

Characterization of actinobacterial isolates for plant growth promotional traits

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Abstract: Actinobacteria are beneficial soil microbes known for promoting plant growth. They solubilize phosphate, fix nitrogen and produce siderophores for nutrient uptake. These bacteria synthesize phytohormones like IAA and gibberellins, aiding root and shoot development. They enhance plant tolerance to abiotic stresses such as drought and salinity. Actinobacteria also produce antibiotics that suppress plant pathogens and promote plant immunity. Their diverse traits make them valuable for sustainable agriculture and eco-friendly crop management. The present study aimed to characterize the fifteen actinobacterial isolates for the PGP traits. Most isolates demonstrated the ability to produce indole-3-acetic acid (IAA), gibberlic acid and siderophores. IAA production ranged from 6.02 to 17.89 µg/ml and gibberlic acid production ranged from 0.69 to 1.58 µg/ml. Siderophore production, an important indirect biocontrol mechanism under iron-limiting conditions. Actinobacteria are positive for both oxidase and catalase tests, indicating their aerobic nature and ability to manage oxidative stress. These enzymatic activities reflect their metabolic versatility and environmental resilience. Such traits contribute to their effectiveness as plant growth-promoting bacteria.

Key words: Actinobacteria, IAA, Siderophore

Introduction

Actinobacteria are widely distributed in both terrestrial and aquatic environments, with a predominant presence in soil, exhibiting characteristics of both bacteria and fungi, they are highly valued in biotechnology for their ability to produce secondary metabolites. Notably, actinobacteria are responsible for generating approximately 10,000 bioactive compounds, accounting for about 45% of all known microbial bioactive metabolites. Among them, *Streptomyces* species stand out as a prolific source of diverse and beneficial natural products, offering significant potential for various biotechnological applications (Anandan *et al.*, 2016). Actinobacteria are Gram-positive bacteria characterized by a high guanine and cytosine (G+C) content in their genomic DNA. They lack a distinct cell wall structure similar to that of fungi, they form non-septate, slender mycelia. When cultured, their colonies typically exhibit a powdery texture and adhere firmly to agar surfaces, often developing hyphae and spore-like structures resembling fungal conidia or sporangia. Like other bacteria, actinobacteria possess peptidoglycan in their cell walls and contain 70S ribosomes, distinguishing them as true prokaryotes. They are widely distributed in soil, freshwater and marine environments, thriving particularly in low-pH soils, although they are notably sensitive to strong acidic conditions (Anandan *et al.*, 2016). Actinobacteria, a versatile group of soil microorganisms, play a crucial role in promoting plant growth through direct mechanisms such as synthesizing plant growth-promoting substances, fixing nitrogen and producing phytohormones. Additionally, they contribute indirectly by acting as potent biocontrol agents, stimulating overall plant development (Jeffrey, 2003).

Material and methods

The present investigation was undertaken to evaluate the plant-beneficial traits of actinobacterial isolates. A total of

fifteen actinobacteria were sourced from the Microbial Genetics Laboratory, Department of Microbiology, University of Agricultural Sciences (UAS), Dharwad, during the academic year 2022-2023.

Indole-3-acetic acid (IAA) production was determined using the colorimetric method described by Gordon and Weber (1951). The gibberellic acid production (GA) of the actinobacterial isolates/strains were determined by using the method described by Paleg (1965). Siderophore production by actinobacterial strains was determined by chrome azurol S (CAS) assay (Schwyn and Neilands, 1987). Actinobacterial cultures were inoculated on CAS agar and incubated for 7 days at 28 ± 0.2°C. Yellow to orange zone around the actinobacterial colonies indicated siderophore production.

Oxidase test was performed according to Kovacs, 1956. A strip of filter paper soaked in 1 percent N,N,N,N-tetramethyl-p-phenylene diamine dihydrochloride solution was taken and a loopfull of culture was smeared on it. The positive reaction was indicated by an intense deep purple colour and the negative reaction was indicated by no colour formation. Catalase test was performed according to Cowan and steel, 1970. A loopful of 5 days old culture of actinobacterial isolates/strains were smeared on clean glass slide and 2-3 drops of 3 per cent hydrogen peroxide solution was added and observed for effervescence (bubble formation) Effervescence formation indicated positive reaction for catalase test.

Results and discussion

Fifteen actinobacterial isolates were subjected to biochemical tests including oxidase, catalase and functional characterization include IAA production and gibberlic acid production.

Fourteen out of fifteen actinobacterial isolates tested positive for oxidase activity. Purple colour developed within 30 seconds upon application of 2–3 drops of 1 percent N,N,N₂,N₂-tetramethyl-p-phenylenediamine dihydrochloride solution indicated positive result for oxidase test. The enzyme plays a key role in microbial respiration and energy production, enabling microbes to maintain metabolic functions under biotic stress, such as pathogen attack. Oxidase-positive microbes can enhance plant defense through sustained colonization, contributing to their role as effective biocontrol agents (Sreedevi *et al.*, 2013). Fourteen actinobacterial isolates/strains exhibited catalase activity, evidenced by effervescence upon the addition of 2–3 drops of 3 percent hydrogen peroxide solution. Catalase positive strain helps to reduce oxidative stress in the host. Catalase breaks down harmful hydrogen peroxide produced during plant defense responses. This ability enhances microbial survival and supports plant health under pest or pathogen stress (Glick, 2012). Eight actinobacterial isolates showed positive for siderophore production, as indicated by yellow to orange halo zone around colonies grown on Chrome Azurol S agar when incubated at 28°C for 5–7 days. Siderophores are high-affinity metal chelators, particularly for iron. They are water-soluble, extracellular fluorescent compounds that facilitate iron uptake from the soil. Beyond their beneficial role in nutrient acquisition, siderophores also play a significant role in suppressing plant diseases. They achieve this by inhibiting the growth of phytopathogenic bacteria and fungi (Ghazy and El-Nahrawy, 2021).

Table 1. Biochemical properties and siderophore production by actinobacterial strains/isolates

Actinobacterial isolates/strains	Catalase test	Oxidase test	Siderophore production
<i>Streptomyces parvus</i> AUDT248	+	+	-
<i>Streptomyces rimosus</i> AUDT502	+	+	+
<i>Streptomyces tanashiensis</i> AUDT573	+	+	+
<i>Streptomyces lavendulae</i> AUDT617	+	+	-
<i>Streptomyces racemochromogenes</i> AUDT626	+	+	+
<i>Streptomyces spectabilis</i> AUDT656	-	-	-
<i>Streptomyces sclerogranulatus</i> AUDT690	+	+	-
<i>Streptomyces polychromogenes</i> AUDT824	+	+	+
<i>Streptomyces hyderabadensis</i> DBT64	+	+	+
<i>Streptomyces xiaminesis</i> DBT80	+	+	-
AUDT596	+	+	-
AUDT811	+	+	+
DBT84	+	+	-
DBT90	+	+	+
DBT96	+	+	+

The synthesis of phytohormones like indole-3-acetic acid (IAA), a primary auxin, promotes cell elongation, root initiation and vascular differentiation, thereby enhancing root architecture and nutrient uptake (Fanai *et al.*, 2024). The amount of IAA produced by the 15 actinobacterial isolates/strains ranged from 6.02 to 17.89 µg/ml broth. The highest IAA production was observed in *Streptomyces racemochromogenes* AUDT626 (17.89 µg/ml) which was on par with *S. polychromogenes* AUDT824 (17.27 µg/ml) and the moderate IAA producers included *Streptomyces tanashiensis* AUDT573 (16.69 µg/ml) and AUDT502 (16.38 µg/ml). The lowest IAA production was noted in *S. spectabilis* AUDT656 (6.02 µg/ml), DBT84 (6.65 µg/ml) and *S. lavendulae* AUDT617 (7.27 µg/ml), which were significantly lower than the top IAA producers (*Streptomyces racemochromogenes* AUDT626 and *S. polychromogenes* AUDT824). The amount of GA produced by the 15 actinobacterial isolates/strains ranged from 0.69 to 1.58 µg/ml broth. The highest GA production was recorded by *S. polychromogenes* AUDT824 (1.58 µg/ml), followed closely by *S. racemochromogenes* AUDT626 (1.53 µg/ml) were significantly higher compared to all other isolates and the moderate GA production was recorded by *Streptomyces parvus* AUDT248 (1.49 µg/ml) and AUDT811 (1.45 µg/ml). *S. spectabilis* AUDT656 showed the lowest GA production (0.69 µg/ml), which was significantly lower than the highest GA producers.

Conclusion

The biochemical and functional characterization of fifteen actinobacterial isolates revealed their strong potential as plant growth-promoting agents. Most isolates tested positive for oxidase and catalase test, highlighting their resilience under oxidative and biotic stress conditions. Eight isolates positive for siderophore production, supporting their role in iron acquisition and biocontrol. All isolates were capable of producing IAA and gibberellic, with significant variation in

Table 2. IAA and GA production by actinobacterial isolates/strains

Actinobacterial isolates/strains	IAA (µg/ml)	GA (µg/ml)
<i>Streptomyces parvus</i> AUDT248	12.43	1.49
<i>Streptomyces rimosus</i> AUDT502	16.38	1.41
<i>Streptomyces tanashiensis</i> AUDT573	16.69	1.53
<i>Streptomyces lavendulae</i> AUDT617	7.27	0.98
<i>Streptomyces racemochromogenes</i> AUDT626	17.89	1.53
<i>Streptomyces spectabilis</i> AUDT656	6.02	0.69
<i>Streptomyces sclerogranulatus</i> AUDT690	8.63	1.11
<i>Streptomyces polychromogenes</i> AUDT824	17.27	1.58
<i>Streptomyces hyderabadensis</i> DBT64	12.53	1.40
<i>Streptomyces xiaminesis</i> DBT80	12.38	0.98
AUDT596	13.21	1.11
AUDT811	15.19	1.45
DBT84	6.65	1.15
DBT90	12.48	1.11
DBT96	8.47	1.19
S.Em±	0.43	0.05
C.D @ 1%	1.52	0.17

production levels. *Streptomyces racemochromogenes* AUDT626 and *S. polychromogenes* AUDT824 showed the highest IAA and GA production. Low IAA and GA production were recorded in *S. spectabilis* AUDT656. These traits underline the

adaptability and multifunctionality of actinobacteria in promoting plant health and productivity. Overall, the isolates exhibit promising potential for use in sustainable agriculture as biofertilizers and biocontrol agents.

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