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CHARACTERIZATION OF MULTIPLE PLANT GROWTH PROMOTION TRAITS OF ENDOPHYTIC BACTERIA ISOLATED FROM *CATHRANTHUS ROSEUS*

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Abstract:

Endophytic bacteria can be defined as those bacteria that are present inside the plant tissue without causing any harm to the host plant. Endophytes help in plant growth promotion and can also act as biocontrol agents. With increasing environmental awareness, developing endophytic bacteria as a biofertilizer is a new alternative. Considering this objective, the present research work was carried out. In current study, fresh plants of Cathranthus roseus were collected from the Patanjali nursery Haridwar and endophytic bacteria were isolated from its roots. Total Twenty one endophytic bacteria were obtained which were preliminary identified through morphological and biochemical characterization. Nine different bacteria were selected for PGP Test such as IAA production, HCN, phosphate solubilization, ammonia production and antifungal activity. However, out of nine, two isolates were found to be most potent and showed maximum PGP traits, which can be used for further studies.

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INTRODUCTION:

The term endophyte (Gr. endon, within; phyton, plant) was first coined by De Bary in 1866. Endophytes are defined as microorganisms such as bacteria and fungi that are located within plant tissues, without causing harm to the host plant [1-3]. Bacterial endophytes have been known for more than hundred years. In year 1926 presence of bacteria residing within healthy plants was first reported [4-5]. Endophytic bacteria can be found at many sites in the plant, such as seeds, fruits, ovules, stems, leaves, roots, tubers, and as well inside legume nodules [6-7]. Innumerable studies suggest that in most of the plants population of endophytic bacteria is greater in roots as compared to shoots and leaves [8]. Once bacteria enter the plant tissue, they either remain localised at the point of entry or spread throughout the plant. In addition to this compatible host plant is also required for successful colonization of endophytic bacteria within the plant tissues. The importance of bacterial endophyte had been reported as plant growth promoters as they have often provide nutrients like nitrogen and phosphorous, protection from effects of abiotic stress [9-10]. Availability of phosphorus to plants is influenced by microorganisms through the process of mineralization and demineralization. Endophytic bacteria offer a biological rescue system capable of solubilizing the insoluble inorganic phosphorus of soil and make it available to the plants [11-12]. These bacteria have ability to convert insoluble phosphate to an accessible form, like orthophosphate, is an important trait in a plant growth promoting bacteria for increasing plant yield [13].

Endophytic bacteria also promote plant growth in terms of increased germination rates, biomass, leaf area, chlorophyll content, nitrogen content, protein content, hydraulic activity, roots and shoot length. Selections of bacterial endophytes isolates from plants are considered as possible measures for improving crop health [14].

Medicinal plants have been recognized as a repository of endophytes with novel metabolites of pharmaceutical and agriculture importance. *Catharanthus roseus* is a medicinal plant belongs to the family Apocynaceae. The plant is also known by the names such as *Vinca rosea*, *Ammocallis rosea* and *Lochnera rosea*. In India it is known as 'Sadabahar' meaning 'always in bloom' and is used for worship The plant has been put to traditional use for the treatment of a wide variety of diseases like diabetes, cancer & high blood pressure, asthma, anti-inflammatory, dysentery, brain stimulatory actions, anti-angiogenesis effects, anti-malarial also potent anti-microbial activities [15]. It has been reported to possess anticancer, anti-diabetic (roots and leaves), hypolipidemic, and antioxidant activity. *C. roseus* being a source of many important secondary metabolites can also be source of beneficial endophytic bacteria.

The aim of the present research work is to isolate efficient bacterial from the roots of *C. roseus* that have the capability to be used as plant growth promoting and biocontrol agents.

MATERIAL AND METHODS:

Collection of plant material

For the isolation of endophytic bacteria, healthy *C.roseus* plants were collected from the Patanjali nursery, Haridwar. Samples were placed in clean plastic bags, brought to the laboratory and used for further experimental purpose.

Isolation of bacterial endophytes

The roots of *Cathranthus roseus* plant were washed with tap water to remove adhering soil particles and the majority of microorganisms present on the surface. Roots were then washed with 0.1% HgCl, then washed with 70% ethanol for 1 min and rinsed thoroughly with sterile distilled water. The samples were again washed with 3% Sodium hypochlorite for -5 min and again washed with sterile distilled water; this final water wash was collected for sterility test. The outer layer of the cortex of the root was removed using sterilized sharp knife. Small pieces 1cm were taken from the inner cortical region of the cuttings these pieces were placed on nutrient agar. Plates were sealed using parafilm tape and incubated at 28C in order to recover the maximum possible colonies of bacterial endophytes. The observation was made after 24hrs and48 hrs respectively [7].

Preliminary characterization of bacterial isolates.

The bacterial isolates were identified based on the characters of the Gram Staining, morphological characterstics and biochemical test such as Starch hydrolysis, Catalase test, Citrate test, Urease test, Indole production [16].

In vitro screening of isolates for different plant growth promoting activities

Indole-3-acetic acid (IAA) Production

The bacterial isolates were cultured on Luria Bertani (LB) broth and incubated at 28 °C for 24 h at 120 rpm. After incubation Salkowaski method was used to determined IAA production colorimetrically. Production of IAA was confirmed by the development of pink colour [17].

Phosphate Solubilization

For qualitative analysis, phosphate solubilization ability of isolated strains was detected by spotting

them separately on Pikovskaya's agar plates. These plates were then incubated at 28 ± 1 °C for 3 days and observed for appearance of clearing zone around the colonies [18].

HCN Determination

The collected isolates were cultured in liquid media supplemented with 4.4 g/l glycine to detect HCN production. [19].

Ammonia Production.

Each strain was tested for the production of ammonia in peptone water. Overnight broth cultures were inoculated in 10 ml peptone water and incubated at 30o C for 48–72 h. Nessler's reagent (0.5 ml) was added to each tube. Development of brown to yellow colour was recorded as a positive test for ammonia production [20].

Antifungal Activity

The selected isolates were screened for their capacities to inhibit *Fusarium oxysporum* on potato dextrose plates. Antifungal activity was observed by zone of growth inhibition in the area where the bacteria were inoculated on the agar plate.

RESULT AND DISCUSSION:

In the present study total 21 endophytic bacteria were isolated from the roots of *Cathranthus Roseus*. Among all isolates 9 different bacterial strains were selected on the basis of gram reaction and their morphological characteristics such as colour, margin, and opacity Table 1. Preliminary characterization of the isolated bacteria showed different biochemical properties such as Starch hydrolysis, Catalase test, Citrate test, Urease test, Indole production Table 2. Later these bacteria were screened for plant growth promoting activity. Four bacteria out of nine showed maximum plant growth promoting activity. One of the most important PGPR trait is IAA production most influences the plant growth. Four isolates were positive for IAA production CR5, CR6, CR9 and CR10. Bacteria that are capable of solubilizing phosphate from insoluble compound are very beneficial to plant nutrition. CR5, CR9 and CR10 isolates showed phosphate solublization in the plate-based assay with a clear halo zone around the colony. HCN production reported as a defence regulatory against phytopathogens CR5, CR9 and CR10 isolates gave positive HCN test by changing colour of whatsmann paper containg picric acid from yellow to orange. Production of ammonia is also one of the important plant growth promoting traits of bacteria. The isolates incubated in peptone water developed brown to yellow colour when Nessler,s reagent was added, indicating a positive test All the tested isolates showed positive result for ammonia production except CR17. Antagonistic activity of the bacterial isolates was evaluated in terms of inhibition zone diameter as an indicator of the reduction in the growth of pathogenic fungi *Fusarium oxysporium* CR5and CR9 (Table 3).

Table 1: Morphological characteristics of endophytic bacteria

STRAIN	Gram reaction	Colour	Margin	Opacity	Texture
CR1	+ive rods	white	irregular	opaque	Smooth
CR3	+ive rods	colurless	regular	transparent	Rough
CR5	+ive rods	creamish	irregular	opaque	Smooth
CR6	+ive cocci	white	irregular	opaque	Rough
CR7	-ive rods	colourless	irregular	transparent	Smooth
CR9	-ive rods	green	regular	translucent	Smooth
CR10	-ive cocci	white	regular	opaque	Rough
CR17	+ive cooci	creamish	regular	opaque	Smooth
CR20	+ive rods	creamish	regular	transparent	Rough

Table 2: Biochemical characteristics of endophytic bacteria

Strain	H_2S	Starch	Catalase	Citrate	Indole
CR1	+	-	+	-	+
CR3	-	-	+	-	-
CR5	-	-	+	-	-
CR6	-	+	+	-	-
CR7	-	-	+	+	-
CR9	-	-	+	+	-
CR10	-	+	+	-	+
CR17	-	-	+	-	-
CR20	+	+	-	-	-

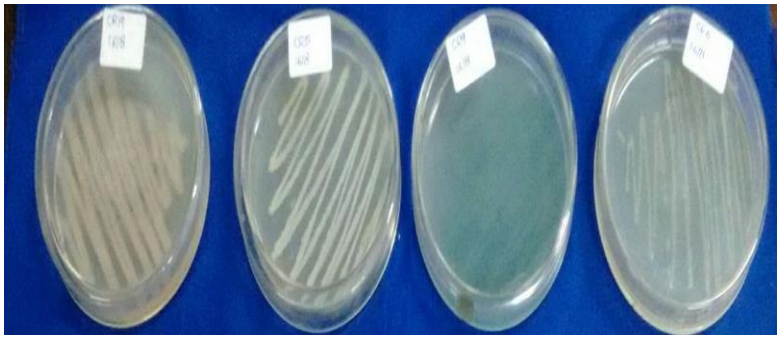
+ = positive, - =negative

Table 3: Plant growth promoting traits shown by endophytic bacteria

Strain	IAA	HCN	Phosphate Solubilisation	Ammonia Production	Antifungal Activity
CR1	-	-	-	+	-
CR3	-	-	-	+	-
CR5	+	+	+	+	+
CR6	+	-	-	+	-
CR7	-	-	-	+	-
CR9	+	+	+	+	+
CR10	+	+	+	+	-
CR17	-	-	-	-	-
CR20	-	-	-	+	-

+ = positive, - =negative

(A)



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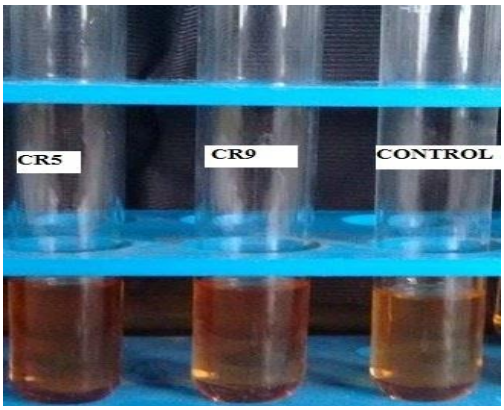

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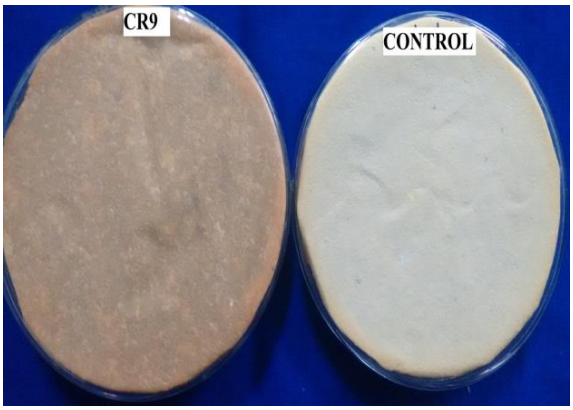
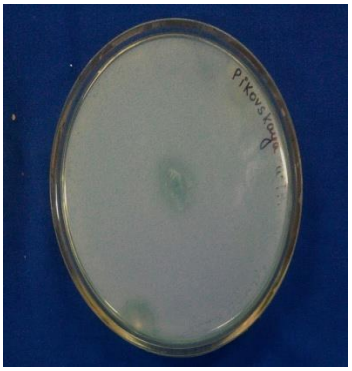


(D)


(E)


(F)


(G)

Fig 1: (A) Isolation of endophytes (B) Different isolates (C) IAA production (D) HCN production (E) Ammonia production (F) Phosphate solubilization (G) Antifungal activity.

CONCLUSION:

The results of the present study suggest that endophytic bacteria isolates CR5 and CR9 showed effective PGP traits. These selected isolates may be used as biofertilizer in sustainable and ecological agricultural systems. However further Invitro studies are necessary in order to evaluate the efficacy of endophytic bacteria in soil ecosystems.

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