BIODIVERSITAS
Volume 26, Number 7, July 2025
Pages: 3294-3301

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260721

Bioprospecting and molecular identification of *Streptomyces* on karst ecosystems in the coastal area of Gorontalo, Indonesia, as plant growth-promoting rhizobacteria

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Manuscript received: 7 November 2024. Revision accepted: 28 June 2025.

Abstract. *Retnowati Y, Katili AS, Kandowangko NY. 2025. Bioprospecting and molecular identification of* Streptomyces *on karst ecosystems in the coastal area of Gorontalo, Indonesia, as plant growth-promoting rhizobacteria. Biodiversitas 26: 3294-3301.* Agricultural systems that rely on synthetic pesticides, chemical fertilizers, herbicides, and monoculture farming systems contribute to declining soil fertility and affect the soil microbial community. In contrast, organic farming systems incorporate Plant Growth-Promoting Rhizobacteria (PGPR) as a potential solution. Therefore, this study aimed to explore the prospecting of *Streptomyces* from the rhizosphere of plants in the karst ecosystem PGPR on the coastal area of Gorontalo by using a molecular approach to identify promising strains. The success of this study in isolating and identifying three promising *Streptomyces* strains is a testament to the robustness of this study. Soil sampling was conducted at three locations at a 15-30 cm depth. The PGPR potential of *Streptomyces* was determined based on phosphate-solubilizing activity, Indole acetic acid production, antagonism to the root pathogenic fungus, and tolerance to fungicide capability. This study successfully isolated three *Streptomyces* from *Jatropha curcas* rhizosphere. In line with these findings, 16S rRNA sequencing showed the affinity of the strain to the genus *Streptomyces* on 98.39 to 99.65% of homology, including *Streptomyces* sp. strain KRJc-01*, Streptomyces rochei* strain KRJc-02, and *Streptomyces pratensis* strain KRJc-03. The initial in vitro assay showed the potential ability of *Streptomyces* sp. in phosphate-solubilizing activity, indole acetic acid production, antagonistic against root-pathogenic fungi, and fungicide tolerance capability. Antagonistic activity against the root-pathogenic fungus of *Fusarium oxysporum* is supported by PKSs and NRPS gene cluster. In general, these findings suggest that *Streptomyces* sp. from the rhizosphere of plants in the karst ecosystem within Gorontalo has significant potential as PGPR, contributing to sustainable crop improvement.

Keywords: Karst ecosystem, plant growth-promoting rhizobacteria, rhizosphere, *Streptomyces*

INTRODUCTION

Plants are frequently subjected to various environmental stresses that can affect their growth and development, and one of the most significant stress factors is the macronutrient deficiency in the soil. Among these essential macronutrients, phosphorus plays an important role in plant growth and development (Fonseca et al. 2020). However, its limited availability in the soil poses a significant challenge to crop productivity. Although phosphorus is abundant in the lithosphere, the plant-available form, inorganic orthophosphate (Pi), is poorly soluble and diffuses slowly in the soil. As a result, phosphorus deficiency is a common issue in agricultural land and natural ecosystems. Soil microbiota influences phosphorus availability, which can either compete with plants for phosphorus or form beneficial relationships, such as mycorrhizae, to enhance phosphorus acquisition efficiency. The application of chemical fertilizers is not an ideal solution, as only 15-25% of the applied phosphorus is absorbed by plants. Meanwhile, the remainder is washed away by surface and groundwater, immobilized, and leaves residues in the soil. This inefficiency contributes to soil degradation and water eutrophication, further increasing environmental pollution concerns (Soumare et al. 2021).

Plant growth is often challenged by root pathogens, which attack plants to extract nutrients. Roots play a vital role in plant health by absorbing essential macro- and micronutrients from the soil. Some pathogens are obligate parasites, surviving only on living plants, while others persist on both living and dead plant tissues. Next, to manage these pathogens, modern agriculture heavily relies on synthetic fungicides. However, their excessive use has led to serious environmental concerns, including the emergence of resistant pathogens and the accumulation of fungicide residues in the soil. These residues disrupt the soil microbial community, degrading beneficial microbes responsible for nutrient cycling and plant nutrient supply. To mitigate these issues, Plant Growth-Promoting Rhizobacteria (PGPR) offer an eco-friendly alternative by enhancing nutrient availability and supporting plant health. Certain soil microbes form mutualistic relationships with plant roots, improving nutrient uptake and overall growth (Ahemad et al. 2014; Faried et al. 2018). Additionally, some rhizosphere microbes act as biocontrol agents, protecting plants from pathogens. Among them, *Streptomyces* stands out as a promising PGPR candidate for sustainable agricultural applications. *Streptomyces* are soil-native bacteria that play an important role in the biogeochemical cycles of elements. This bacterial is also known as a producer of secondary metabolites, including antibiotics (Katili and Retnowati 2017; Retnowati et al. 2018; Rangseekaew and Pathom-aree 2019), anticancer (Alhawsawi et al. 2023) and Plant-Growth Promoting (PGP) (Damam et al. 2016; Franco-Correa and Chavarro-Anzola 2016; Ali et al. 2021; Retnowati et al. 2024). *Streptomyces* in the soil is found independently or interacts with the plant root system to form a symbiotic mutualism (Damam et al. 2016). Several types of *Streptomyces* in the root system have the potential to produce PGP in the form of IAA hormones, phosphate solubilizing, and siderophores (Damam et al. 2016; Terra et al. 2021; Chouyia et al. 2022). The existence of PGP-producing *Streptomyces* that colonize the root system significantly benefits plants, particularly those growing in marginal soils or environments where essential nutrients are present in inaccessible forms.

Streptomyces was distributed on several types of soil. Retnowati et al. (2017) reported that antibiotic-producing actinomycetes were found on the mangrove rhizosphere of the karst ecosystem within mangrove Torosiaje Gorontalo. The actinomycetes are also found specifically distributed in plant rhizosphere on the karst ecosystem (Syahril et al. 2023; Retnowati et al. 2024). Subsequently, Karst ecosystems are characterized by extreme-marginal soils with low soil fertility. Gorontalo has a wide area of karst ecosystems, but the *Streptomyces* potential has been extensively studied. Therefore, this study aimed to explore phosphate-solubilizing *Streptomyces* endemic to the plant rhizosphere in the karst ecosystem in the coastal area of Gorontalo. Investigations on *Streptomyces* as phosphate solubilizers have been widely reported, but information about *Streptomyces* associated with plant rhizosphere in karst ecosystems is still very limited.

MATERIALS AND METHODS

Study area

Rhizosphere-soil sampling was collected at three locations of the karst ecosystem on the coastal area of Gorontalo, Indonesia: Dulanga beach (0°29'40.29"N 123° 1'43.66"E), Kurunai beach (0°28'41.73"N 123° 5'23.50"E), and Otanaha Fortress (0°32'53.64"N 123° 0'30.29"E) (Figure 1). A rhizosphere-soil sample was carried out from the dominant plant at each location with about 15-30 cm of soil depth.

Procedures

Isolation and purification of Streptomyces

Isolation of *Streptomyces* was carried out with pre-treatment of the soil sample. 5 g of soil samples were suspended in 45 mL of sterile physiological saline (0.9% NaCl), boiled at 70°C for 15 minutes, and finally homogenized with a rotary shaker at 200 rpm for 30 minutes (Alwi et al. 2020). Isolation of *Streptomyces* was carried out using a selective medium of Raffinose-histidine Agar (RHA) supplemented by 50 mg/L of cyclohexamide. The soil suspension was carried out with serial dilution up to 10^{-3}. Approximately 200 µL of each dilution was inoculated on RHA medium based on the surface plate method. The plate was incubated at 30ºC between 14 to 28 days. Colonies showing different morphology characteristics were purified on the ISP2 medium.

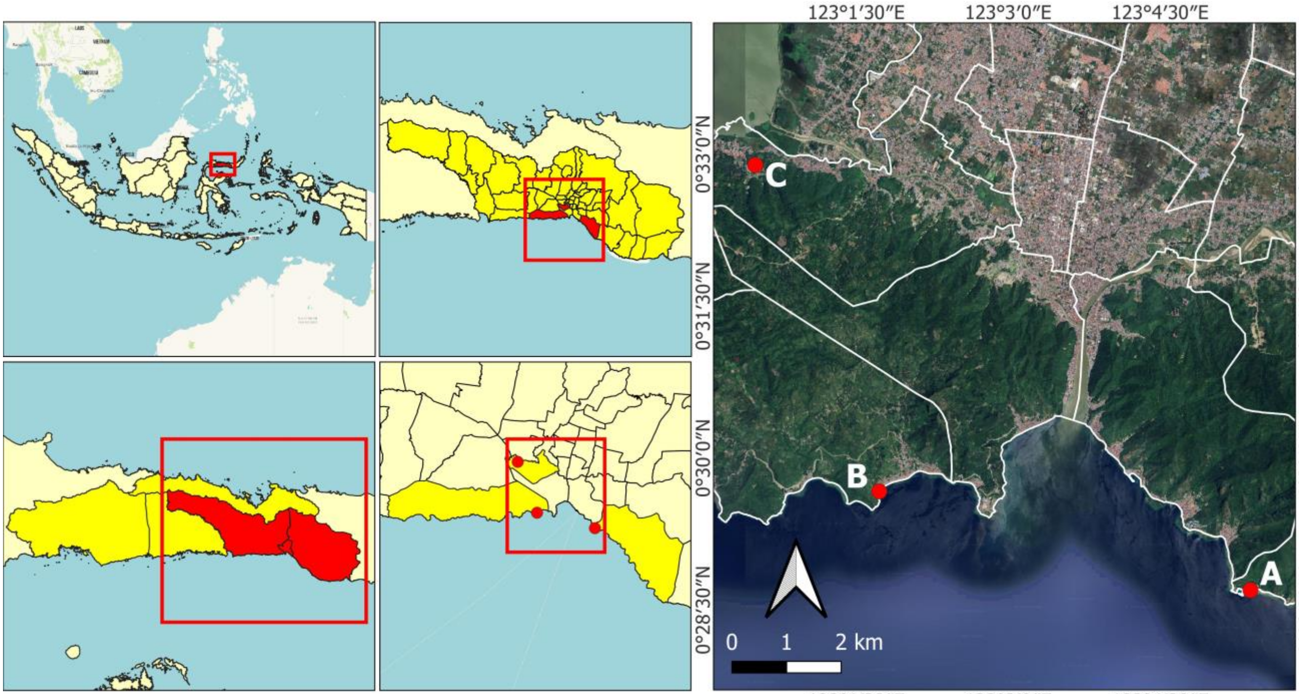

Figure 1. Rhizosphere-soil sampling location on the karst ecosystem of Gorontalo, Indonesia. A. Kurunai beach (0°28'41.73"N 123° 5'23.50"E), B. Dulanga beach (0°29'40.29"N 123° 1'43.66"E), C. Otanaha Fortress (0°32'53.64"N 123° 0'30.29"E)

Identification of Streptomyces

The identification of *Streptomyces* up to the species level was carried out by a molecular approach based on the 16S rRNA gene sequence. The genomic DNA of *Streptomyces* was extracted by following the protocol of the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) (Retnowati et al. 2023). The 16S rRNA gene was amplified using 2X MyTaq HS Red Mix (BIO-25048) and universal primers: 27F (5'-`AGA GTT TGA TCM TGG CTC AG`-3') and 1492R (5'-`GGT TAC CTT GTT ACG ACT T`-3') (Retnowati et al. 2024). The PCR protocol included an initial denaturation, denaturation, annealing, extension, and a final extension, as described by Retnowati et al. (2017). The resulting 16S rRNA amplicon was purified using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research) and sequenced through bi-directional sequencing. Homology analysis of the 16S rRNA sequences was performed using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast. cgi) to compare the sequences with those in GenBank. Sequence alignment was conducted with ClustalW, and a phylogenetic tree was constructed using the neighbor-joining method in Molecular Evolutionary Genetics Analysis (MEGA) XI software (Retnowati et al. 2017).

Phosphate solubilizing assay

Streptomyces isolate assayed the activity of solubilizing phosphate on the Pikovskaya medium (10 g L^{-1} glucose, 5 g L^{-1} Ca_3PO_4, 0.5 g L^{-1} $(NH_4)2SO_4$, 0.2 g L^{-1} KCl, 0.1 g/L^{-1} $MgSO_4.7H_2O$, 0.1 g L^{-1} $MnSO_4.H_2O$, 0.5 g L^{-1} yeast extract, and 0.01 g L^{-1} $FeCl_3.6H_2O$ at pH 7.0). The phosphate-solubilizing activity was determined based on clear zone formation on the Pikovskaya medium (Faried et al. 2018).

Indole acetic acid production assay

Streptomyces isolates were grown on Nutrient Broth supplemented with 0.1 g of L-tryptophan. These isolates were shaken on a 150 rpm shaker incubator for 6 × 24 hours at room temperature. The supernatant and pellet cells were separated by centrifugation at 7000 rpm for 30 minutes. About 4 mL of Salkowsky reagent was placed on 1 mL of supernatant and then incubated for 24 hours in a dark place. The color change to the pink of the supernatant was observed (Pattern and Glick 2002).

Antagonistic in vitro assay against Fusarium oxysporum *and antibiotic-gene detection*

Antagonistic in vitro assay of *Streptomyces* against *F. oxysporum* was carried out on Potato Dextrose Agar (PDA) medium based on cork borer. *Streptomyces* were scratched lengthwise on the surface of the PDA medium, and the fungi antagonists were inoculated in the direction perpendicular to the *Streptomyces*. The plate was incubated for 14 days at room temperature. The antagonistic activity was shown by clear zone formation surrounding the *Streptomyces* isolate.

The cluster gene of antibiotic production of *Streptomyces* was targeted on Polyketide Synthases (PKSs) including Type I and Type II and Non-Ribosomal Poliketide Synthethase (NRPS) cluster genes. PKSs are responsible for synthesizing polyketide chains from acyl-CoA molecules as the building blocks, while NRPSs synthesize peptide chains from amino acids. PCR amplification of PKSs cluster genes was performed as previously described by Retnowati et al. (2018). The amplification of Type I PKS genes was conducted by primers of K1F (5′-`TSA AGT CSA ACA TCG GBC A`-3′) and M6R (5′-`CGC AGG TTS CSG TAC CAG TA`-3′), while Type II PKS gene was amplified using a set of primer of KSα (5′-`TSG CST GCT TGG AYG CSA TC`-3′) and KSβ (5′-`TGG AAN CCG CCG AAB CCT CT`-3′). The detection of the NRPS cluster gene was carried out by a primer set of A3F (5′-`GCS TAC SYS ATS TAC ACS TCS GG`-3′) and A7R (5′-`SAS GTC VCC SGT SCG GTA S`-3′). The size of PCR products was 1200-1400 bp (K1F/M6R), 600 bp (KSα/KSβ), and 700-800 bp (A3F/A7R). The PCR products were visualized by electrophoresis in 2% agarose gels.

Tolerance assay of Streptomyces *isolates to fungicide*

Streptomyces isolates that showed solubilizing phosphate and IAA-producing abilities were further assessed for fungicide tolerance. Three kinds of fungicides, such as benlate, thiramo, and captive at varied concentrations, i.e., 25, 50, 75, and 100 µg L^{-1}, were assayed. The growth of *Streptomyces* was observed quantitatively in the range 0-3 based on the colony diameters. The number 0 indicates no growth; 1 indicates little growth; 2 indicates moderate growth; and 3 indicates good growth.

Data analysis

The data for the bioprospecting of *Streptomyces* were analyzed based on descriptive quantitative analysis. The ability of *Streptomyces* to solubilize phosphate, indole acetic acid production, antagonistic to *F. oxysporum*, and tolerance to fungicide were described to represent the *Streptomyces* potential as PGPR. The 16S rRNA sequence data of strain *Streptomyces* was analyzed based on comparison to the NCBI GenBank. The cluster gene of secondary metabolite production was described based on the presence of a DNA band of cluster gene on agarose gel electrophoresis on 1200-1400 bp (K1F/M6R), 600 bp (KSα/KSβ), and 700-800 bp (A3F/A7R).

RESULTS AND DISCUSSION

Physicochemical conditions of rhizosphere soil on the karst ecosystem of Gorontalo

The rhizosphere soil sample at the three locations showed varying physico-chemical conditions (soil pH, temperature, and humidity) and vegetation. The rhizosphere soils at the three locations tended to be neutral, with Soil acidity values ranging from pH 6.5 to 8 and rhizosphere soil temperatures ranging from 28 to 32°C. Humidity tends to be the same, namely Dry^+ (Table 1).

Table 1. Physico-chemical condition of plant-soil rhizosphere on karst ecosystem of Gorontalo, Indonesia

Sampling location	**Physicochemical conditions**			**Dominant vegetation**
	pH	**Temp (°C)**	**Humidity**	
Dulanga Beach	6.5-8	28-32	Dry^+	*Jatropha curcas* L.
Kurunai Beach	6.5-7	27-28	Dry^+	*Cascabela thevetia* (L.) Lippold
Otanaha Mountains	7	28-31	Dry^+	*Ligustrum sinense* Lour.

Table 2. The nearest phylogenetic relative of *Streptomyces* isolates, based on the 16S RNA sequencing

Code of *Streptomyces* isolate	**Nearest phylogenetic neighbor**	**Homology (%)**	**E -value**	**Genus**
KRJc-01	*Streptomyces* sp. (OQ275015.1)	99.65	0	*Streptomyces*
KRJc-02	*Streptomyces rochei* (KP872947.1)	99.37	0	*Streptomyces*
KRJc-03	*Streptomyces pratensis* (PP456304.1)	98.39	0	*Streptomyces*

Table 3. The antagonistic activity of *Streptomyces* isolates from *Jatropha curcas* L. rhizosphere on the karst ecosystem of Gorontalo

Kinds of *Streptomyces*	**Clear zone diameter (cm)**
Streptomyces sp. strain KRJc-01	0.5
Streptomyces rochei strain KRJc-02	1
Streptomyces pratensis strain KRJc-03	4

Table 4. The tolerance fungicide activity of *Streptomyces* isolates from *Jatropha curcas* rhizosphere on the karst ecosystem of Gorontalo

Kinds of *Streptomyces*	**The diameters of the colony (mm)**		
	Banlate (100 ppm)	**Captive (100 ppm)**	**Thiramo (100 ppm)**
Streptomyces sp. strain KRJc-01	24.01	12.42	33.02
Streptomyces rochei strain KRJc-02	33.97	24.92	31.94
Streptomyces pratensis strain KRJc-03	34.39	25.64	39.46

Rhizospheric-*Streptomyces* on the karst ecosystem of Gorontalo

Three *Streptomyces* isolates were successfully selectively isolated from the rhizosphere of *Jatropha curcas* called KRJc-01, KRJc-02, and KRJc-03. In contrast, *Streptomyces* was not found in the rhizosphere of Ginjel and *Ligustrum sinense*. Homology search in NCBI using the BLAST tool confirmed the identity of the isolates (Table 2). According to the sequence data of 16S rRNA gene, the third isolate was closely related to the genus *Streptomyces* (Figure 2).

The solubilizing-phosphate and IAA-producing activity of *Streptomyces* sp.

Streptomyces sp. isolated from *J. curcas* rhizosphere showed activity to solubilize phosphate and produce IAA. The clear zone around the *Streptomyces* colony on the Pikosvkaya medium indicated the ability to solubilize phosphate. The results showed a clear zone diameter of about 1.54 to 2.98 mm. *Streptomyces* isolates also showed activity to produce IAA, indicated by the medium's pink color.

Antagonistic activity of *Streptomyces* against *Fusarium oxysporum*

Streptomyces sp. isolated from *J. curcas* rhizosphere of the karst ecosystem Gorontalo showed antagonistic activity against the root pathogen of *F. oxysporum*. The antagonistic activity was indicated by clear zone formation surrounding the *Streptomyces* colony with varied clear zone diameters. *Streptomyces pratensis* strain KRJc-03 showed the highest antagonistic activity, followed by *Streptomyces rochei* strain KRJc-02 and *Streptomyces* sp. strain KRJc-01 (Table 3).

The differences in the antagonistic activity of *Streptomyces* against the root pathogen of *F. oxysporum* were reinforced with genetic data. The detection gene of PKSs and NRPS genes showed that *S. pratensis* strain KRJc-03 possesses PKSI, PKS II, and NRPS genes. *S. rochei* strain KRJc-02 and *Streptomyces* sp. strain KRJc-01 were only detected with NRPS cluster genes, respectively (Figure 3).

Tolerance fungicide of *Streptomyces*

Streptomyces sp. isolated from the *J. curcas* rhizosphere of a karst ecosystem showed tolerance to the fungicides Benlate, Captive, and Thiramo up to 100 ppm on growth ability around 12.42 to 39.46 mm colony diameters (Table 4).

Discussion

The karst ecosystem in the coastal area of Gorontalo is a defining feature of the geographical landscape. Physicochemical measurements show relatively uniform soil characteristics, including pH, temperature, and moisture. However, the key finding of this research is the variations in the dominant plant species at each location, which are influenced by the dominant vegetation cover in these areas. The dominant vegetation cover in these areas is believed to influence the biological conditions within the soil in each location, suggesting that microbial diversity and biological activity in the soil may vary across the three areas. This vegetation cover is believed to play a significant role in shaping the biological conditions within the soil in each location, potentially leading to variations in microbial diversity and biological activity. Kooch et al. (2024) reported that changes in vegetation cover in mountainous areas are known to impact various fractions of organic matter and other soil properties, subsequently affecting microbial and enzyme activity involved in nutrient cycling. This is supported by findings from Sui et al. (2022), reporting that forests restored with different tree species can exhibit variations in soil quality and characteristics despite having the same climate. Furthermore, broadleaf and coniferous forests show unique diversity and composition of fungal communities. Similarly, Chandra et al. (2016) reported differences in soil physicochemical properties and their relationship with microbial characteristics in temperate and dry deciduous forest areas in India. Mixed-species forests exhibited significantly higher enzyme activity, with soil microbial characteristics and unique soil physicochemical properties interlinked, significantly influenced by specific forest types and climate variables.

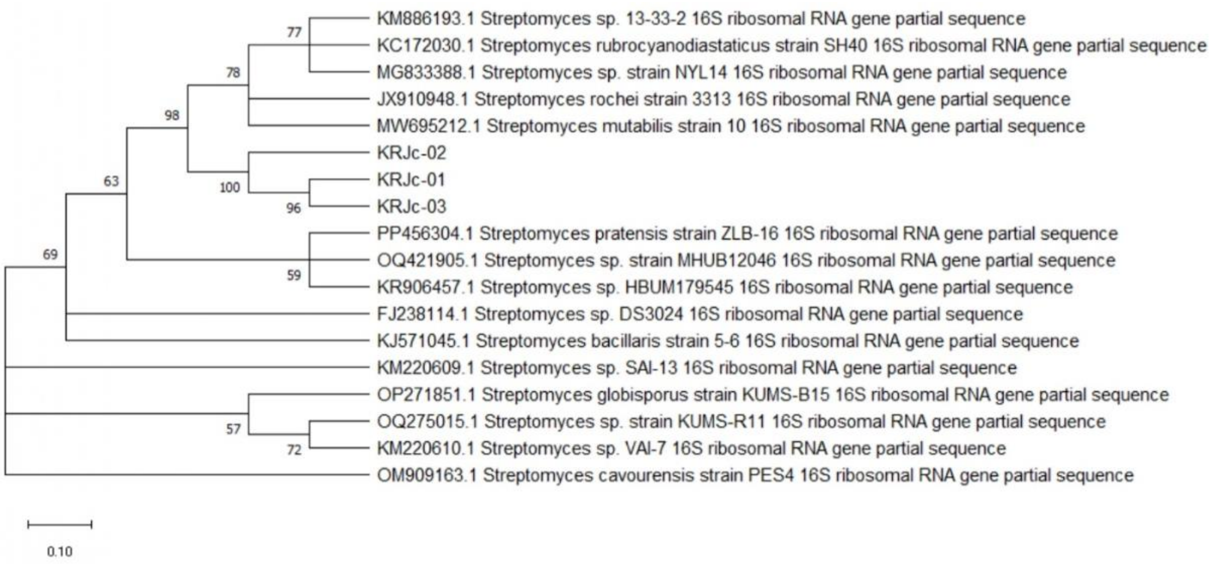

Figure 2. Neighbor-joining phylogenetic tree inferred from 16S rRNA gene sequence of *Streptomyces* sp.. The phylogenetic tree shows the phylogenetic relationship of isolates with related genera

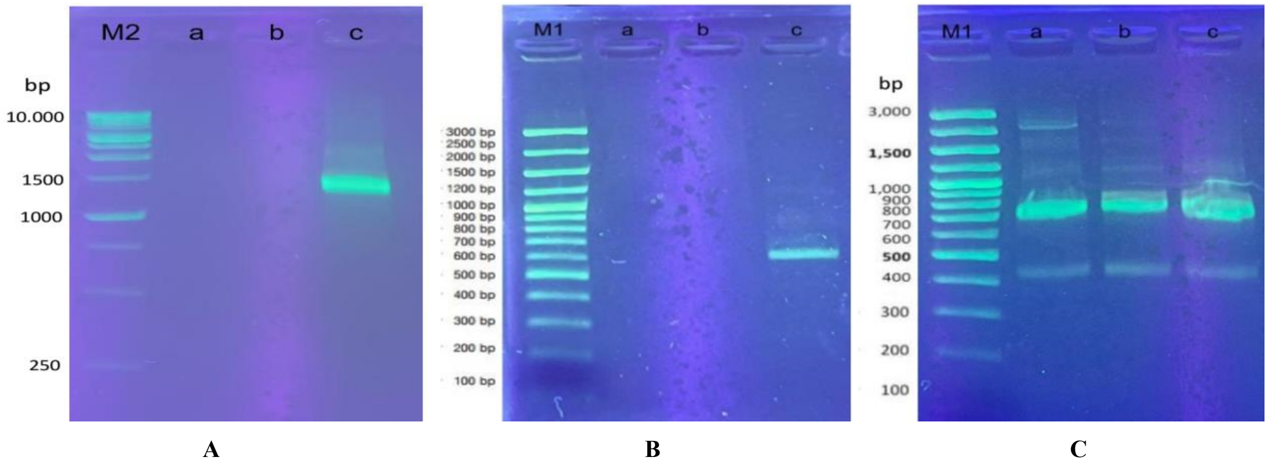

Figure 3. Detection of PKSs and NRPS cluster gene on genome of *Streptomyces* isolated from the rhizosphere of plants in the karst ecosystem, Gorontalo: A: *Streptomyces* sp. strain KRJc-01; B. *Streptomyces rochei* strain KRJc-02; C. *Streptomyces pratensis* strain KRJc-03. a: Type I PKS gene, b: PKS Type II gene, c: NRPS gene, M1: Marker 100 plus DNA Ladder, M2: Marker 1 kb DNA ladder

The karst ecosystem in the coastal area of Gorontalo, with its specific dominant plant characteristics at each location, demonstrates differences in microbial communities, particularly *Streptomyces*, in the rhizosphere of plants. Our observations show that *Streptomyces* is only associated with the rhizosphere of *J. curcas* in the coastal Dulanga karst ecosystem. The rhizosphere, an area around the roots composed of soil components, roots, and microorganisms, is influenced by various factors, including soil type and moisture levels, soil amendments, pH, root proximity to soil, plant species, plant age, and root exudates. These factors contribute to the diverse composition of microorganisms in the rhizosphere. Different plant species often host some variable microflora in the rhizosphere area. Qualitative and quantitative differences in microflora are attributed to variations in rooting habits, tissue composition, and excreted products. However, one of the most critical factors responsible for the rhizosphere effect is the availability of a variety of organic substances in the root area through root exudates/excretions. The quantitative and qualitative differences in rhizosphere microflora compared to general soil are primarily driven by the influence of root exudates. Since the chemical composition of root exudates varies widely, their impact on microflora also differs significantly. The nature and quantity of chemical substances exuded are dependent on plant species, plant age, inorganic nutrients, temperature, light intensity, O_2/CO_2 levels, root injury, and other factors.

Streptomyces are soil microorganisms commonly found in various soil types, including karst soil. These microorganisms form a mutualistic symbiosis with the plant rhizosphere in karst ecosystems. The rhizosphere is an environmental zone around the roots. In karst ecosystems, *Streptomyces* are capable of solubilizing phosphate, supported by their ability to synthesize the enzyme phosphatase. An investigation reports the presence of phosphate-solubilizing *Streptomyces*, as shown by a clear zone on the Pikovskaya medium. Nurkanto (2007) reported that *Streptomyces* and other genera, including *Nocardia* sp., *Micromonospora* sp., *Actinoplanes* sp., *Microbiospora* sp., *Microtetraspora* sp., and *Streptosporangium* sp., showed phosphate solubilizing abilities in soil under acidic or alkaline conditions. Similarly, Zhou et al. (2024) reported that using gene detection methods to identify those involved in organic soil P mineralization (G6PD, suhB, phoD, ppx) and P absorption and transformation systems (pstS, pstA, pstB, pstC), showed that approximately 9.45%-25.23% of the microbial community in the rhizosphere belongs to Actinobacteria, including the genus *Streptomyces*. These microorganisms exhibit phosphate-solubilizing capabilities within plant rhizospheres in karst ecosystems.

The presence of *Streptomyces* in the plant rhizosphere can also induce plant growth by producing the growth hormone IAA. Research findings show that *Streptomyces* from the *J. curcas* rhizosphere in the karst ecosystem can produce IAA, which is common natural auxin phytohormone with an indole ring, and it is a product of L-tryptophan metabolism in microorganisms. Various plant growth-promoting bacterial genera, including Actinomycetes and fungi, enhance plant growth through IAA production through L-tryptophan-dependent mechanisms. Myo et al. (2019) reported that *Streptomyces fradiae* has significant potential to promote plant growth and produce IAA. In addition to plant synthesis, IAA can also be produced by rhizosphere microbes, such as *Burkholderia* spp. and *Serendipita* spp. (Venneman et al. 2020; You et al. 2020; Mosaddeghi et al. 2021). The enrichment of *Burkholderia* and *Serendipita* may be another source of IAA production. Additionally, auxin signaling has been shown to play a crucial role in balancing plant growth and defense responses induced by beneficial microbes (Stringlis et al. 2018). Ge et al. (2024) also reported that further root transcriptome analysis indicated that S769 treatment significantly enhances the expression of MAPK signaling pathways, plant hormone signal transduction, and plant-pathogen interactions, particularly genes associated with PR-1 and ethylene, as well as genes related to auxin production and reception.

The presence of *Streptomyces* in the plant rhizosphere is fundamentally beneficial for plant health through the coordination of plant microbiome and rhizosphere interactions. Observations show that *S. pratensis* strain KRJc-03, isolated from the *J. curcas* rhizosphere in the karst ecosystem of the coastal area of Dulanga, exhibits antagonistic activity against the pathogenic fungus *F. oxysporum*. According to a study by Du et al. (2018), *Streptomyces ahygroscopicus* var. *gongzhulingensis* (strain 769), originally isolated from black soil in Northeast China, shows antagonistic effects on various phytopathogens (such as *F. oxysporum* and *Alternaria solani*) and promotes growth in multiple crops, including rice and soybean. Ge et al. (2024) also reported that treatment with *Streptomyces ahygroscopicus* strain 769 (S769) results in substantial changes in the composition of bacterial and fungal communities, inducing a highly interconnected microbial association network in the rhizosphere. Zhang et al. (2021) also reported that *Streptomyces* sp. BITDG-11 isolated from the primitive ecological nature reserve of 'Yingge' mountain in Hainan province, China showed antagonistic activity against *F. oxysporum* TR4.

The antagonistic capability of *S. pratensis* strain KRJc-03 against pathogenic fungi is supported by the presence of PKS and NRPS genes cluster in the genome of *S. pratensis* strain KRJc-03, which are believed to encode bioactive compounds responsible for the antagonistic activity. The PKSs and NRPS genes cluster encode for a number of enzymes related to the biosynthesis of polyketide and nonribosomal peptide secondary metabolites. These two secondary metabolites are the main secondary metabolites in the genus *Streptomyces*, because half to three-quarters of the secondary metabolite biosynthesis gene clusters in each streptomycetal genome generally include Polyketide Synthase (PKS) and/or Nonribosomal Peptide Synthetase (NRPS) genes. Each *Streptomyces* strain contains several dozen biosynthesis gene clusters for polyketides and nonribosomal peptides in its genome (Komaki and Tamura 2023). The presence of PPKSs and NRPS cluster genes in the genome of *S. pratensis* strain KRJc-03 indicates the capability to produce the secondary metabolites of the polyketide and non-ribosomal peptide groups with antifungal biological activity against *F. oxysporum*. Although there has been no screening of metabolite types in this study, the molecular data answer the suspicion that actinomycetes from the karst ecosystem of Gorontalo have the potential to be a source of secondary metabolites with biological activity. Komaki and Tamura (2023) also reported that the result of whole genome of *Streptomyces cellostaticus* NBRC 12849^T analysis showed the genome encoded 12 PKS, one NRPS and eight hybrid PKS/NRPS gene clusters, and among the 21 gene clusters, products of 10 gene clusters were annotated to be an annimycin congener, fuelimycins, lankamycin, streptovaricin, spore pigment, flaviolin, foxicin, blasticidin, lankacidin and an incarnatapeptine. Retnowati et al. (2018) reported that *Streptomyces qinglanensis* and *Streptomyces sanyensis* isolated from the mangrove ecosystem of Gorontalo possess the PKS and NRPS gene cluster in both genome and both bacteria showed antagonistic activity against pathogenic microbial. Zhang et al. (2021) reported that genome sequencing of *Streptomyces* BITDG-11 chromosome contained large numbers of conserved biosynthesis gene clusters encoding terpenes, non-ribosomal peptides, polyketides, siderophores, and ectoines.

Streptomyces spp. isolated from the rhizospheric karst ecosystem of Gorontalo showed tolerance to several types of fungicides. The ability to tolerate is shown by the ability to grow on a medium containing 100 ppm of banlate, captive and thiramo fungicides. The ability to tolerate fungicides is important as one of the requirements possessed by actinomycetes as plant growth-promoting rhizobacteria. Fungicides that are widely applied in agriculture can accumulate in the soil, and can affect the structure of microbial communities in the soil. Yin et al. (2023) explained some common molecular mechanisms developed by microbes for tolerance and resistance to fungicides including i) mutations in the fungicide target, which causes a decrease in the affinity of the fungicide for the target protein; ii) overexpression or duplication of the fungicide target gene, which causes an increase in the production of the target protein; iii) overexpression of ATP-Binding Cassette (ABC) or Major Facilitator Superfamily (MFS) transporters encoding efflux pumps; and iv) epigenetic changes such as chromatin or histone modifications that alter gene expression, resulting in microbial adaptation to fungicide stress. Likewise, according to De Simeis and Serra (2021), actinomycetes, which are a group of antibiotic-producing bacteria, also develop resistance mechanisms to various types of bioactive compounds, through the mechanisms of permeability alteration, target modification/ amplification, and drug inactivation.

In conclusion, *Streptomyces* sp. isolated from *J. curcas* in the karst ecosystem in the coastal area of Gorontalo has a high potential as PGPR. The strain showed the characteristics to solubilize phosphate, produce IAAs, tolerate fungicides, and be antagonistic to fungal-root pathogens. These characteristics were crucial for bacteria to be developed as biofertilizers to improve crop growth.

ACKNOWLEDGEMENTS

The study was funded by LPPM UNG via PNBP-Universitas Negeri Gorontalo, Gorontalo, Indonesia, according to DIPA-UNG through *Riset Akseleratif Lektor Kepala* under contract No. 840/UN47.D1/PT.01.03/2024, and the author is grateful to Anisa, Citra Leonita, Danial, Syahrul, Tharisa, Andre, and Susilo as field assistants.

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