



# Bioprospecting of actinomycetes isolated from Malaysia agriculture soil

Jeffrey, Lim Seng Heng<sup>1\*</sup>, Aman Nejis, Norzaimawati<sup>1</sup> & Hamzah, Halizah<sup>1</sup>

<sup>1</sup>Biological Control Programme, Agrobiodiversity and Environment Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, MALAYSIA.

\*Corresponding author: [shlim@mardi.gov.my](mailto:shlim@mardi.gov.my)

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**Abstract:** Actinomycetes are well-recognized for their ability to produce diverse bioactive compounds with applications in sustainable agriculture. This study aimed to investigate the biodiversity of soil-derived actinomycetes and evaluate their potential for producing plant growth-promoting substances and antimicrobial metabolites. A total of 23 actinomycete isolates were obtained from soil samples, with a population density of  $4.5 \times 10^6$  CFU g<sup>-1</sup>, and exhibited variation in spore coloration, indicating taxonomic diversity. Screening for phytohormone traits revealed that 60.9% of isolates produced indole-3-acetic acid (IAA), while 43.5% synthesized gibberellic acid (GA<sub>3</sub>). Additionally, 47.8% and 26.1% of isolates demonstrated antimicrobial activity against *Ralstonia solanacearum* and *Colletotrichum gloeosporioides*, respectively. Statistical analysis using ANOVA and Tukey's HSD test confirmed significant differences among isolates ( $P < 0.05$ ). Notably, isolate 5 exhibited the highest overall performance across multiple assays, suggesting strong multifunctional potential. The results highlight the presence of actinomycetes with combined plant growth-promoting and biocontrol properties, which are highly desirable for agricultural applications. Overall, this study underscores the importance of soil actinomycetes as promising candidates for the development of eco-friendly bioinoculants. Further studies involving molecular identification, characterization of bioactive compounds, and field validation are recommended to fully exploit their biotechnological potential.

**Keywords:** Actinomycetes, Soil microbial diversity, Plant growth-promoting actinomycetes, Bioactive compounds, Biocontrol agents

## 1. Introduction

Actinomycetes is a Gram-positive, filamentous bacteria that are pervasive across terrestrial ecosystems and are widely acknowledged for their profound ecological and biotechnological relevance. They are widely associated to the decomposition of complex organic substrates and the regulation of nutrient turnover, thereby contributing substantially to soil structure, fertility, and long-term ecosystem stability. Beyond their ecological roles, actinomycetes are distinguished by their exceptional capacity to synthesize structurally diverse secondary metabolites, which exhibit potent antimicrobial, antifungal, and enzymatic activities (Hara, 2024; Rui et al., 2025). This unparalleled metabolic versatility has positioned actinomycetes as a central focus for bioprospecting efforts aimed at identifying novel bioactive compounds with applications in medicine, industry, and particularly sustainable agriculture.

Tropical environments such as Malaysia represent highly promising reservoirs for the discovery of novel actinomycetes taxa due to their extraordinary biodiversity and heterogeneous ecological gradients. Malaysian soils including those from primary forests, intensively managed agricultural systems, and mangrove ecosystems harbour unique physicochemical conditions that support diverse and often unexplored actinomycete assemblages. Studies have demonstrated that isolates obtained from Malaysian mangrove soils exhibit pronounced antimicrobial and enzymatic activities, underscoring their potential as sources of agriculturally relevant metabolites (Nor Hasan et al., 2024). These findings reinforce the importance of systematic exploration across varied soil habitats to fully elucidate the metabolic and functional potential of actinomycetes communities.

The agricultural relevance of actinomycetes has gained increasing attention, particularly in the context of global efforts to reduce dependence on synthetic agrochemicals. Actinomycetes contribute to plant health through the production of bioactive compounds that suppress phytopathogens, modulate plant immune responses, and enhance overall plant vigour. Numerous species have been characterized as effective biocontrol agents and biofertilizers, offering environmentally benign alternatives to conventional pesticides and fertilizers (Diab et al., 2024; Kariyanna & Panda,

2025). Kariyana & Panda (2025) noted that, *Streptomyces griseoflavus* and *S. atrovirens* produces indole-3-acetic acid (IAA), while *S. olivaceoviridis* and *S. rochei* synthesize gibberellic acid that can enhance plant growth, and resilience.

The agricultural impact of actinomycetes is both multifaceted and far-reaching. Their application has been shown to improve crop productivity, enhance plant tolerance to biotic and abiotic stresses and promote soil microbial diversity. Continued discovery of novel bioactive metabolites from actinomycetes provides innovative strategies for disease suppression and stress mitigation (Diab et al., 2024; Rui et al., 2025). *Streptomyces* sp. AN090126 exhibits strong inhibitory activity against *Ralstonia solanacearum* and *Xanthomonas euvesicatoria* (Khanh et al., 2022), while *Streptomyces* sp. HSL-9B effectively suppresses *Colletotrichum gloeosporioides*, the causal agent of mango anthracnose (Zhou et al., 2022). As global demand for sustainable food production intensifies, the strategic utilization of actinomycetes as natural, biological resources becomes increasingly indispensable.

This study aims to characterize the biodiversity of actinomycetes inhabiting Malaysian soils and to evaluate their potential to produce agriculturally relevant bioactive compounds for future applications in sustainable crop management.

## 2. Materials and Methods

### 2.1 Sample collection and isolation

Soil samples were collected from a depth of approximately 15 cm beneath the rhizosphere of *Syzygium samarangense* (Jambu air) in Serdang, Malaysia. The samples were air-dried under ambient laboratory conditions for three days to reduce the abundance of fast-growing, non-sporulating microorganisms. Subsequently, 10 g of soil sample was transferred into a sterile conical flask containing 100 mL of sterile distilled water. The suspension was incubated at room temperature ( $28 \pm 2$  °C) for 1 h on an orbital shaker, followed by vigorous agitation at high speed to ensure thorough dispersion of soil particles.

A 150  $\mu$ L aliquot of the resulting soil suspension was aseptically spread onto Starch Casein Agar (SCA) plates and incubated at  $28 \pm 2$  °C for seven days to facilitate the selective growth of actinomycetes (Jeffrey et al., 2024). Distinct actinomycete colonies were carefully picked using sterile toothpicks and transferred onto fresh SCA plates. These plates were incubated for an additional seven days under the same conditions. Pure isolates were subsequently maintained on SCA at  $28 \pm 2$  °C for until needed.

### 2.2 Bioactivity screening

Screening for functional plant growth promoting traits was conducted using three selective media. Nitrogen-fixing ability was screened using Jensen's Agar (sucrose 2%,  $K_2PO_4$  0.1%,  $MgSO_4 \cdot 7H_2O$  0.05%, NaCl 0.05%,  $FeSO_4$  0.01%,  $Na_2Mo$  0.0005%,  $CaCO_3$  0.2%, agar 1.5% in 1L sdH<sub>2</sub>O), while phosphate-solubilizing ability was evaluated using Pikovskaya's Agar (Himedia, India). Potassium-solubilizing activity was determined on Aleksandrow Agar (Himedia, India). Selected actinomycete isolates were spot-inoculated onto each of the three media. Growth on Jensen's Agar was interpreted as indicative of potential nitrogen fixation, whereas the formation of a clear halo zone surrounding colonies on Pikovskaya's Agar and Aleksandrow Agar signified phosphate and potassium solubilization, respectively. All assays were performed in triplicate to ensure reproducibility.

Antimicrobial activity was evaluated using a modified agar plug diffusion method based on Bauer et al. (1966), with adaptations described by Barakate et al. (2002). Actinomycete cultures grown on Starch Casein Agar (SCA) were excised using a sterile 5 mm cork borer to obtain agar plugs containing actively growing mycelia. These plugs were placed onto lawn cultures of pathogenic bacteria, and plates were incubated for three days. Antagonistic activity was quantified by measuring the diameter of the resulting inhibition zones.

For antifungal activity, a mycelial plug of the test fungus was positioned at the centre of a Potato Dextrose Agar (PDA) plate, while two actinomycete agar plugs were placed equidistantly on opposite sides of the plate. The development of a halo zone between the actinomycetes isolates and the fungal colony showed antifungal activity between the cultures. The pathogenic test organisms used in this study were *Ralstonia solanacearum* and *Colletotrichum gloeosporioides*.

All experiments were conducted in triplicate. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test.

### 2.3 Screening for indole-3-acetic acid (IAA) production

Indole-3-acetic acid (IAA) production was studied using a modified protocol by Iqbal and Hasnain (2013). Actinomycetes cultures were incubated at  $30 \pm 2$  °C for 48 hrs, after which the cell density was standardized to approximately  $10^6$  CFU mL<sup>-1</sup> using a hemocytometer. A 2 mL aliquot of each culture was transferred into sterile microcentrifuge tubes and centrifuged at 10,000 rpm for 30 minutes to obtain cell-free supernatants. The resulting supernatant was then mixed with Salkowski's reagent in a 2:1 ratio and incubated in the dark for 30 minutes to allow chromophore development. The appearance of a pink color was indicated positive reaction of IAA synthesis by the actinomycete isolates. Quantification was performed by measuring absorbance at 520 nm using a Nanodrop spectrophotometer, and IAA concentrations were estimated from a standard curve prepared with analytical grade synthetic IAA (Sigma).

## 2.4 Screening for gibberellic acid (GA<sub>3</sub>) production

Gibberellic acid (GA<sub>3</sub>) production by the actinomycetes isolates was assessed by using a modified version of method described by Panday and Desai (2014). Quantification was performed by preparing a standard calibration curve using analytical grade GA<sub>3</sub> (Sigma). Absorbance readings of the culture supernatants were done using a Nanadrop spectrophotometer at 254 nm, and GA<sub>3</sub> concentrations were interpolated from the standard curve.

## 2.5 Molecular identification

Genomic DNA (gDNA) extraction of Actinomycetes isolate 5 was conducted using the MagAttract Microbial DNA Kit following the manufacturer’s recommended procedure (Qiagen, 2025a). Identification of Actinomycetes isolate 5 was performed through amplification of the 16S rRNA gene region using specific primers targeting the V4 and V5 variable regions. Each PCR reaction mixture consisted of 15.5 µL sterile distilled water, 2.5 µL of 10× PCR buffer, 2.0 µL of 25 mM MgCl<sub>2</sub>, 0.6 µL dNTP mix, 0.4 µL Taq DNA polymerase, 1.0 µL each of 20 pmol forward primer COM1 (5'-CAGCAGCCGCGGTAATAC-3') and reverse primer COM2 (5'-CCGTCAATTCCTTTGAGTTT-3') as described by Congestri et al. (2020), and 2.0 µL DNA template per reaction. PCR amplification was performed using a thermal cycler with an initial denaturation step at 94°C for 3 min, followed by 35 amplification cycles consisting of denaturation at 94°C for 45 s, annealing at 62°C for 45 s, and extension at 72°C for 2 min. A final extension step was carried out at 72°C for 10 min.

The amplified PCR products were separated by electrophoresis on a 1.0% agarose gel at 80 V for 50 min. Gel visualization was performed using a Bio-Rad Gel Documentation System. The PCR amplicons were subsequently purified using the QIAquick PCR & Gel Cleanup Kit according to the manufacturer’s instructions (Qiagen, 2025b). Purified amplicons were submitted to Apical Scientific Sdn. Bhd., Selangor for sequencing using an ABI PRISM® 377 DNA Sequencer (Applied Biosystems). The resulting nucleotide sequences were analyzed and compared with reference sequences available in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) as described by Altschul et al. (1990).

## 3. Results

### 3.1 Enumeration of actinomycetes

The colony forming units (CFU) of actinomycetes isolated from the soil were determined to be  $4.5 \times 10^6$  CFU g<sup>-1</sup>. Based on the results obtained, a total of 23 actinomycete isolates were isolated from the soil, comprising 9 isolates with white spores, 10 with grey spores (Figure 1), and 4 with yellow spores (Table 1). The variation in spore coloration among the isolates indicates the diversity of actinomycetes present in the soil.

**Table 1: Actinomycetes and their spores color**

Strain number	Spores color
J01, J05, J06, J10, J12, J17, J18, J20, J21	White
J03, J04, J07, J09, J11, J13, J14, J19, J22, J23	Grey
J02, J08, J15, J16	Yellow



**Fig. 1: Actinomycetes grown on SCA producing grey spores.**

### 3.2 Bioprospection of phytohormones and antimicrobial properties

The capacity of actinomycetes to synthesize a broad spectrum of bioactive metabolites has been widely recognized across diverse ecological studies. In the present investigation, 21.7% of the isolates exhibited both nitrogen-fixing and phosphate-solubilizing capability (Table 2). Potassium solubilization was observed in 13.0% of the isolates. Additionally, 60.9% of the 23 actinomycete isolates produced indole-3-acetic acid (IAA), and 43.5% showed ability of synthesizing gibberellic acid (GA<sub>3</sub>) (Table 2). Actinomycetes isolate 5 produced the highest IAA at 30.777 µg/mL, while Actinomycetes isolate 13 produced the highest GA<sub>3</sub> (20.067 µg/mL). Antimicrobial screening revealed that 26.1% of the isolates inhibited the growth of *Ralstonia solanacearum*, whereas 17.4% suppressed the growth of *Colletotrichum gloeosporioides* (Table 3). Among all isolates, Actinomycete isolate no. 5 displayed the most extensive and robust bioactivity profile underscoring its potential as a promising candidate for further usage as biofertilizer or biopesticide.

**Table 2: Phytohormones abilities of isolated actinomycetes**

Isolate no.	N-fixer	P-solubilizer	K-solubilizer	Phytohormone activities (µg/mL)	
				IAA	GA <sub>3</sub>
1				10.200 ± 0.200 <sup>h</sup>	0.000
2				11.220 ± 0.300 <sup>h</sup>	8.733 ± 0.208 <sup>q</sup>
3				7.933 ± 0.057 <sup>i</sup>	10.333 ± 0.351 <sup>p</sup>
4				0.000	0.000
5	√	√		30.733 ± 0.306 <sup>a</sup>	16.266 ± 0.231 <sup>m</sup>
6				13.133 ± 0.231 <sup>g</sup>	0.000
7				13.333 ± 0.379 <sup>g</sup>	16.067 ± 0.306 <sup>m</sup>
8		√		19.633 ± 0.321 <sup>c</sup>	0.000
9				0.000	0.000
10				0.000	0.000
11	√	√		25.300 ± 0.608 <sup>b</sup>	0.000
12				0.000	10.467 ± 0.451 <sup>o</sup>
13	√		√	23.913 ± 0.557 <sup>c</sup>	20.067 ± 0.208 <sup>l</sup>
14				0.000	0.000
15		√		21.267 ± 0.231 <sup>d</sup>	0.000
16				0.000	4.367 ± 0.115 <sup>j</sup>
17			√	17.403 ± 0.173 <sup>f</sup>	7.500 ± 0.100 <sup>k</sup>
18	√			0.000	0.000
19				20.802 ± 0.173 <sup>de</sup>	13.633 ± 0.153 <sup>n</sup>
20		√	√	21.433 ± 0.513 <sup>d</sup>	0.000
21				0.000	0.000
22				0.000	17.033 ± 0.058 <sup>m</sup>
23	√			20.067 ± 0.058 <sup>e</sup>	0.000

Means followed by different letters are significantly different at P < 0.05 based on Tukey's HSD test.

**Table 3: Antimicrobial abilities of isolated actinomycetes**

Isolate no.	Antimicrobial activities (mm ± SE)	
	<i>Ralstonia solanacearum</i>	<i>Colletotrichum gleosporioides</i>
1	0.00	19.00 ± 1.00 <sup>e</sup>
2	0.00	14.33 ± 0.58 <sup>f</sup>
3	0.00	0.00
4	18.67 ± 1.15 <sup>c</sup>	0.00
5	27.33 ± 0.58 <sup>a</sup>	18.33 ± 1.53 <sup>e</sup>
6	0.00	0.00
7	0.00	0.00
8	0.00	0.00
9	0.00	0.00
10	0.00	0.00
11	00.00	0.00
12	20.33 ± 0.29 <sup>b</sup>	0.00
13	20.33 ± 0.58 <sup>b</sup>	13.67 ± 1.15 <sup>f</sup>
14	0.00	0.00
15	18.33 ± 0.58 <sup>c</sup>	0.00
16	0.00	0.00
17	0.00	0.00
18	0.00	0.00
19	0.00	0.00
20	25.00 ± 1.00 <sup>a</sup>	0.00
21	0.00	0.00
22	0.00	0.00
23	0.00	0.00

Means followed by different letters are significantly different at P < 0.05 based on Tukey’s HSD test.

### 3.3 Identification of actinomycetes

Actinomycetes isolate number 5 was identified as *Streptomyces tendae* (Table 4).

**Table 4: Identification of potential actinomycetes using 16S rRNA**

Isolate no	ID & Sequence	E-value	Similarity (%)
5	<i>Streptomyces tendae</i>  ccctggaac ggggtctaata accggataaa gagcctcgca ggcactctgag aggttcgaaa gctccggcgg tgcaggatga gcccgcgcc taccagcttg ttggtgaggt aatggctcac caaggcgacg acgggtagcc ggcctgaaag ggcgaccggc cacactggga ctgagacacg gcccagactc ctacgggagg cagcagtggg gaattattgca caatgggca aagcctgatg cagcgacgcc gcgtgagggga tgacggcctt cgggttgtaa acctctcca gcaggaaga agcgaagtgc acgtacctg cagaagaagc gccggctaac tacgtgccag cagccgcggt aatacgtagg gcgaagcgt tgcgggaat tattgggctg aaagagctcg tagggcgctt gtcagctcgg ttgtgaaagc cgggggctta acccgggctc tgcagctgat acgggcaggc tagagttcgc taggggagat cggaattcct ggtgtagcgg tgaatgcgc agatcagc aggaacaccg gtggcgaagg cggatctctg ggccgatact gacgctgagg agcgaagcgc tggggagcga acaggattag ataccctggt agtccacgcc gtaaacggtg ggcactaggt	0.0	99.55

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gtgggcaaca ttccacgttg tcegtgccgc agctaacgca ttaagtcccc cgctgggga
gtacggccgc aaggctaata ctcaaaggaa ttgacggggg cccgcacaag cggcgggagca
tgtgtcttaa ttcgacgcaa cgcaagaac cttaccaagg cttgacatac accggaaaac
atcaagatcg ctgcgcccc ttgtggtcgg tgtacagtgg tgcattggctg

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#### 4. Discussions

The screening of 23 actinomycete isolates for plant growth promoting bacteria (PGPB) traits revealed relatively low but significant occurrences of nitrogen fixation (N-fix), phosphate solubilization (P-sol), and potassium solubilization (K-sol) activities. Only 21.7% of isolates exhibited N-fix and P-sol capabilities, while 13.0% demonstrated K-sol activity, indicating that multifunctional nutrient-solubilizing traits are not widespread among soil actinomycetes. This observation is consistent with previous findings that only a subset of actinomycetes possesses functional genes involved in nitrogen fixation and mineral solubilization, as these processes are metabolically specialized and environmentally regulated (Yadav et al., 2018).

Nitrogen fixation ability actinomycetes was detected in five isolates, suggesting their potential contribution to biological nitrogen input in soil systems. Actinomycetes capable of fixing atmospheric nitrogen play an important role in enhancing soil fertility, particularly in nutrient-deficient soils (Aluvihara et al., 2023). However, the relatively low frequency observed in this study aligns with reports that nitrogen-fixing actinomycetes are less common compared to other diazotrophic bacteria, due to the high energy requirement of the nitrogenase enzyme system (Yadav et al., 2018). Despite this limitation, such isolates are valuable for sustainable agriculture as they can reduce dependency on synthetic nitrogen fertilizers.

Phosphate solubilization was also observed in five isolates, reflecting the ability of certain actinomycetes to convert insoluble phosphorus into bioavailable forms through the production of organic acids and phosphatases. This trait is critical for improving phosphorus uptake in plants, as a large proportion of soil phosphorus exists in insoluble forms. The findings are in agreement with most studies conducted, showing that actinomycetes contribute to phosphorus cycling and can significantly enhance plant growth under phosphorus-limited conditions (Yadav et al., 2018).

In contrast, potassium solubilization was the least common trait, detected in only three isolates (13.0%). This lower occurrence is expected, as potassium solubilization requires specific metabolic pathways involving the production of organic acids that release potassium from mineral sources such as feldspar and mica. Previous studies have also reported that potassium-solubilizing microorganisms, including actinomycetes, are less frequently isolated compared to nitrogen-fixing and phosphate-solubilizing microbes (Meena et al., 2020). Nevertheless, their role in improving potassium availability is crucial for plant physiological processes, including enzyme activation and stress tolerance.

The screening of 23 actinomycete isolates for phytohormone production demonstrated that a considerable proportion of isolates possess the ability to synthesize indole-3-acetic acid (IAA) and gibberellic acid (GA<sub>3</sub>), although with notable variability. In this study, 60.9% of isolates produced IAA, whereas only 43.5% produced GA<sub>3</sub>, indicating that IAA production is more commonly observed among actinomycetes. This finding is consistent with previous studies reporting that IAA is one of the most frequently produced phytohormones by actinomycetes due direct mechanism of root colonization of IAA by actinomycetes but GA<sub>3</sub> is the later secondary by product of actinomycetes (Vurukonda et al., 2018; Ahlawat et al., 2022).

The quantitative analysis showed that IAA production ranged from 7.933 to 30.733 µg/mL, with Actinomycetes isolate 5 exhibiting the highest production of IAA. Omar et al. (2022) noted 2 different strain of *Streptomyces* sp having a very significant difference in their IAA production. *Streptomyces* strain HM3 produces 22 µg/mL of IAA while *Streptomyces* strain HM10 produces 146 µg/mL of IAA (Omar et al., 2022). This shows that ability of actinomycetes to produce IAA was high. This relatively high number of IAA producing isolates in this study suggests that Malaysian soil actinomycetes are promising candidates for phytohormones production.

For the study of GA<sub>3</sub> production, it was detected that fewer isolates and lower concentrations (4.37–20.07 µg/mL) of GA<sub>3</sub> was produced, with Actinomycetes strain 13 showing the highest results. The lower frequency of GA<sub>3</sub> production observed among actinomycetes is consistent with reports that gibberellic-acid production occurs only in selected actinomycete taxa, including *Frankia*, *Streptomyces*, *Actinoplanes*, and *Micromonospora* (Silva et al., 2022). Boykova et al. (2023), stated that *Streptomyces* sp. strain P56 produces IAA (24 µg/mL) but not GA<sub>3</sub> when screened for phytohormones production ability. This once again reinforced our results that GA<sub>3</sub> producing actinomycetes is not widely found.

The screening of 23 actinomycete isolates for antagonistic activity against *Ralstonia solanacearum* and *Colletotrichum gloeosporioides* showed that a proportion of isolates possesses inhibitory potential against both bacterial and fungal plant pathogens. In this study, 6 isolates (26.1%) exhibited activity against *R. solanacearum*, whereas only 4 isolates (17.4%) showed inhibition against *C. gloeosporioides*, indicating that antibacterial activity was more prevalent than antifungal activity. This observation is consistent with previous reports that actinomycetes, particularly *Streptomyces* spp., are well-known producers of antibacterial compounds due to their rich repertoire of secondary metabolites such as antibiotics and lytic enzymes (Barka et al., 2015).

The inhibition zones against *R. solanacearum* ranged from 18.33 to 27.33 mm, with Actinomycetes isolate 5 showing the highest activity (27.33 mm). This strong antibacterial activity suggests the production of potent bioactive compounds capable of suppressing phytopathogenic bacteria. Previous studies have demonstrated that actinomycetes produce a wide range of antimicrobial substances, including polyketides and peptides, which are effective against soil-borne pathogens such as *R. solanacearum* (Zhuang et al., 2020). In a study done by Saputra et al. (2019), they showed that *Streptomyces* sp. strain S-4 was able to inhibit *R. solanacearum* with the diameter of 4.53 mm which is much smaller than what we observed in this study. In a different study conducted by Liao et al. (2024), they showed that *Streptomyces rapamycinicus* isolated was able to produce a 47.9 mm halo zone when tested with *R. solanacearum*. These findings highlight the potential of actinomycetes particularly the genus of *Streptomyces* as biocontrol agents for managing bacterial wilt disease.

In contrast, antifungal activity against *C. gloeosporioides* was less frequently observed in this study. The highest antifungal activity was recorded by Actinomycetes strain 1 (19.00 mm), followed by isolates 5 (18.33 mm). This result was almost similar to the halo zone produce by Actinomycetes strain BVEZA1 02 (16.00 mm) (Trinidad-Cruz et al., 2021). The relatively lower occurrence of antifungal activity is in agreement with previous findings that not all actinomycetes produce antifungal metabolites, as these require specific biosynthetic gene clusters and are environmental triggers (Barka et al., 2015).

Two isolates (isolates 5 and 13) demonstrated antagonistic activity against both pathogens, indicating broad-spectrum antimicrobial potential. Such isolates are particularly valuable in agriculture, as they can simultaneously control multiple plant diseases. The presence of dual-activity isolates supports previous studies showing that certain actinomycetes possess multifunctional biocontrol mechanisms, including the production of diverse secondary metabolites for the competition for nutrients and space (Ebrahimi-Zarandi et al., 2022; Zailan et al., 2024).

## 5. Conclusion

This study demonstrates the significant potential of soil-derived actinomycetes as sources of bioactive compounds for sustainable agriculture. A total of 23 isolates were obtained, exhibiting considerable diversity in both morphological and functional characteristics. Among these, 9 isolates produced white spores, 10 produced grey spores, and 4 exhibited yellow spores. Functional screening revealed that 5 isolates possessed nitrogen-fixing ability, 5 demonstrated phosphate-solubilizing capacity, and 4 showed potassium-solubilizing activity. In addition, 14 isolates were capable of producing indole-3-acetic acid (IAA), while 11 produced gibberellic acid (GA<sub>3</sub>). Antagonistic assays indicated that 6 isolates inhibited *Ralstonia solanacearum* and 4 inhibited *Colletotrichum gloeosporioides*. The most promising isolate giving positive results to all the bioactivities screening was Actinomycetes isolate 5, which was identified as *Streptomyces tendae*. Overall, these findings highlight not only the biodiversity of soil actinomycetes but also their multifunctional potential as effective bioinoculants, supporting their application as environmentally friendly alternatives in integrated and sustainable agricultural systems.

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## Conflict of Interest

The authors declare no conflicts of interest

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