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ORIGINAL RESEARCH ARTICLE

Indole Acetic Acid, Gibberellic Acid and Siderophore Production by PGPR Isolates from Rhizospheric Soils of *Catharanthus roseus*

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ABSTRACT

In the present study, four Plant growth promoting rhizobacterial (PGPR) strains *viz., Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens and Bacillus megaterium* were isolated from rhizosphere region of *Catharanthus roseus* in 20 different locations of Cuddalore district, Tamil Nadu, India. The twenty isolates were further tested for the production of Indole acetic acid (IAA), Gibberellic acid (GA_3) and Siderophore production and all the isolates were found to produce IAA and GA_3 but *Azospirillum lipoferum* CRAS-2 strain (74.2 µg 25 ml^{-1} of broth and 7.10 µg 25 ml^{-1} of broth) produce maximum amount in Nitrogen free malate broth. *Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens* and *Bacillus megaterium* produced catechol and salicylate siderophore but *Pseudomonas fluorescens* produced the maximum amount in King's B broth (Catechol 9.42 µg ml^{-1} and Salicylate 9.84 µg ml^{-1}).

Key words: PGPR, *Catharanthus roseus*, Indole acetic acid, Gibberellic acid and Siderophore.

1. INTRODUCTION

Catharanthus roseus (L.) G. Don. (Madagascar periwinkle) is a perennial tropical plant belonging to the family Apocynaceae that produces more than 100 monoterpenoid indole alkaloids (MIAs) including two commercially important cytotoxic dimeric alkaloids used in cancer chemotherapy [1] Periwinkle, native to Madagascar is now found in many tropical and sub-tropical regions of the world. The plant contains anti-cancer alkaloids, vincristine (VCR) and vinblastine (VLB) and antihypertension alkaloid, ajmalicine.

Plant growth promoting rhizobacteria have been identified in influencing the growth and yield of many plants. Plant growth promoting rhizobacteria increases the plant growth directly or indirectly [2,3]. Direct mechanisms by PGPR includes, nitrogen fixation, production of phytohormones such as indole acetic acid, Gibberellic acid, Siderophore production, lowering of ethylene concentration and solubilization of phosphorous, whereas, antibiotic production, depletion of iron from rhizosphere, synthesis of antifungal metabolites, synthesis of antifungal cell wall lysis enzymes, competition for the sites on the roots and induced systemic resistance, are included in the indirect mechanisms of plant growth promotion by PGPR.

Microorganisms inhabiting rhizosphere of various plants are likely to synthesize and release auxin as secondary metabolites because of the rich supplies of substrates exuded from the roots compared with nonrhizospheric soils [4]. Plant morphogenic effects may also be a result of different ratios of plant hormones produced by roots as well as by rhizosphere bacteria [5]. IAA (indole-3-acetic acid) is the member of the group of phytohormones and is generally considered the most important native auxin [6]. It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division, and differentiation, and gene regulation [7]. Diverse bacterial species possess the ability to produce the auxin phytohormone IAA. Gibberellins are known to be a component of light signaling [8] phytochromes and GA_3 act in coordination to regulate multiple aspects of Arabidopsis development such as flowering and hypocotyls elongation [9, 10]. Production of plant hormones by *Azotobacter* [11] and *Azospirillum* [12] as well as the concomitant changes in plant growth and development were observed. Out of the five major classes of hormones, indole acetic acid (IAA), gibberellic acid (GA_3), kinetin, abscisic acid and

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ethylene, the first three kinds of phytohormones were known to produced by *Azospirillum, Pseudomonas* [13,15] and *Bacillus megaterium* [16,17] whereas indole acetic acid, gibberellic acid and cytokinnin are produced by *A. chroococcum* [18,19]. Siderophores are low molecular weight bio-molecules secreted by micro-organisms in response to iron starvation for acquisition of iron from insoluble forms by mineralization and sequestration [20]. Although some siderophores are known to chelate other ions, their specificity and avidity for iron is the most consistent feature [21]. Siderophore producing PGPR like *Pseudomonas* sp. [22], *Azotobacter* [23], *Bacillus megaterium* [16] plays the vital role in stimulating plant growth and controlling several plant disease [24]. They function as a biocontrol agent by depriving the pathogen from iron nutrition, thus resulting in increased yield of crop [25]. The Present work focuses on potential effects of cultural conditions on growth and Indole acetic acid, Gibberellic acid and Siderophore production of Plant Growth Promoting Rhizobacterial strains (*Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens and Bacillus megaterium*).

2. MATERIALS AND METHODS

Sample Collection and Isolation of PGPR strains

The soil samples were collected from five different places of Cuddalore district *viz*., Chidambaram, Annamalai Nagar, Vallampadukai, Bhuvanagiri and Ammapettai in Tamil Nadu, India. The commercially well grown medicinal plant of *Catharanthus roseus*, with intact roots was uprooted from the field and excess soil was removed. The soil adhered to root surface and in between root was collected and used as rhizosphere soil for the PGPR isolation. *Azospirillum lipoferum* isolates were isolated from Nitrogen free malate medium, *Azotobacter chroococcum* isolates were isolated from Waksman's No. 77 medium, *Pseudomonas fluorescens* isolates were isolated from King's B medium *Bacillus megaterium* isolates were isolated from Pikovskaya's medium.

Biochemical characterization of the test isolates

All the 20 isolates of *Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens and Bacillus megaterium* were biochemically characterized for Gram reaction, motility, Nitrite reduction, IMViC tests, Citrate utilization, Catalase test, oxidase test, starch hydrolysis, and gelatin liquefaction as per the standard methods [26].

Estimation of indole acetic acid (IAA)

Indole acetic acid (IAA) production was detected as described by [27]. All the test isolates were screened for IAA production [28].

Estimation of Gibberellic acid (GA_3)

The gibberellic acid production by plant growth promoting rhizobacteria was determined by the method of Borrow *et al*. [29].

Estimation of Siderophore Production

Siderophore production by the plant growth promoting bacteria was estimated by the method described by Reeves *et al*. [30].

3. RESULTS AND DISCUSSIONS

A total of 20 isolates of *Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens* and *Bacillus megaterium,* were isolated from rhizospheric soils and tentatively identified on the basis of biochemical tests and sugar fermentation behavior as described in Bergey's manual of Determinative Bacteriology (**Table 1**). These bacterial isolates were screened for their ability to produce plant growth regulators IAA, GA_3 and Siderophore production. All the five isolates of *Azospirillum lipoferum* produced IAA and GA_3 and the quantity ranged from 71.64 to 74.24 µg 25 ml^{-1} broth for IAA and 6.45 to 7.10 µg 25 ml^{-1} broth for GA_3. The isolate CRAS-2 produced the maximum amount of IAA 74.24 µg of 25 ml^{-1} and 7.10 µg 25 ml^{-1} broth for GA_3 of nitrogen free malate broth followed by CRAS-4 which produced 73.32 µg of IAA 25 ml^{-1} and 6.84 µg 25 ml^{-1} broth for GA_3 All the *Azospirillum* isolates produced both catechol and salicylate type of siderophores. The catechol type of siderophore produced by *Azospirillum lipoferum* isolates ranged from 3.23 to 4.21 µg ml^{-1} of culture broth and salicylate type ranged from 3.26 to 7.12 µg ml^{-1}. The isolate CRAS-2 produced the highest quantity of 4.21 and 7.83 µg ml^{-1} of catechol type and salicylate type of siderophores respectively followed by other isolates (**Table 2**). The potential of *Azospirillum* strains to produce Indole-3-acetic acid and gibberellic acid under *in vitro* conditions was reported by Bashan *et al*. [31] and Pedraza *et al*. [32].

All the five isolates of *Azotobacter chroococcum* produced IAA and GA_3 and the quantity ranged from 70.1 to 72.7 µg 25 ml^{-1} broth for IAA and 6.21 to 6.80 µg 25 ml^{-1} broth for

GA_3. The isolate CRAB-2 produced the maximum amount of 72.7 of IAA 25 ml^{-1} and 6.80 µg 25 ml^{-1} broth for GA_3 of waksman' base broth followed by CRAB-4 which produced 72.2 µg of IAA 25 ml^{-1} and 6.64 µg 25 ml^{-1} broth for GA_3. Among the five *Azotobacter chroococcum* isolates, the isolate CRAB-2 recorded the maximum production of both catechol and salicylate type of siderophores (3.16 and 5.12 µg ml^{-1}) followed by other isolates (**Table 3**). Our findings of IAA and GA_3 production in *Azotobacter* isolates are in same with those of other researchers [11,33].

All the five isolates of *Pseudomonas fluorescens* produced IAA and GA_3 and the quantity ranged from 68.6 to 72.1 µg 25 ml^{-1} broth for IAA and 6.14 to 6.64 µg 25 ml^{-1} broth for GA_3. The isolate CRPS-2 produced the maximum amount of 72.1 of IAA 25 ml^{-1} and 6.64 µg 25 ml^{-1} broth for GA_3 followed by CRPS-1 which produced 70.9 µg of IAA 25 ml^{-1} and CRPS-4 produced 6.34 µg 25 ml^{-1} broth for GA_3. The siderophore production by *Pseudomonas fluorescens* CRPS-2 recorded the maximum siderophore production of 9.42 and 9.84 µg of catechol and salicylate type ml^{-1} change this respectively followed by CRPS-4. The minimum amount of siderophore production was recorded with the isolate CRPS-1of about 8.12 and 8.19µg ml^{-1} (**Table 4**). Our test isolates showed a similar high level of IAA and GA_3 production to those recorded by other researchers [34,35].

Among the five *Bacillus megaterium* isolates, the isolate CRBA-2 produced the maximum amount of 50.3 µg IAA 25 ml^{-1} and 4.50 µg 25 ml^{-1} broth for GA_3 followed by CRBA-4 isolate which produced 49.7 µg IAA 25 ml^{-1} and 4.04 µg 25 ml^{-1} broth for GA_3. Among the five *Bacillus megaterium* isolates, the isolate CRBA-2 recorded the maximum production of both catechol and salicylate type of siderophores (7.12 and 8.96 µg ml^{-1}) followed by other isolates (**Table 5**).The IAA and GA_3 producing potential of *Azotobacter* isolates was more than that of *Pseudomonas* and *Bacillus* isolates. It is also to note that *Pseudomonas* isolates were better than *Bacillus* for IAA and GA_3 production. The production of IAA and GA_3 by *Azotobacter*, *Bacillus* and *Pseudomonas* in the rhizosphere of wheat and vegetables and their plant growth augmenting role was well explained by Verma *et al.* [36] and Tank and Saraf [37] in various plant rhizosphere. Indole-3-acetic acid is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement [38].

All the isolates of *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* produced appreciable quantities of siderophore. Among the plant growth promoting bacteria, *P. fluorescens* CRPS-2 secreted highest amount of both catechol and salicylate type of siderophores followed by *Bacillus* isolates, *Azospirillum* and *Azotobacter* isolates. Production of siderophore by plant growth promoting bacteria and its role in Fe mobilization was reported by several workers [39-44]. All the plant growth promoting bacterial isolates tested in the present study produced more amount of salicylate type than catechol type of siderophore. The potential of *Pseudomonas* to produce siderophore was more than that of *Bacillus, Azospirillum* and *Azotobacter.*

The findings of the present investigation highlighted that IAA, GA_3 and siderophore producing rhizobacteria from rhizospheric soils could be easily isolated and may be exploited after strain improvement for research. However, further studies using PGPR strains of these isolates are needed to explore the exact contribution of IAA, GA3 and siderophore production in the promotion of plant growth as well as the contribution of other PGPR traits.

Table 1: Biochemical characteristics of PGPR isolates

Name of the test	*Azospirillum lipoferum (5 isolates)*	*Azotobacter chroococcum (5 isolates)*	*Pseudomonas fluorescens (5 isolates)*	*Bacillus megaterium (5 isolates)*
Gram reaction	-	-	-	+
Indole test	-	+	-	-
MR/ VP	-	+	+ /-	- /+
Citrate Utilization	+	+	+	+
Catalase	+	+	+	+
Nitrite reduction	+	+	+	+
Oxidase test	+	+	+	-
Hydrolysis of Starch	+	+	+	+
Hydrolysis of Gelation	+	-	-	+
Motility	+	+	+	+
Sugar fermentation Sucrose	-	+	+	+
Mannitol	+	+	-	+
Lactose	+	+	-	+
Dextrose	+	+	+	+

+ = Positive; - = Negative

Table 2: Indole acetic acid, Gibberellic acid and Siderophore production by *Azospirillum lipoferum* isolates from rhizosphere of *Catharanthus roseus*

S. No	Name of the isolates	Indole acetic acid (µg 25 ml^{-1} of broth)	Gibberellic acid (µg 25 ml^{-1} of broth)	Siderophore content (µg ml^{-1})	
				Catechol type	Salicylate type
1	CRAS - 1	72.8	6.75	3.37	6.45
2	CRAS - 2	74.2	7.10	4.21	7.83
3	CRAS - 3	72.6	6.45	3.74	6.87
4	CRAS - 4	73.3	6.84	3.92	7.12
5	CRAS - 5	71.6	6.68	3.23	6.62

Table 3: Indole acetic acid, Gibberellic acid and Siderophore production by *Azotobacter chroococcum* isolates from rhizosphere of *Catharanthus roseus*

S. No	Name of the isolates	Indole acetic acid (µg 25 ml^{-1} of broth)	Gibberellic acid (µg 25 ml^{-1} of broth)	Siderophore content (µg ml^{-1})	
				Catechol type	Salicylate type
1	CRAB - 1	71.8	6.50	2.43	4.26
2	CRAB - 2	72.7	6.80	3.16	5.12
3	CRAB - 3	71.9	6.21	2.64	4.67
4	CRAB - 4	72.2	6.64	2.76	4.85
5	CRAB - 5	70.1	6.41	2.18	4.42

Table 4: Indole acetic acid, Gibberellic acid and Siderophore production by *Pseudomonas fluorescens* isolates from rhizosphere of *Catharanthus roseus*

S. No	Name of the isolates	Indole acetic acid (µg 25 ml^{-1} of broth)	Gibberellic acid (µg 25 ml^{-1} of broth)	Siderophore content (µg ml^{-1})	
				Catechol type	Salicylate type
1	CRPS - 1	70.9	6.25	8.12	8.19
2	CRPS - 2	72.1	6.64	9.42	9.84
3	CRPS - 3	69.8	6.17	8.27	8.28
4	CRPS - 4	70.6	6.34	8.73	8.92
5	CRPS - 5	68.6	6.14	8.64	8.75

Table 5: **Indole acetic acid, Gibberellic acid and Siderophore production by *Bacillus megaterium* isolates from rhizosphere of *Catharanthus roseus***

S.No	Name of the isolates	Indole acetic acid (µg 25 ml^{-1} of broth)	Gibberellic acid (µg 25 ml^{-1} of broth)	Siderophore content (µg ml^{-1})	
				Catechol type	Salicylate type
1	CRBA - 1	48.6	4.35	6.37	7.65
2	CRBA - 2	50.3	4.50	7.12	8.96
3	CRBA - 3	49.1	4.18	6.43	7.37
4	CRBA- 4	49.7	4.32	6.84	8.12
5	CRBA - 5	47.8	4.04	6.72	7.28

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