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Characterization and Evaluation of Endophytic Bacteria from the Ethno-Medicinal Plant *Gynura cripidioides* (Gende) of North Eastern Himalayan Region, India

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Abstract

Bacterial endophytes are bacteria that reside internally within plants, flourishing in a distinct environment that protects them from external adversities and changes in environmental circumstances, unlike microbes that live outside. Their entry into plant tissues occurs through specific 'hotspot' areas, such as the root system. After gaining entry, the plants use a variety of secondary metabolites, structural component synthesis, plant immunity, resource competition with pathogens, antioxidant activities and phenylpropanoid metabolism to reduce the effects of both biotic and abiotic stressors. From the Gende (*Gynura cripidioides*; Family: Asteraceae) that was removed from the Pasighat region in the East Siang District of Arunachal Pradesh, India, endophytic bacteria were recovered. This study set out to evaluate and characterise endophytic bacteria for their cpability to enhance plant growth through various means, including phosphate solubilization, IAA production, siderophore production, growth on nitrogen-free media, exo-polysaccharide production, *in-vitro* evaluation and antagonistic activity analysis.

Keywords: Bio-control, Endophytes, IAA production, PGPR, Siderophore production

Introduction

In recent years, significant efforts have been directed towards uncovering innovative biological approaches to manage harmful microbes. Among these strategies, the utilization of bacterial endophytes has garnered considerable attention due to their multifaceted applications. Bacterial endophytes are known to enhance plant growth and productivity (Compant *et al.*, 2005), act as biocontrol agents (Sturz *et al.*, 1999; Wilhelm *et al.*, 1997) and produce a broad spectrum of natural products beneficial in agricultural, medicinal and other sectors (Hallman *et al.*, 1997; James, 2000).

The plant kingdom harbors a wide array of endophytic bacteria. Sturz and Christie (1996) have documented that these bacteria can form non-pathogenic associations with their host plants, exhibit allelopathic properties and function as allelopathic agents. These associations are not merely benign but confer several benefits to the host plants. They can enhance disease resistance (Sturz *et al.*, 1999), enhance stress tolerance (Sziderics *et al.*, 2007) and boost soil fertility by means of nitrogen fixation and phosphate solubilization (Dong *et al.*, 1995; James, 2000).

Moreover, Newman and Reynolds (2005) posited that bacterial endophytes might be more effective than environmental bacteria in promoting plant health due to their ability to occupy an intrinsic niche within the host plant, thereby reducing competitive pressures during colonization. This unique endophytic lifestyle allows them to respond intimately with their host plants, resulting in less rivalry for nutrients and superior protection from negative environmental conditions compared to bacteria

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in the rhizosphere and phyllosphere (Petrini, 1991; Weyens *et al.*, 2013).

The microbial diversity present in global forests remains vast and largely unexplored. These microorganisms possess unique genetic and biological characteristics and are known to produce antibacterial compounds. Living as endophytes within their host plants, these microbes could potentially offer substantial benefits to their hosts.

Materials and Methods

Isolation

Endophytic bacteria were extracted from plant samples collected near Pasighat in Arunachal Pradesh's East Siang district, India, during the period of 2015-2016. Whole Gende plants (*Gynura crispidioides*) were carefully uprooted by hand while they were in their pre-anthesis stage. The procedures for isolating, enumerating, selecting and storing the endophytic bacteria were meticulously followed as described by Pandey *et al.* (2015; 2016).

Overview of Isolates

Morphological Description

All isolates underwent morphological characterisation using established protocols. Examining the colony's surface, edge, elevation, margin, colour, cell shape, gramme response, endospore staining and motility was part of this process. These features were then used to tentatively group and document the isolates.

Biochemical Characterization

Catalase Activity

Endophytic bacterial cultures were transferred from nutrient agar slants to a glass slide and mixed with a few drops of distilled water. A 3% hydrogen peroxide solution was applied to the slide, resulting in the formation of bubbles and effervescence (Aneja, 2005).

Oxidase Test

An inverted posture was maintained at 30 °C for 48 hours after the addition of endophytic isolates to a solution of trypticase soy agar medium. Following incubation, the streaking area was treated with two to three drops of para-amino dimethyl aniline oxalate solution. Within thirty seconds, the colour changed from pink to maroon to purple, indicating a positive response (Cappuccino and Sherman, 1996).

Nitrate Reduction Test

Endophytic bacterial isolates were placed into test tubes containing nitrate broth, which is a solution of 3 g L^{-1} beef extract, 5 g L^{-1} peptone and 1 g L^{-1} potassium nitrate. The broth, with a pH of 7.0, was incubated at 30°C for two weeks. Post-incubation, sulfanilic acid and alpha-naphthylamine were added in equal parts to the broth. The appearance of a crimson color revealed the conversion of nitrate to nitrite (Cappuccino and Sherman, 1996).

Synthesis of Hydrogen Sulfuride

Using a kit from Himedia Laboratories Pvt. Ltd., Mumbai, India, endophytic bacterial isolates generated hydrogen sulphide. Between the plug and tube wall, a lead acetate paper strip (DD 034) was placed above the medium; endophytic bacterial isolate was added to the peptone water (M028). Incubated at 35 °C for 18 to 24 hours. The production of H_2S was indicated by the black lead acetate paper strip at the bottom.

Indole Production

Tryptone-containing glucose broth containing 10 g L^{-1} of endophytic bacteria isolates was cultured for 48 hours at 30 °C. Kovacs reagent (0.3 ml) was added and thoroughly mixed after the incubation period. Soon the top layer of alcohol became red, indicating the presence of indole (Seeley and Van Demark, 1981).

The Methyl Red and Voges-Proskauer Testing

After two sets of endophytic bacterial isolates were added to Methyl Red and Voges-Proskauer (MR-VP) broth (pH 6.6-7.1), which contained 7 g L^{-1} peptone, 5 g L^{-1} K_2HPO_4 and 5 g L^{-1} glucose; the combination was cultured at 30 °C for 48 hours. To conduct the Methyl Red Test, a small quantity of an alcoholic methyl red solution (0.1 g methyl red in 100 ml ethyl alcohol, followed by 60 ml of water) was mixed with the first set of MR-VP broth. A red hue indicates a positive result for this test. For the Voges-Proskauer test, the second set of MR-VP broth was combined with an α-naphthol solution (5 g α-naphthol in 100 ml ethyl alcohol) and gently stirred for 15 minutes. The production of acetyl methyl carbinol and a red color signifies a positive VP test (Seeley and Van Demark, 1981).

Citrate Usage Assessment

A modified Seeley and Van Demark (1981) method were used on Simmons' citrate agar to conduct the citrate utilisation test for endophytic bacteria. 1 g of $(NH_4)H_2PO_4$, 1 g of K_2HPO_4, 5 g of NaCl, 2 g of sodium citrate, 2 g of $MgSO_4$, 15 g of agar and 8 g of bromothymol blue made up the agar composition $litre^{-1}$, which had a pH of 6.9. Except for the phosphates, all the ingredients were dissolved in 900 ml of water. The final volume was one litre after the phosphates were individually dissolved in 100 ml of water.

Endophytic Bacterial Isolates Ability for Fermentation of Carbohydrates

The ability of endophytic bacterial isolates to ferment a range of carbohydrates, including polysaccharides, disaccharides (like lactose and sucrose) and monosaccharides (like glucose), was studied. Durham tubes were put inside the tubes to monitor the gas generation during fermentation. The fermentation medium had a pH of 7.3, in addition to 10 g L^{-1} peptone, 5 g L^{-1} carbohydrate, 15 g L^{-1} sodium chloride, 0.018 g L^{-1} phenol red and 0.50% of glucose, sucrose, lactose, mannitol and mannose.

A pH indicator, phenol red turns yellow below pH 6.8 from organic acid generation. After being injected into sterile fermentation tubes containing broths containing glucose, sucrose, lactose, mannitol and mannose, endophytic isolates were cultured for 48 hours at 30 °C. There was no vaccination

administered to the control tubes. The production of gas and acid was shown by a colour shift and the appearance of bubbles in contrast to the control (Aneja, 2005).

Urease Test

To perform the urease test, endophytic bacterial isolates are grown on urea broth (g L^{-1}; yeast extract 0.1, potassium phosphate, monobasic 9.1, dibasic 9.5, urea 20, phenol red 0.01, pH 6.8). The mixture is then dissolved and sterilised by filtering all the components in one litre of distilled water. Mix and pour 3 ml of yellow-orange color prepared broth into sterile tubes. Refrigerate prepared broth at 4-8 °C until needed. Avoid heating or autoclaving the medium, which decomposes urea. Inoculate broth with heavy day-old pure culture inoculums. Shake culture inoculums gently in broth to suspend bacteria. Loosen cap and culture broths at 35 °C for 24 hours. Urease-containing isolates will produce ammonia, which raises the pH of the broth and turns it yellow to red or deep pink of phenol red during incubation. Color change indicates urease-producing endophytic bacterium isolate (Stuart *et al.*, 1945).

Plant-Beneficial Characteristics of Isolated Endophytic Bacteria

Phosphate Solubilization

Following the methodology described by Mehta and Nautiyal (2001), endophytic bacterial isolates were assessed for their phosphate solubilization capability using the NBRI-BPB (National Botanical Research Institute-Bromo Phenol Blue) medium. Every litre of the medium have contained 10 g of glucose, 5 g of $Ca_3(PO_4)_2$, 0.25 g of $MgSO_4 \cdot 7H_2O$, 5 g of $MgCl_2 \cdot 6H_2O$, 0.20 g of KCl, 0.10 g of $(NH_4)_2SO_4$, 0.025 g of BPB and 20 g of agar.

The pH of the medium was 7. Approximately 25 ml of the medium were placed into each Petri plate after it had been prepared and autoclaved. Each plate was then spot inoculated with fresh cultures of endophytic bacteria and it was then cultured for two to three days at 28 degrees Celsius (~ ±1). The bacterial colonies' clear zones were evaluated following incubation to show that the isolates had dissolved phosphate. The formula was used to compute the phosphate solubilization index:

$$\text{Phosphate Solubilization Index} = \frac{A}{B} \times 100$$

Where,

A = Total diameter (including the colony and the halo zone);

B = Diameter of the colony itself.

Ability to Produce Exo-Polysaccharide

The plate test was used to evaluate endophytic bacterial isolates' ability to produce exo-polysaccharides in 5% sucrose-containing nutritional media. The medium was composed of the following components: 3 g of beef extract, 50 g of sucrose, 5 g of peptone, 5 g of NaCl and 15 g of agar. The medium had a pH of 7. After streaking the isolates on these plates, they were cultivated for four to seven days at 30 °C. According to reports, the production of exo-polysaccharides was suggested by the emergence of a thick, sticky fluid covering the streaking area (Anu Rajan, 2012).

Synthesis of Indole Acetic Acid

A nutrient-dense broth with 5 µg ml^{-1} of L-tryptophan was introduced to the bacterial culture and incubated at 28±2 °C for five days to evaluate indole acetic acid (IAA) synthesis. The cultures were centrifuged at 3,000 rpm for 30 minutes following incubation. Subsequently, 2 ml of the supernatant was amalgamated with Salkowski's reagent, consisting of 1 ml of 0.5 M $FeCl_3$ and 50 ml of 35% perchloric acid. The manifestation of a crimson hue signified IAA synthesis. The optical density (OD) was assessed at 535 nm with a spectrophotometer, and the IAA concentration was documented in µg ml^{-1}.

Siderophore Production

Schwyn and Neilands (1987) devised this method to quantify the quantity of siderophores generated by endophytic bacteria. Plates containing Chrome Azurol S (CAS) agar were spotted with pure bacterial cultures and they were cultured at 30 °C for two to six days. The existence of a yellow halo around the bacterial colonies suggested that siderophores were being synthesised.

Evaluation and Investigation of Bacterial Endophytes' Ability to Oppose Pathogens in vitro

The dual culture plate technique on potato dextrose agar (PDA) was employed to evaluate the antagonistic effects of endophytic bacterial isolates against the fungal pathogens *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, as outlined by Dennis and Webster (1971). At 28 °C for 24 hours, a 5 mm mycelial disc of the fungal pathogen was put in the centre of a petri plate. The pathogen disc was then streaked with a loopful of endophytic bacterial culture on both sides at an equal distance on the same plate. The controls were PDA plates that contained just the pathogen disc. The zone of inhibition, which is defined as the separation between the endophytic bacterial culture and the fungal pathogen, was assessed after one week. For every experiment, three copies were conducted. The bacterial endophytes were classified based on their percent growth inhibition (PGI) and growth inhibition category (GIC), which were evaluated according to their antagonistic efficacy.

The formula for calculating the percent growth inhibition (PGI) caused by pathogen-associated bacterial endophytes is:

$$\text{PGI (\%)} = \frac{K_R - R_1}{K_R} \times 100$$

Where,

K_R = Measurement in mm between the inoculation site and the colony edge of the fungal pathogen;

R_1 = Amount of time (mm) that elapses between the fungal growth colony border and the point of inoculation.

Based on the effectiveness of percent growth inhibition (PGI), bacterial endophytes were divided into five growth inhibition groups: zero, up to 25%, 50%, 75%, 100% and so on are the many levels of growth inhibition that can occur.

Results and Discussion

Isolation and Characterization of Endophytic Bacterial Isolates

From various sections of *Gynura crispidioides*, particularly leaves, stems and roots, thirteen endophytic bacterial isolates were collected. Morphological characterization of these isolates revealed a diverse range of colony characteristics. For example, isolates PG1 and PG2 from the leaves exhibited smooth, circular colonies with entire margins and yellowish-white pigmentation, while isolate PG8 from the stem had contoured, filamentous colonies. Such morphological diversity suggests the presence of different bacterial species with potentially varied functional roles within the host plant (Table 1).

Table 1: Phenotypic characterization of endophytic bacterial isolates isolated from *Gynura cripidioides*

Sl. No.	Isolates	Colonial surface	Colony edge	Elevation of colonies	Margin	Discoloration	Plant part
1	PG1	Smooth	Circular	Round	Entire	Yellowish white	Leaf
2	PG2	Smooth	Circular	Round	Entire	Yellowish white	Leaf
3	PG3	Smooth	Circular	Round	Entire	white	Leaf
4	PG4	Smooth	Circular	Round	Entire	white	Leaf
5	PG5	Smooth	Circular	Round	Entire	white	Leaf
6	PG6	Smooth	Circular	Round	Entire	white	Leaf
7	PG7	Smooth	Circular	Round	Entire	Yellowish white	Stem
8	PG8	Contoured	Filamentous	Flate	Filamentous	White	Stem
9	PG9	Smooth	Circular	Raised	Entire	Yellowish	Root
10	PG10	Smooth	Circular	Raised	Entire	Yellowish	Root
11	PG11	Smooth	Circular	Raised	Entire	White	Root
12	PG12	Smooth	Circular	Raised	Entire	White	Root
13	PG13	Smooth	Circular	Raised	Entire	White	Root

Biochemical characterization further differentiated these isolates. Several isolates' positive findings on the oxidase test suggested the existence of cytochrome c oxidase in these bacteria (Table 2). Positive in numerous isolates, the nitrate reduction test evaluates the capacity of bacteria to convert nitrate to nitrite or other nitrogenous chemicals, implying their possible function in nitrogen cycle inside the plant. Moreover, all isolates tested positive on the catalase test, which detects the presence of the catalase enzyme breaking down hydrogen peroxide, therefore proving their ability to reduce oxidative stress.

The metabolic adaptability of these endophytic bacteria is shown by the formation of hydrogen sulfide (H_2S), seen in isolates PG6, PG7, PG8 and PG13, and the urease activity, seen in isolates PG1, PG4, PG6 and PG13. These biochemical characteristics are significant since they may change the interaction between the bacteria and their host plant, therefore influencing plant health and growth (Table 2).

Plant-Beneficial Characteristics of Isolated Endophytic Bacteria

Phosphate Solubilization

Phosphorus is an essential mineral for plant development, although its availability in soil is frequently constrained due to immobilization. The capacity of endophytic bacteria to solubilize phosphate can markedly improve plant nutrient absorption (Table 3). NBRI-BPB medium was employed to evaluate the phosphate solubilizing activity of the isolates in this work. The findings showed that most isolates have this capacity as shown by the development of obvious halo zones surrounding the bacterial colonies.

Isolate PG12 demonstrated the highest phosphate solubilization index of 283.3, followed by isolates PG2 (280.0), PG5 (271.4) and PG9 (271.4) (Table 4). The solubilization of phosphate by these isolates suggests that they could be quite important in increasing the availability of phosphorus to *Gynura crispidioides*, hence supporting plant development. In agricultural soils where phosphorus availability limits crop production, this capacity is very beneficial.

Exo-Polysaccharide Production

Bacterial biofilm production depends on exo-polysaccharides, which can help plants resist infections and environmental stress. Visually evaluating the isolates' generation of exo-polysaccharides after 24 and 48 hours at 30 °C. All isolates produced varying amounts of polysaccharides, with significant production observed in isolates PG1, PG2, PG3, PG4, PG5, PG6, PG7, PG8, PG10, PG11, PG12 and PG13 (Table 5).

Table 2: Biochemical characteristics of the endophytic bacteria

Sl. No.	Isolates	Oxidase	Nitrate	H_2S	Catalase	Indole	MR test	VP test	Citrate Utilization	Urease
1	PG1	-	+	-	+	-	+	-	-	+
2	PG2	-	+	-	+	-	+	-	-	-
3	PG3	+	-	-	+	-	+	-	-	-
4	PG4	-	+	-	+	-	+	-	-	+
5	PG5	+	+	-	+	-	+	-	-	-
6	PG6	+	-	+	+	-	-	-	-	+
7	PG7	+	-	+	+	-	+	-	-	-
8	PG8	+	-	+	+	-	-	-	-	-
9	PG9	+	+	-	+	-	+	-	-	-
10	PG10	+	+	-	+	-	+	-	-	-
11	PG11	+	+	-	+	-	+	-	-	-
12	PG12	+	-	+	+	-	+	-	-	-
13	PG13	+	+	+	+	-	+	-	-	-

[Note: '+' : positive result; '-' : negative result]

Table 3: Carbon source utilisation by the endophytic bacterial isolates

Sl. No.	Isolates	Glucose		Sucrose		Lactose		Mannitol		Mannose	
		Colour	Gas	Colour	Gas	Colour	Gas	Colour	Gas	Colour	Gas
1	PG1	+	-	+	-	+	-	+	-	+	-
2	PG2	+	-	+	-	+	-	+	-	+	-
3	PG3	+	-	+	-	+	-	+	-	+	-
4	PG4	+	-	+	-	+	-	+	-	+	-
5	PG5	-	-	-	-	-	-	+	-	+	-
6	PG6	+	-	+	-	-	-	+	-	+	-
7	PG7	+	-	+	-	-	-	+	-	+	-
8	PG8	+	-	+	-	-	-	+	-	+	-
9	PG9	-	-	-	-	-	-	-	-	-	-
10	PG10	+	-	+	-	-	-	+	-	+	-
11	PG11	+	-	+	-	-	-	-	-	-	-
12	PG12	+	-	+	-	-	-	+	-	+	-
13	PG13	+	-	+	-		-	+	-	+	-

The samples were analysed for gas and acid generation (a positive result is shown by a '+'; a negative result is indicated by a '-') after being cultured by endophytic bacteria for 24 hours at 30 °C

The capacity to produce a thick, sticky polysaccharide matrix suggests these isolates can form biofilms, which could improve their survival and persistence inside the plant. Endophytic bacteria's biofilm development can also strengthen stress resistance and shield the plant from pathogenic incursion.

Indole Acetic Acid (IAA) Production

IAA, a significant auxin, drives plant growth and development by altering several physiological processes. A nutritional broth including L-tryptophan was employed to determine IAA generation by the isolates. All isolates showed notable IAA synthesis, with the highest production observed in isolate PG13, which produced 178 µg ml^{-1}, followed by PG5 (174 µg ml^{-1}) and PG11 (169 µg ml^{-1}) (Table 5).

The capacity of these isolates to generate notable quantities of IAA implies that by affecting root development, cell elongation and differentiation, they can promote the growth of *Gynura crispidioides*. This auxin production is a key trait for plant growth-promoting bacteria and can have substantial benefits for agricultural practices.

Siderophore Production

Siderophores are iron-chelating compounds that play a crucial role in iron acquisition and pathogen inhibition.

Chrome Azurol S (CAS) agar plates were utilized to assess siderophore synthesis by the isolates. Of the nine isolates that generated siderophores, PG1, PG2, PG4, PG6, PG7 and PG8 showed substantial production (Table 5).

Table 4: Showing phosphate solubilization activity of endophytic bacteria

Sl. No.	Isolates	Phosphate solubilization on NBRI-BPB medium			
		Diameter of Colony (mm)	Diameter of halo zone (mm)	Total diameter (Colony + Halo zone)	Phosphate Solub. Index
1	PG1	8.0	13.0	21.0	262.5
2	PG2	10.0	18.0	28.0	280.0
3	PG3	9.0	15.0	24.0	266.6
4	PG4	6.0	9.0	15.0	250.0
5	PG5	7.0	12.0	19.0	271.4
6	PG6	5.0	No zone	5.0	Nil
7	PG7	5.0	No zone	5.0	Nil
8	PG8	7.0	No zone	7.0	Nil
9	PG9	7.0	12.0	19.0	271.4
10	PG10	6.0	10.0	16.0	266.6
11	PG11	6.0	10.0	16.0	266.6
12	PG12	6.0	11.0	17.0	283.3
13	PG13	8.0	13.0	21.0	262.5

[Note: '+' : positive result; '-' : negative result]

A yellow halo surrounding the bacterial colonies suggested siderophore production. This quality is crucial for many physiological processes since it can improve iron availability to the host plant. Additionally, siderophore production can inhibit pathogenic microbes by sequestering iron, hence reducing its availability to pathogens.

Evaluation and Analysis of Antagonistic Activity against Pathogens

Using the dual culture plate technique, we evaluated the antagonistic actions of endophytic bacterial isolates on the fungal pathogens *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. The findings revealed that the isolates varied in their capacity to suppress the mycelial growth of these pathogens.

Antagonistic Activity against Sclerotium rolfsii

At 55.0%, isolate PG3 showed the greatest mycelial growth suppression, therefore classifying it under growth inhibition category (GIC) 3 (Table 6). Falling under GIC 2, isolates PG1, PG2, PG4, PG6, PG9, PG10, PG11 and PG12 demostrated moderate antagonistic activity with PGI values ranging between 35.0% and 50.0%. These isolates showed a notable capacity to suppress *Sclerotium rolfsii* growth, implying their possible application as biocontrol tools against this pathogen.

Isolates PG5, PG7, PG8 and PG13 exhibited lower antagonistic activity with PGI values between 10.0% and 25.0%, categorized under GIC 1. Although these isolates showed lower inhibition, their combined use with more effective isolates could enhance biocontrol efficacy.

Antagonistic Activity against Sclerotinia sclerotiorum

Isolate PG10 displayed the most inhibition for *Sclerotinia*

Table 5: Exo-polysaccharide, IAA and Siderophore production by the endophytic bacteria

Sl. No.	Isolates	Polysaccharide production		IAA production		Siderophore Production
		24 hours	48 hours	O.D. at 535 WL	IAA production (µg ml^{-1})	
1	PG1	++	+++	0.113	151.00	+++
2	PG2	++	+++	0.079	106.00	+++
3	PG3	++	+++	0.118	158.00	++
4	PG4	++	+++	0.093	124.00	+++
5	PG5	++	+++	0.130	174.00	+
6	PG6	++	+++	0.105	140.00	+++
7	PG7	++	+++	0.096	128.00	+++
8	PG8	++	+++	0.097	130.00	+++
9	PG9	++	+++	0.109	146.00	-
10	PG10	++	+++	0.099	132.00	+
11	PG11	+	++	0.126	169.00	-
12	PG12	++	+++	0.122	163.00	++
13	PG13	++	+++	0.133	178.00	-

[Note: Ability of endophytic bacteria for polysaccharide and siderophore production was estimated visually by scoring growth of the colonies and halo zone respectively in the plates; '-' : No activities, '+' : Slight activities, '++' : Moderate activities, '+++' : High activities]

sclerotiorum at 75.0%, followed by PG2 (72.5%) and PG1 (70.0%); all categorised under GIC 3. Strong antagonistic activity has been shown by these isolates which suggests their possible use as efficient biocontrol agents against this pathogen (Table 6).

Table 6: Showing *in vitro* antagonistic activity evaluation of endophytic bacteria and their percent growth inhibition (PGI)

Sl. No.	Isolates	% Growth Inhibition (PGI)	
		Against *Sr*	Against *Ss*
1	PG1	48.5	70.0
2	PG2	48.5	72.5
3	PG3	55.0	52.5
4	PG4	45.0	55.0
5	PG5	25.0	55.0
6	PG6	35.0	47.5
7	PG7	25.0	37.5
8	PG8	22.5	47.5
9	PG9	40.0	27.5
10	PG10	50.0	75.0
11	PG11	47.5	55.0
12	PG12	45.0	50.0
13	PG13	10.0	27.5

[Note: '+' : positive result; '-' : negative result]

Isolates PG4, PG5 and PG11 also showed significant inhibition with PGI values of 55.0%; while PG3, PG6, PG7, PG8, PG9, PG12 and PG13 have exhibited moderate to low inhibition. The varying levels of antagonistic activity observed among the isolates suggest that a combination of these bacteria could provide a broader spectrum of pathogen control.

The outcomes of this investigation underlined the possibility of endophytic bacterial isolates from *Gynura crispidioides* in fostering plant growth and offering biocontrol against fungal diseases. Among the remarkable qualities the isolates showed were phosphate solubilization, exo-polysaccharide production, IAA synthesis and siderophore production, all of which benefit the plant health and growth (Wang *et al.*, 2022). Their antagonistic action against the fungal pathogens shows their capacity to properly stop pathogen growth, hence lowering crop disease incidence. Including such endophytic bacteria into agricultural procedures can reduce dependence on chemical fertilizers and pesticides, hence promoting sustainable and ecologically friendly farming methods.

Furthermore, the combination of isolates with complementary traits could enhance their overall efficacy. For example, isolates with strong phosphate solubilization and IAA production could be paired with those showing robust antagonistic activity, providing both growth promotion and pathogen suppression. This integrated approach could lead to the development of multifunctional bioinoculants that offer comprehensive benefits to crops (Sturz *et al.*, 1999; Hallman *et al.*, 1997).

Conclusion

A major source of chemically distinct and bioactive chemicals are natural microorganisms known as endophytes. They are extensively employed in industrial, agricultural, medical and pharmaceutical applications. Using endophytes produces faster and higher yields of bioactive chemicals than treating many mature plants to generate low yields of final goods. By creating chemicals *in vitro* and under carefully regulated conditions, endophytes lessened the chance of plant extinction. In addition, endophytes shorten the time and labour costs needed to generate a vast array of beneficial biological active chemicals. Utilising endophytes allows plants to develop novel compounds that were absent from their prior state. Secondary metabolites are produced by endophytes and are utilised in the manufacturing of medicinals, insecticides and other naturally occurring compounds of significant industrial value. Furthermore, implicated in immune induction, environmental interaction and adaptation are secondary metabolites. Antimicrobial compounds are produced by endophyte-related plants to prevent pathogen invasion of their tissues or sections.

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