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Short Communication

ENDOPHYTES OF *CATHARANTHUS ROSEUS*: A POTENTIAL SOURCE OF PLANT GROWTH PROMOTERS AND ANTIMICROBIAL COMPOUNDS

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ABSTRACT

The present study was undertaken to isolate bacterial and fungal endophytes from medicinal plant- *Catharanthus roseus* (Periwinkle). A total of 13 endophytic bacterial isolates were obtained. The bacterial isolates were screened for the production of plant growth promoters (Indole Acetic Acid, nitrogen fixation & phosphate solubilization) as well as antimicrobial agents against potent human pathogens. Among the isolates, 11 bacterial isolates produced Indole-3-acetic acid (concentration ranging 11-74 µg/ml), 3 isolates were able to fix nitrogen and 3 solubilize insoluble tri-calcium phosphate *in vitro*. In primary screening, the extracts of 5 endophytic isolates appeared to have antimicrobial activity against 3 pathogens *(E.coli*, *Staphylococcus* sp., *Vibrio* sp.) in vitro assayed by agar well diffusion method.

Keywords: Endophytes, IAA, Nitrogen fixation, Phosphate solubilization, Antimicrobial agent

1. INTRODUCTION

Endophytes are the plant inhabiting, endosymbiotic microorganisms that live within the host plant tissues without causing any apparent disease but promoting protection from environmental stress and pathogen competition [1, 2].

Endophytes establish a mutualistic association with their host. One of the major contributions of endophytes toward plant growth is the production of Indole-3-aectic acid (auxin), which promotes cell division and elongation, root growth, apical dominance etc. In addition to this, endophytes also exhibit properties like phosphate solubilization, nitrogen fixation and so on [3].

Recently fungal endophytes have been explored for diverse application owing to production of extracellular enzymes. Cellulose and amylose account for 50% of dry weight of plant biomass. The cellulase and amylase producing fungal endophytes carry out depolymerization of cellulose and amylose in plant matter to fermentable sugars, so as to obtain nutrition from host plant [4] and eliciting defense response against plant pathogens [5].

Endophytes have been recognized as potential source of novel bioactive secondary metabolites [6], & hence Owen et al. [7] proposed that they are "the chemical synthesizer inside plant" which creates a "barrier effect" to out compete & prevent herbivores, insects & pathogens from taking hold.

Catharanthus roseus (Nayantara) is medicinal herb used in treatment of various diseases like cancer, diabetes, menstrual regulators and hypotension. Endophytes associated with medicinal plants are of interest as producers of novel bioactive metabolites, plant growth promoters and enhanced resistance to various pathogens and herbivores [8].

Present study illustrates the details on isolation and fungal endophytes from *C. roseus* and their potential to produce plant growth promoters and antimicrobial compounds.

2. MATERIAL AND METHODS

2.1. Collection of plant sample

Intact healthy *Catharanthus roseus* (Local Name: Nayantara) plants were collected from local area and taken aseptically to the laboratory.

2.2. Surface Sterilization and isolation of endophytes

Collected plant materials are then washed and treated with sterile water, tween 20, sodium hypochlorite, various grades of alcohol and ultimately dried in sterile filter paper. They are then grinded and proper dilutions were prepared with sterile saline water. Finally various concentrations were plated in nutrient agar plates.

2.3. Screening of the isolates for the production of growth promoters

2.3.1. IAA production

The bacterial isolates were inoculated into 20ml of IAA production media supplemented with 0.5% (v/v) of L-tryptophan & incubated for 10 days at 28°C. After incubation, the culture was centrifuged at 3000 rpm for 20 min & the supernatent was used for analyzing IAA production. One ml supernatant was mixed with 2 ml of freshly prepared Salkowski reagent & tubes were incubated in dark for 30 min. The development of red colour was observed as positive result. Standard graph was prepared from known concentration of IAA & the amount of IAA produced by isolates was measured at 530 nm by spectrophotometer [9].

2.3.2. Phosphate Solubilization

The endophytic bacterial isolates were screened for phosphate solubilization by inoculating into Pikovskaya medium. The media was inoculated with isolates & incubated at 30°C for 7 days & were observed for the formation of clear halo around the colony due to the utilization of tricalcium phosphate present in the medium.

2.3.3. Nitrogen Fixation

The bacterial isolates were inoculated into slant prepared with glucose nitrogen free mineral medium containing bromothymol blue solution and incubated at 30°C for 7 days & were observed for the appearance of prussian blue color.

2.4. Screening of Endophytic Bacteria for the production of Antimicrobials

Isolated endophytic bacteria from *Catharanthus roseus* were cultured in 5 ml nutrient broth medium at 32°C for 5 days in a rotary shaker (150 rpm). After 5 days culture medium was centrifuged at 8000 rpm for 8 mins & filtrate was used for the screening of antimicrobial activity by agar-diffusion technique on Luria-Bertani agar media that was previously seeded with test pathogens like *E. Coli*, *Staphylococcus* sp. and *Vibrio* sp. Sterile broth was set as control. Formation of any inhibition zone was recorded.

3. RESULTS AND DISCUSSION

A total of 13 bacterial endophytic isolates were obtained from surface sterilized leaves, stem and root of *Catharanthus roseus* (Table 1). The strains are mostly gram positive and rod in shape. 35 bacterial endophytes were isolated from 4 medicinal plants like *Catharanthus roseus*, *Ocimum sanctum, Mentha arvensis,* and *Stevia rebaudiana* using various surface sterilizing agents [10]. A total of 48 endophytic bacteria were isolated from surface-sterilized tissues of the medicinal plant *Lonicera japonica*, which is grown in eastern China [11].

Table 1: Endophytes Isolated From *Catharanthus roseus*

Plant	No. of isolates			
	Stem	Rroot	Leaves	Total
Catharanthus roseus	4	6	3	13

Table 2: IAA Production by Bacterial Endophytes of *C. roseus*

Isolate	IAA Production	Concentration of IAA (µg/ml)
CRL1	-	-
CRL2	+	40
CRL3	+	74
CRS1	+	48
CRS2	+	27
CRS3	+	68
CRS4	+	11
CRR1	-	-
CRR2	+	51
CRR3	+	57
CRR4	+	63
CRR5	+	52
CRR6	+	29

Under natural conditions, endophytes promote plant growth by using various mechanisms [12]. These include phosphate solubilization activity [13], indole acetic acid (IAA) production [14] and the production of a siderophore [15]. All the isolates were screened for the production of Indole acetic acids. After 10 days of incubation, except isolate CRL1 (Leaf Isolate) and CRR1 (Root isolate) 11 bacterial isolates produced indole-3-acetic acid (Table 2). Isolate CRL3-B showed maximum IAA production 74µg/ml followed by CRS3 (68 µg/ml) and CRR4 (63 µg/ml). One of the major contributions of endophytes towards plant growth is the production of auxin-like molecules was also reported [16]. Indole 3 acetic acid (IAA) being an auxin can stimulate both rapid responses like cell elongation and long term responses like cell division and differentiation in plants [17]. Indole-3-acetic acid (IAA) is shown to be produced by many root associated bacteria including *Enterobacter* sp., *Pseudomonas* sp., and *Azospirillium* sp. [18]. Due to its important role in plants, the level as well as distribution of IAA in plant tissue and endophytic production of IAA has gained a great deal of attention [19]. Pseudomonas sp. isolated from the rhizome of *Zingiber officinale* was also reported to produce IAA [20].

Table 3: Phosphate Solubilization and Nitrogen Fixation by Endophytic Bacteria of *C. roseus*

Isolate	Phosphate Solubilizing Activity		Nitrogen Fixation
	Activity	Mean zone of Solubilization (cm)	
CRL1-B	-	-	-
CRL2-B	-	-	-
CRL3-B	-	-	-
CRS1-B	-	-	+
CRS2-B	+	1.7	+
CRS3-B	+	2.07	-
CRS4-B	-	-	-
CRR1-B	-	-	-
CRR2-B	-	-	-
CRR3-B	-	-	-
CRR4-B	+	2.1	+
CRR5-B	-	-	-
CRR6-B	-	-	-

Table 4: Antimicrobial activity by Endophytes of *C. roseus*

Endophytes	*E.coli*		*Staphylococcus sp.*		*Vibrio sp.*	
	Inhibition Zone (cm)	Sensitivity	Inhibition Zone (cm)	Sensitivity	Inhibition Zone (cm)	Sensitivity
CRL1-B	-	Negative	-	Negative	-	Negative
CRL2-B	-	Negative	-	Negative	-	Negative
CRL3-B	-	Negative	-	Negative	-	Negative
CRS1-B	-	Negative	-	Negative	-	Negative
CRS2-B	-	Negative	-	Negative	-	Negative
CRS3-B	-	Negative	-	Negative	-	Negative
CRS4-B	-	Negative	-	Negative	-	Negative
CRR1-B	-	Negative	-	Negative	-	Negative
CRR2-B	-	Negative	-	Negative	-	Negative
CRR3-B	-	Negative	-	Negative	-	Negative
CRR4-B	1.4	Sensitive	1.2	Sensitive	1.03	Sensitive
CRR5-B	-	Negative	-	Negative	-	Negative
CRR6-B	1.2	Sensitive	1.4	Sensitive	1.03	Sensitive

Screening of the isolates for phosphate solubilization by the endophytes showed that 3 isolates were able to solubilize insoluble tri-calcium phosphate present in Pikovskaya medium, as indicated by clear halo around the colony (Table 3) and 3 of them can fix atmospheric nitrogen. CRS2 and CRR4 have both phosphate solubilizing capability and nitrogen fixing ability render the strains as agriculturally potent one. *Bacillus* and *Paenibacillus* strains isolated from *Lonicera japonica* showed high phosphate solubilizing capability and can induce growth in wheat plant [11]. Two root endophytes (CRR4 & CRR6) were found producing antimicrobial activity against three human pathogenic bacteria *E,coli, Staphylococcus sp. and Vibrio sp* and both appeared to have broad antimicrobial activity (Table 4). Similarly endophytes isolated from *Andrographis paniculata* showed antimicrobial activity against human and fish pathogens [21]. Again endophytes of *Polygonum cuspidatum* showed antimicrobial activity against various fungal as well as bacterial pathogens [22].

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