salt, or evaporite (Goldscheider et al. 2019). The thin soil layer, limited nutrient availability-except for calcium and magnesium-extreme weather conditions, and intense heat, especially during the dry season, make karst areas less hospitable to most plant species (Aprilia et al. 2021). Different plants possess varying capacities for adaptation, resulting in the distinct and specialized vegetation found in karst environments (Liu et al. 2021). The appearance and composition of vegetation in these areas differ markedly from those in other ecosystems, with many plant species found in karst areas being endemic (Liu et al. 2022).

Karst ecosystems, with their unique characteristics, serve as habitats for various types of microorganisms that play a crucial role in the biogeochemical cycle. These microbes, through their significant contribution, enhance soil properties, thereby promoting vegetation succession (Li et al. 2019). Numerous studies have been conducted on microbial communities within karst ecosystems. In these environments, soil microbes are limited by the availability of carbon and phosphorus, which regulate pH through acidification processes and influence microbial diversity. The level of microbial diversity in karst soils can serve as an indicator for soil biomonitoring (Yun et al. 2016; Chen et al. 2019; Santillán et al. 2021). Furthermore, microbes in karst ecosystems have potential commercial applications as Plant-Growth-Promoting Rhizobacteria (PGPR), offering benefits for agriculture and plantations (Franco-Correa and Chavarro-Anzola 2015; Damam et al. 2016; Retnowati et al. 2024).

Actinomycetes are native soil microbes distributed across various habitats. The karst ecosystem is one such environment where Actinomycetes function as decomposers and contribute to the biogeochemical cycle. In karst ecosystems, Actinomycetes can exist either independently or in association with plants, acting as endophytes within plant tissues or interacting with the root system. Retnowati et al. (2018) reported that Actinomycetes found in the mangrove rhizosphere of the Gorontalo karst ecosystem exhibited bioactive compound-producing activity. Their presence in plant root systems also plays a key role in nutrient provision. Actinomycetes isolated from plant rhizospheres in karst ecosystems demonstrate phosphate-solubilising activity, converting unavailable phosphate into forms accessible to plants (Damam et al. 2016; Pan and Cai 2023). In addition, some Actinomycetes in karst environments produce the growth hormone indole-3-acetic acid (IAA), which promotes plant growth (Saadouli et al. 2022). However, the diversity and potential of Actinomycetes are influenced by environmental conditions and the vegetation types in the ecosystems.

In recent years, the application of beneficial Actinomycetes as Plant Growth-Promoting Rhizobacteria (PGPR) has become increasingly relevant in agriculture (Sreevidya et al. 2016; Sathya et al. 2017). Actinomycetes are known to form mutualistic associations with many plants (Ndeddy and Babalola 2016). They are capable of inducing phytohormonal modifications, producing exopolysaccharides, synthesizing antibiotic substances, fixing atmospheric nitrogen, producing soluble iron compounds (siderophores), and solubilizing inorganic phosphates (Vurukonda et al. 2016). Moreover, Actinomycetes act as plant growth stimulators by generating phytohormones such as indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC), cytokinins, and gibberellins (GA) (Ndeddy and Babalola 2017). These exceptional properties of PGPR contribute to the efficient stimulation of seed germination (Chukwuneme et al. 2020; Teo et al. 2021).

Zea mays with high productivity, is one of the most important cereal crops, following wheat and rice. However, crop yields have significantly declined due to decreasing soil fertility, largely caused by the prolonged use of commercial and inorganic fertilizers. The growth of maize plants depends heavily on the availability of nitrogen, phosphorus, zinc, and other essential elements in the soil. During the early growth phase, maize requires adequate nutrients-particularly nitrogen (N) and phosphorus (P)-to develop strong root systems and healthy seedlings. In the vegetative growth stage, nutrient uptake is especially high, with a significant demand for nitrogen, phosphorus, potassium (K), and micronutrients such as zinc (Zn) and manganese (Mn) (Batool 2023). The application of Plant Growth-Promoting Rhizobacteria (PGPR) presents an alternative solution for supplying essential nutrients to support maize growth. This study focuses on exploring Actinomycetes associated with plants in the karst ecosystem and evaluating their potential to promote maize seed germination. It is anticipated that this research will identify promising actinomycete isolates with PGPR properties capable of enhancing the growth and productivity of agricultural and plantation crops.

MATERIALS AND METHODS

Study area

Rhizosphere-soil sampling was collected at four locations of the karst ecosystem in the coastal area of Gorontalo, Indonesia, including the maize plantation area of Pahu at 0.807477, 122.436845; Potanga area at 0.54378, 123.032486; Tanjung Kramat area at 0.505562, 123.050775; and Lombongo forest area at 0.548138, 123.180695 (Figure 1).

Procedures

Rhizospheric soil sample

Soil sampling was conducted at four locations in the karst ecosystem of Gorontalo, Indonesia. It was based on a purposive sampling of the seedling-dominant plant at each location. The soil sampling at the maize plantation area of Pahu was divided into three zones based on gradient elevation (i) Top; (ii) Middle; and (iii) Low elevation. Rhizospheric soil was collected at 10 to 30 cm of soil depth for three replications. Three replicate soil samples from the same kinds of plant at each location were mixed equally to create a composite soil sample.

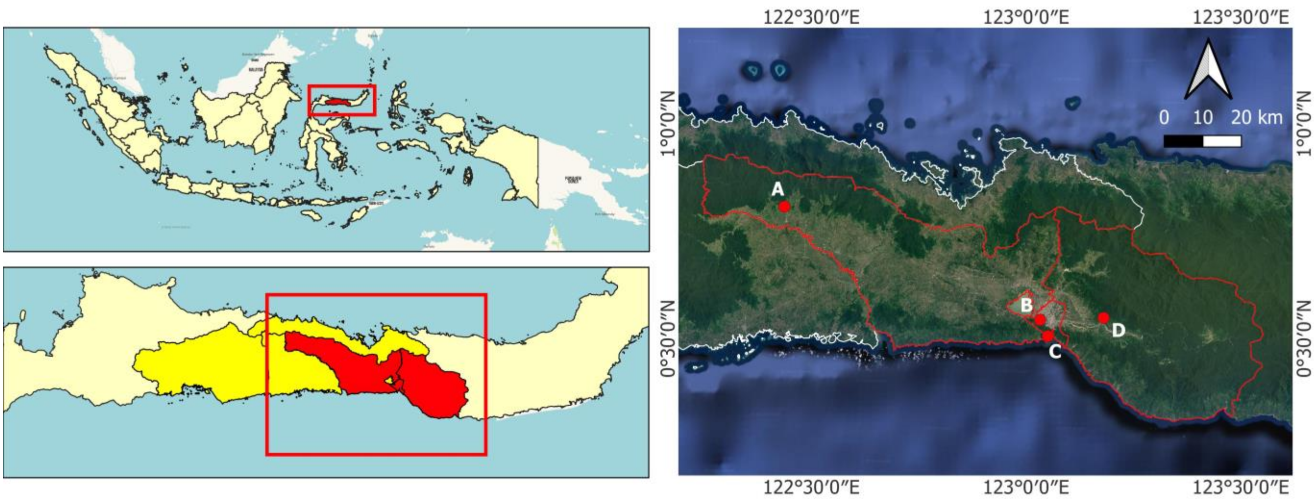

Figure 1. Rhizospheric soil sampling location at karst ecosystem of Gorontalo coastal area. A. Pahu, Gorontalo District; B. Potanga and C. Tanjung Kramat, Gorontalo City; D. Lombongo forest, Bone Bolango, Gorontalo, Indonesia

Isolation and purification of rhizospheric Actinomycetes

Rhizosphere soil samples (approximately 5 g) were suspended in 45 mL of sterile physiological saline (0.9% NaCl). The soil suspension was boiled in a water bath for 15 minutes at 70°C and then homogenized using a rotary shaker at 200 rpm for 30 minutes (Retnowati et al. 2017). Serial dilutions of the soil suspension were performed up to threefold, and 100 µL of the final dilution was spread onto Starch Casein Agar medium supplemented with 50 mg L^{-1} of cycloheximide. The plates were incubated for 14 days at 30°C. The Actinomycetes displayed varying colony morphologies and were purified, and the isolated Actinomycetes were subsequently used for further assays.

Molecular identification of Actinomycetes isolates

A pure culture of the isolates was grown in Starch Casein Broth medium for 7×24 hours in a shaker incubator set at 200 rpm. The pellet cells were separated from the supernatant by centrifugation for 15 minutes at 500 rpm. Genomic DNA was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) from the pellet cells following the manufacturer's protocol (Retnowati et al. 2023). The 16S rRNA gene amplicons were generated using the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') with 2X MyTaq HS Red Mix (BIO-25048) (Okolie et al. 2013). The amplification program involved approximately 35 cycles as follows: initial denaturation at 95°C for 3 minutes; denaturation at 95°C for 15 seconds; annealing at 52°C for 30 seconds; extension at 72°C for 45 seconds; and final extension at 72°C for 2 minutes. The genomic DNA was purified using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research) (Retnowati et al. 2023).

The consensus sequence of the 16S rRNA gene for each isolate was obtained using a bi-directional sequencing method (Retnowati et al. 2017). The sequences were compared to the nucleotide database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/blast/) using the Basic Local Alignment Search Tool (BLAST). Nucleotides with at least 99% similarity were identified for each isolate and subsequently downloaded for further analysis to confirm their identity (Retnowati et al. 2023).

Phosphate solubilizing assay

Actinomycetes isolate assayed the activity of solubilizing phosphate on the Pikovskaya medium (10 g/L glucose, 5 g/L Ca_3PO_4, 0.5 g/L $(NH_4)2SO_4$, 0.2 g/L KCl, 0.1 g/L $MgSO_4.7H_2O$, 0, 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 by forming clear zones on the Pikovskaya medium (Widawati 2008). Actinomycetes isolates that showed qualitative phosphate solubilizing ability were followed up with quantitative phosphate solubilizing ability analysis.

Indole Acetic Acid production analysis of Actinomycetes isolates

Actinomycetes isolates were inoculated into Nutrient Broth (NB) supplemented with 0.1 g of L-tryptophan and incubated in a shaker incubator at 150 rpm for 6×24 hours at room temperature. The supernatant and pellet cells were separated by centrifugation at 7,000 rpm for 30 minutes. To test for IAA production, 4 mL of Salkowski reagent was added to 1 mL of the supernatant, followed by incubation in the dark for 24 hours. A color change to pink in the supernatant indicated the presence of IAA (Pattern and Glick 2002). Isolates that qualitatively demonstrated IAA production were subjected to further quantitative analysis. For this, 25 mL of NB supplemented with 0.1 g/L of L-tryptophan was inoculated with the Actinomycetes isolates and incubated in a shaker incubator at 100 rpm and 30°C for 24 hours. The supernatant and pellet cells were then separated by centrifugation at 10,000 rpm for 10 minutes (Sukmadewi et al. 2015). Absorbance measurements were performed using a spectrophotometer at a wavelength of 535 nm. The IAA content was quantified by comparing the absorbance values with a standard IAA solution curve.

Evaluation of Actinomycetes for seed germination induction in Zea mays *var.* doti

The ability of Actinomycetes to induce seed germination in *Zea mays* var. *doti* was assessed using the following indicators: final germination percentage (GT), seed viability, germination rate, root length, shoot length, and the root-to-shoot ratio. The final germination percentage (GT) was calculated as the ratio of germinated seeds to the total number of seeds sown (Al-Ansari and Ksiksi 2016). Seed viability (DK) was estimated following the method described by Ghorbanpour and Hatami (2014), as follows:

DK=Σni/N x 100%

Where, ni: the sum of germinate seed at i-days germination; N: the sum of assayed seed. The calculation of germination rate was observed on the time interval of 7 days following Wawo et al. (2020) by the formula:

Kct=%TN7/etmal 7 + %TM14/etmal 14 +TN21/etmal 21

Where, Kct: % of germination rate; TN: % of germination; Etmal: time-germination interval. The percentage of growth seed enumerated is based on the sum of normal growth seed divided by total seed.

Data analysis

The data on Actinomycetes' seed germination induction capability were analyzed using descriptive quantitative methods. The strains' potential as Plant Growth-Promoting Rhizobacteria (PGPR) was evaluated based on their ability to solubilize phosphate, produce indole-3-acetic acid (IAA), exhibit antagonistic activity against root fungal pathogens, tolerate fungicides, and colonize the rhizosphere. The 16S rRNA sequence data of the Actinomycetes strains were analyzed by comparison with the NCBI GenBank database.

RESULTS AND DISCUSSION

Study areas for rhizosphere soil sampling in the karst ecosystem of Gorontalo

The karst ecosystem in Gorontalo spans several areas with diverse vegetation conditions. The locations selected for rhizosphere soil sampling were the Potanga area (0.54378, 123.032486) and the Tanjung Kramat area (0.505562, 123.050775). Two additional comparison sites were included: the wildlife sanctuary of Lombongo, part of the wildlife sanctuary of Bogani Nani Wartabone (0.548138, 123.180695), and the Maize Plantation of Pahu, located within the Nantu wildlife sanctuary (0.807477, 122.436845). The Tanjung Kramat and Potanga areas are characterized by karst mountains with limestone dominating the soil structure. In contrast, the Lombongo forest area is a natural tropical mountain forest classified as a conservation area. The Pahu area, previously a forested site, has been converted into maize farming land, with slopes ranging between 20° and 40°. The vegetation across the four locations varies significantly, with ten plant species identified as the primary sources of Actinomycetes isolates. Nine plant species were sampled from the Tanjung Kramat and Potanga areas: *Leucaena leucocephala*, *Indigofera tinctoria*, *Mesosphaerum suaveolens*, *Lantana montevidensis*, *Jatropha gossypiifolia*, *Imperata cylindrica*, *Dolichos oliveri*, *Catharanthus roseus*, and *Pneumatopteris pennigera*. In the Lombongo forest area, the focus was on pandanus species, including *Pandanus tectorius* and *Pandanus amaryllifolius*. In the Pahu area, the sampling focused on maize (*Zea mays*).

The physicochemical conditions across the four locations vary (Table 1). These variations are likely due to differences in each site's geographical position, slope, and land use, which play a significant role in shaping the soil's physicochemical characteristics. These variations create opportunities to discover a range of potential Actinomycetes with PGPR properties.

Describe rhizospheric Actinomycetes

In this study, we have successfully isolated 25 actinomycete isolates with distinct morphological characteristics from 12 plant species across four regions in Gorontalo. What's particularly impressive is the adaptability of these Actinomycetes, as their distribution varied across locations and plant species. Nine isolates were obtained from the rhizosphere of *Z. mays*, sixteen from *Pandanus* sp., and 22 isolates from nine other plant species in the karst ecosystem. Our analysis of their potential as Plant Growth-Promoting Rhizobacteria (PGPR), evaluated through phosphate-solubilizing ability and in vitro IAA production, revealed that eight isolates were identified as having PGPR potential. These eight actinomycete isolates exhibited varying capacities for phosphate solubilization and IAA production, both qualitatively and quantitatively (Table 2).

Actinomycetes isolated from the rhizosphere of six plant species in the karst ecosystem of Gorontalo, which exhibited phosphate-solubilizing activity and produced the growth hormone indole-3-acetic acid (IAA), were identified based on 16S rRNA gene sequences. A homology search using the BLAST tool confirmed the identity of the isolates on the NCBI database. The data showed that out of the 8 Actinomycetes isolates, 6 belong to the genus *Streptomyces*, 1 isolate belongs to the genus *Nocardiopsis*, and 1 isolate belongs to the genus *Micromonospora*. This indicates the dominance of *Streptomyces* in the soil sample. All isolates have a homology percentage above 99.5%, indicating a genetic identity that is highly similar to the reference species. An E-value of 0 for all isolates indicates a high level of confidence in the phylogenetic identification (Table 3). Phylogenetic analysis revealed that most of the actinomycete isolates (75%) belong to the genus *Streptomyces* identified as *Streptomyces cavourensis* strain KRZm-02, *Streptomyces* sp. strain KRZm-03, *Streptomyces pratensis* strain KRLl-01, *Streptomyces carpaticus* strain KRIt-01, *Streptomyces* sp. strain KRIt-, and *Streptomyces aquilus* strain KRPa-01. In addition, 12.5% of the isolates were identified as *Nocardiopsis alba* strain KRZm-01 and 12.5% as *Micromonospora* sp. strain KRPt-01 (Figure 2).

Table 1. The physicochemical characteristics of soil at the soil sampling location

Location	Ordinate soil sampling	pH	Humidity (%)
Pahu	0.807477, 122.436845	5.1	4.6
Potanga	0.54378, 123.032486	6.6	1.6
Tanjung Kramat	0.505562, 123.050775	5.43	4.2
Lombongo	0.548138, 123.180695	3.4	6.1

Table 2. Phosphate solubilizing and IAA-producing activity of Actinomycetes

Actinomycetes isolates	Host plant	Phosphate solubilizing (ppm)	IAA-producing (ppm)
KRZm-01	*Zea mays*	1.63	7.94
KRZm-02	*Zea mays*	1.55	4.43
KRZm-03	*Zea mays*	0.96	3.75
KRLl-01	*Leucaena leucocephala*	1.51	0,64
KRIt-01	*Indigofera tectorial*	1.73	0.45
KRIt-02	*Indigofera tectorial*	1.34	0.43
KRPa-01	*Pandanus amaryllifolius*	1.97	2.14
KRPt-01	*Pandanus tectorius*	2.11	2.25

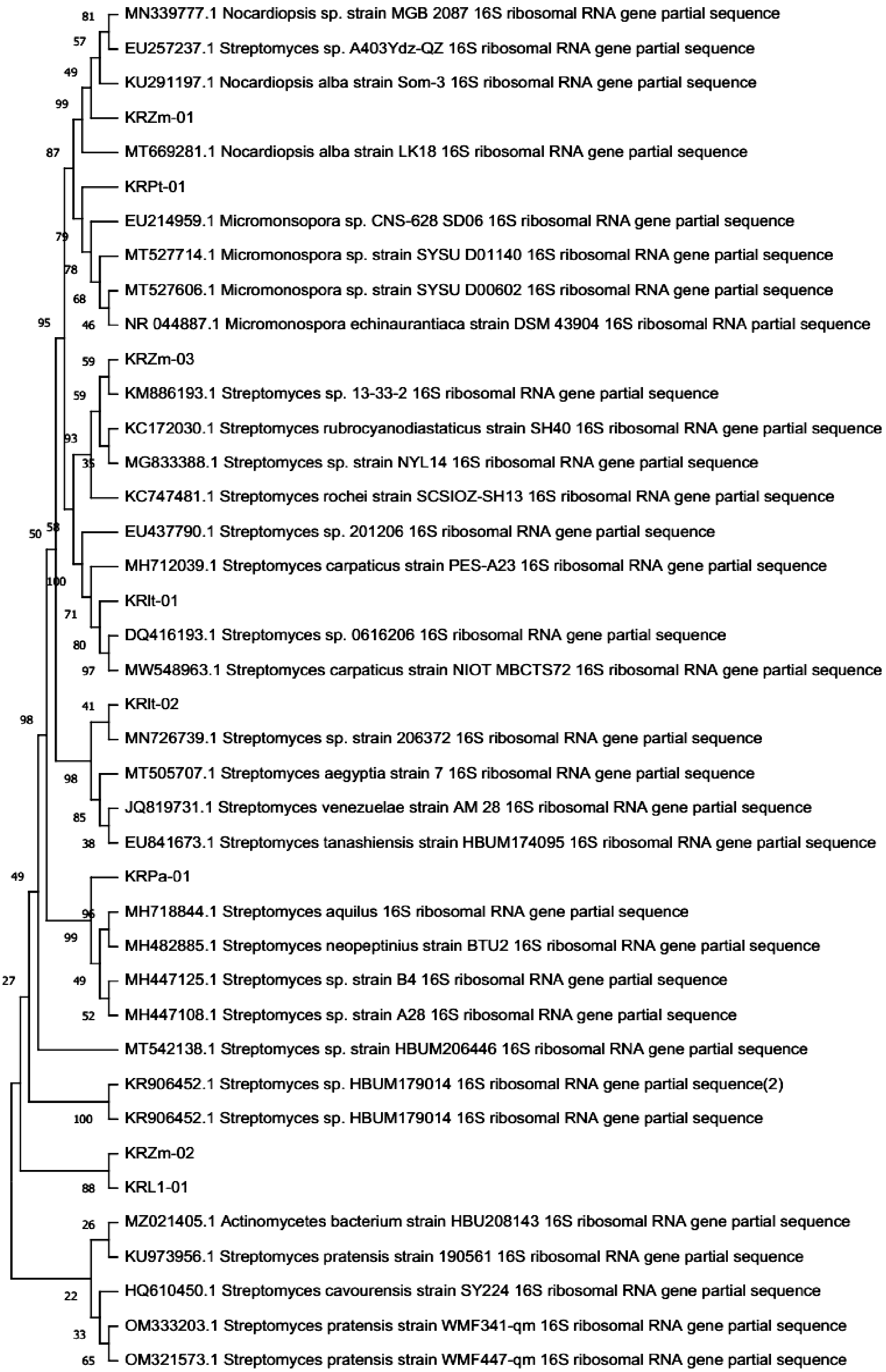

Figure 2. Neighbor-joining phylogenetic tree inferred from 16S rRNA gene sequence of Actinomycetes isolated from the karst ecosystem of Gorontalo. The phylogenetic tree revealed the phylogenetic relationship of isolates with related genera

Assessment of Actinomycetes for maize seed germination induction

The actinomycete isolates with the highest phosphate-solubilizing and IAA-producing activities were further tested for their ability to induce the germination of *Zea mays* var. *doti* seeds. These seeds, obtained from BSIP Gorontalo, belong to a local variety that farmers in the region rarely cultivate. Two actinomycete isolates, identified as *Streptomyces pratensis* strain KRLl-01 and *Streptomyces carpaticus* strain KRIt-01, were evaluated for their ability to promote corn seed germination. The evaluation included several indicators: final germination percentage (GT), seed viability, germination rate, root length, shoot length, and root-to-shoot ratio. The results demonstrated that both isolates successfully induced seed germination over 7 days. *Streptomyces carpaticus* strain KRIt-01 enhanced seed germination by approximately 75.11%, while *Streptomyces pratensis* strain KRLl-01 improved it by about 106.14% compared to untreated seeds (Table 4).

Discussion

Karst ecosystems, characterized by high carbonate content, present a limiting environment for the growth of many plant species. The presence of plants in these ecosystems results from both morphological and physiological adaptations of their root systems to the physicochemical conditions of the environment. Morphological adaptations include the ability of roots to penetrate crevices in karst rocks to access water sources (Whitten et al. 1999). In contrast, physiological adaptations involve the production of organic compounds (root exudates) that function to solubilize phosphate (Farda et al. 2022). The types of plants found in karst ecosystems, each with its unique set of adaptations, influence the diversity and presence of microorganisms in the soil. Nine species of herbaceous, shrubby, and bush plants in the karst ecosystem were found to host 22 actinomycete isolates, each displaying distinct morphological characteristics. The rhizosphere, or root zone, serves as a microhabitat for a variety of microbes. The physicochemical conditions of the soil associated with each plant species are shaped by the physiological activity of their root systems through the production of root exudates. Root exudates consist of complex mixtures of carbon and nutrient sources, such as amino acids, sugars, and other essential nutrients, which promote microbial growth in the soil (Essarioui et al. 2017). Each plant species produces specific exudates, which in turn influence the abundance, distribution, and diversity of soil microorganisms (Saleem et al. 2018).

Microorganisms in karst ecosystems play a crucial role in the biogeochemical cycling of elements. The key ecosystem services in karst are driven by microorganisms that enhance soil properties, facilitate vegetation succession (Zhou et al. 2019), and act as indicators of ecological restoration (Hu et al. 2016). Santillán et al. (2021) found that karst soils in tropical forests contain a diverse microbial community influenced by soil physicochemical and environmental characteristics. Actinomycetes, a group of bacteria known for producing bioactive compounds, offer significant benefits to their host plants. Their presence in the root system can suppress pathogenic microbes, promoting healthier plant growth. Additionally, some Actinomycetes exhibit phosphate-solubilizing ability. In karst ecosystems, where plants may lack the physiological capability to produce phosphate-solubilizing enzymes, Actinomycetes provide essential support. Phosphate, typically bound due to the high calcium content in karst soils, can be converted into a bioavailable form through the enzymatic activity of Actinomycetes, particularly via phosphatase and phytase enzymes (Sharma et al. 2017; Solans et al. 2019).

Table 3. The nearest phylogenetic relative of Actinomycetes isolates based on the 16S RNA sequencing

Code of Actinomycetes isolate	Nearest phylogenetic neighbor	Homology (%)	E -value	Genus
KRZm-01	*Nocardiopsis alba* (KU291197.1)	99.86	0	*Nocardiopsis*
KRZm-02	*Streptomyces cavourensis* (HQ610450,1)	99.89	0	*Streptomyces*
KRZm-03	*Streptomyces* sp. (KM886193.1)	99.64	0	*Streptomyces*
KRLl-01	*Streptomyces pratensis* (OM333203.1)	99.71	0	*Streptomyces*
KRIt-01	*Streptomyces carpaticus* (MH712039.1)	100	0	*Streptomyces*
KRIt-02	Streptomyces sp. (MN726739.1)	99.57	0	*Streptomyces*
KRPa-01	*Streptomyces aquilus* (MH718844.1)	99.64	0	*Streptomyces*
KRPt-01	*Micromonospora* sp.(EU214959.1)	99.66	0	*Micromonospora*

Table 4. The capability of rhizospheric Actinomycetes from the karst ecosystem of Gorontalo to induce seed germination in *Zea mays* var. *doti*

Actinomycetes	Final germination (%)	Seed viability (%)	Germination rate	Root length (mm)	Shoot length (mm)	Root:shoot ratio
Streptomyces carpaticus strain KRIt-01	93.33	93.33	15.55	34.27	15.95	2.14
Control 1 (without *S. carpaticus*)	26.67	26.67	8.88	10.82	6.28	1.72
Streptomyces pratensis strain KRLl-01	66.67	66.67	12.08	21.52	17.49	1.23
Control 2 (without S. *pratensis*)	33.34	33.34	5.86	9.46	5.53	1.71

Actinomycetes isolated from the karst ecosystem of Gorontalo have demonstrated phosphate-solubilizing ability. Another important physiological activity of Actinomycetes is the production of the growth hormone indole-3-acetic acid (IAA). Mulani et al. (2021) reported that phytohormones such as auxins, gibberellins, cytokinins, ethylene, and abscisic acid are predominantly produced by rhizosphere microorganisms, fungi, algae, and Actinobacteria. These physiological functions greatly support plant growth, especially in marginal soils. Myo et al. (2019) found that IAA production by *Streptomyces fradiae* NKZ-259 significantly improved tomato seedling growth. In this study, eight of the 25 actinomycete isolates successfully obtained from the rhizosphere of plants in the karst ecosystem of Gorontalo exhibited both phosphate-solubilizing ability and IAA production. These findings not only support the theory of specific interactions between Actinomycetes in the root system and their host plants but also highlight the importance of collaborative research in advancing our understanding of the role of microorganisms in karst ecosystems.

Actinomycetes isolated from the rhizosphere of plants in the karst ecosystem of Gorontalo, which exhibited phosphate-solubilizing and IAA-producing activities, were successfully identified as members of the genera *Streptomyces*, *Nocardiopsis*, and *Micromonospora*. Soumare et al. (2021) reported that certain actinobacteria, including members of the genera *Nocardia* and *Streptomyces*, demonstrated phosphate-solubilizing activity. Putri and Sumerta (2020) also identified eleven genera of Actinomycetes from karst cave soils on Simeulue Island, including *Streptomyces*. Furthermore, *Streptomyces griseorubens* BC10 and *Nocardiopsis alba* BC11 have been identified as promising candidates for phosphate-solubilizing bacteria (Boubekri et al. 2021). Retnowati et al. (2024) reported that three types of Actinomycetes were successfully isolated from the karst ecosystem and identified as members of the genus *Streptomyces*, along with several non-actinomycete bacterial species. The diversity of Actinomycetes in the karst ecosystem of Gorontalo was found to include only three genera, predominantly dominated by the genus *Streptomyces*. This is suspected to be influenced by the physicochemical properties of the soil in the karst ecosystem. Chen et al. (2019) reported that the limited microbial resources in karst ecosystems determine soil microbial activity. Soil microbes in karst ecosystems are more constrained by carbon and phosphorus than by nitrogen. Further analysis revealed that the patterns of carbon and phosphorus limitations vary across different land uses or lithologies. This is supported by D'Amico et al. (2015), who found that lithology influences soil properties and the structure of microbial communities.

Actinomycetes play an important role in various aspects of human life with their varied metabolic and physiological activities. Those with phosphate-solubilizing and IAA-producing activities hold great potential for development as Plant Growth-Promoting Rhizobacteria (PGPR) to support plant growth. The application of these microbes, particularly in marginal soils, is highly beneficial in providing essential macronutrients for plant development. Yadav et al. (2018) reported that the use of Actinomycetes as PGPR has become increasingly prevalent in agricultural practices. Globally, PGPR are rhizosphere bacteria that enhance plant growth through several mechanisms, including the production of phytohormones, siderophores, volatile organic compounds (VOCs), and toxins that inhibit pathogens, as well as the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. PGPR with antifungal activity also interacts with plants by inducing systemic resistance (ISR), solubilizing phosphate, fixing biological nitrogen (BNF), engineering the rhizosphere, interfering with quorum sensing (QS) signals, and inhibiting biofilm formation (Bhattacharyya and Jha 2012; Kumar and Singh 2020). The phosphate-solubilizing and IAA-producing Actinomycetes isolated from the karst ecosystem of Gorontalo demonstrated the ability to induce seed germination in *Z. mays* var. *doti*. Studies by Amaresan et al. (2018), Cinkocki et al. (2021), and Abd-Alla et al. (2013) have reported that IAA-producing endophytic Actinomycetes, including *Streptomyces atrovirens*, *Streptomyces olivaceoviridis*, *Streptomyces rimosus*, *Streptomyces rochei*, and *Streptomyces viridis*, significantly improved seed germination, root elongation, and plant growth.

The ability of Actinomycetes from the Gorontalo karst ecosystem to induce germination in *Z. mays* var. *doti* demonstrates the capacity of these actinomycete isolates to interact with the root system of *Z. mays* var. *doti*. Microorganisms associated with the rhizosphere sense and interpret signals produced by themselves, other microbes, and plants, and they can influence their host plants through the release of signaling molecules. The main outcomes of this communication include the induction of plant immunity, stress tolerance, overall growth, health, nutrition, and the maintenance of an associated rhizosphere microbiome (Kakkar et al. 2015; Xu et al. 2015). The ability of actinomycete isolates from the rhizosphere of plants in the karst ecosystem to produce IAA (indole-3-acetic acid) is another factor that facilitates the interaction between Actinomycetes and the root system of *Z. mays* var. *doti*. This further supports the recommendation of Actinomycetes from the karst ecosystem as biofertilizers for agricultural ecosystems. However, further testing is needed to evaluate the colonization ability of actinomycete isolates with various types of agricultural crops better to understand their interaction potential across different plant species.

In conclusion, Actinomycetes interact with various plants in the Gorontalo karst ecosystem. Actinomycetes identified as members of the genera *Streptomyces*, *Nocardiopsis*, and *Micromonospora* exhibited characteristics of Plant Growth-Promoting Rhizobacteria (PGPR) through their ability to solubilize phosphate and produced the growth hormone IAA. In particular, *Streptomyces* demonstrated the ability to induce maize seed germination. Therefore, Actinomycetes from the Gorontalo karst ecosystem have the potential to be developed as biostimulants to enhance plant growth.

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