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Plant growth promoting rhizobacteria (PGPR) from rhizosphere soils of Bt and Non Bt cotton

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Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth by direct and indirect mechanisms. Rhizosphere soils of *Gossypium hirsutum* L.from Khammam and Warangal districts of Andhra Pradesh, India were explored for PGPR and a total of 76 bacterial strains were isolated. Among these bacterial isolates 40 were found associated with Bt cotton and 36 being from non Bt cotton plants.Out of these 76 isolates 23 exhibited antifungal activity against *Fusarium oxysporum* f.sp. *vasinfectum* and *Colletotrichum gossypi.* These antifungal strains were screened for different plant growth promoting traits like ammonia, IAA, HCN, phosphate solubilisation, catalase, protease and lipase activity. Isolates from Bt rhizosphere produced high amount of IAA. These isolates were also positive for HCN production. Six isolates were most effective, which may be useful as potential biofertilizers and biocontrol agents.

Key words: Antifungal activity, *Colletotrichum gossypi, Fusarium oxysporum* f. sp. *vasinfectum Gossypium hirsutum* L., PGPR

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are very small portion of bacteria (2-5%) of rhizosphere that promote plant growth. PGPR influence plant growth by direct or indirect modes of action. Direct effects include nutrient uptake by plants, nitrogen fixation, solubilization of minerals such as phosphorus, production of siderophores and synthesis of phytohormones i.e., Indole-3-acetic acid (IAA), gibberellic acid, cytokinins and ethylene(Nelson, 2004). Indirect effects include biological control of plant pathogens and deleterious microbes through the production of antibiotics, lytic enzymes and hydrogen cyanide. Further PGPR promote plant growth by seedling emergence, vigour and yield (Khan, 2006). Application of PGPR to tropical sandy loam semi-arid vertisol soils have been shown to improve the sorghum productivity even under low levels of fertilizer application (Tilak and Manoharachary, 2011).

Cotton, is considered as the 'white gold', and is cultivated worldwide. Bt cotton is introduced in India in 2002 and India occupies second position with 11.7 million hectares under cotton cultivation. It occupies 7% of the global area under cotton cultivation. India's 2012-13 yield is estimated at 475 Kg/hectare (Johnson *et al.,* 2013). Several studies are conducted to assess the risk of Bt cotton on flora and fauna earlier (Zhang *et al.,* 2000, Bai *et al.,* 2003) and varied results are obtained. Sarkar *et al.,* (2009) report no negative effects, while others report adverse effects (Cui and Xia, 2000). Since a decade is over after the introduction of transgenic cotton in India, the impact of Bt cotton on rhizosphere microflora is with reference to the state of Andhra Pradesh is meagre. Therefore, an attempt has been made to study the impact of Bt cotton on PGPR the rhizosphere microbes and their ability for antifungal activity is reported in this paper.

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MATERIALS AND METHODS

Soil sampling and isolation of PGPR

The rhizosphere soil samples of the Bt (var.Neeraja) and non Bt cotton plants during the maximum growth stage were collected in triplicate from agriculture fields of Kakarla village of Khammam and Kapula Kanaparthy village of Warangal districts in A.P., India. Serial dilution plate method (Johnson and Curl, 1972) was adopted for isolating plant growth promoting microorganisms from rhizosphere. All the isolates were maintained at 4 °C in nutrient agar (NA) and King's B (KB) medium. The bacterial strains were screened for plant growth promotion (PGP) traits viz., ammonia, IAA, HCN production, P-solubilization and other attributes.

Antifungal activity

In vitro antibiosis of rhizosphere bacteria against *Fusarium oxysporum* f.sp. *vasinfectum* and *Colletotrichum gossypi* was tested using potato dextrose agar (PDA) medium. Antagonist activity was evaluated for 3 to 5 days after incubation at room (28± 2° C) temperature. The plates were incubated for 4-5 days. The % inhibition was calculated using the formula described by Idris *et. al.*, (2007) which is (R - r) / R × 100 (r: radial growth of the fungal colony opposite to the bacterial colony, R: the radial growth of the pathogen in control).

In vitro screening of rhizosphere bacterial isolates for different plant growth promoting traits

The production of ammonia was determined as per the methodology outlined by Cappuccino and Sherman (1992).The production of IAA was determined by method Gordon and Weber (1951). Indole-3-acetic acid (IAA) was estimated by colorimetric assay (Loper and Schroth, 1986). The bacterial isolates were also screened for the production of hydrogen cyanide (HCN) (Bakker and Schippers, 1987). Isolates were screened on Pikovaskya's (Pikovaskya, 1948) agar medium for phosphate solubilization (Gaur, 1990). After 2-6 days, plates were observed for clearing zones around the bacterial colony. Catalase test was performed by adding three to four drops of H_2O_2 on bacterial culture which was grown for 48 h on trypticase soy agar medium. The effervescence indicated catalase activity (Schaad, 1992). Protease activity was determined by clear zone in protease medium (Chaiharn, 2008). Bacterial cultures were grown on nutrient agar amended with egg yolk. After 24 h of incubation clear zones around the colony indicated positive for lecithinase activity. The plates were flooded with saturated $CuSO_4$ solution and dried at 37°C for 20 min and the appearance of blue greenish colour on the surface around the colony indicated lipolytic activity (Cowan, 1974).

RESULTS AND DISCUSSION

The rhizobacteria have been screened for antifungal activity against *Fusarium oxysporum* f. sp.

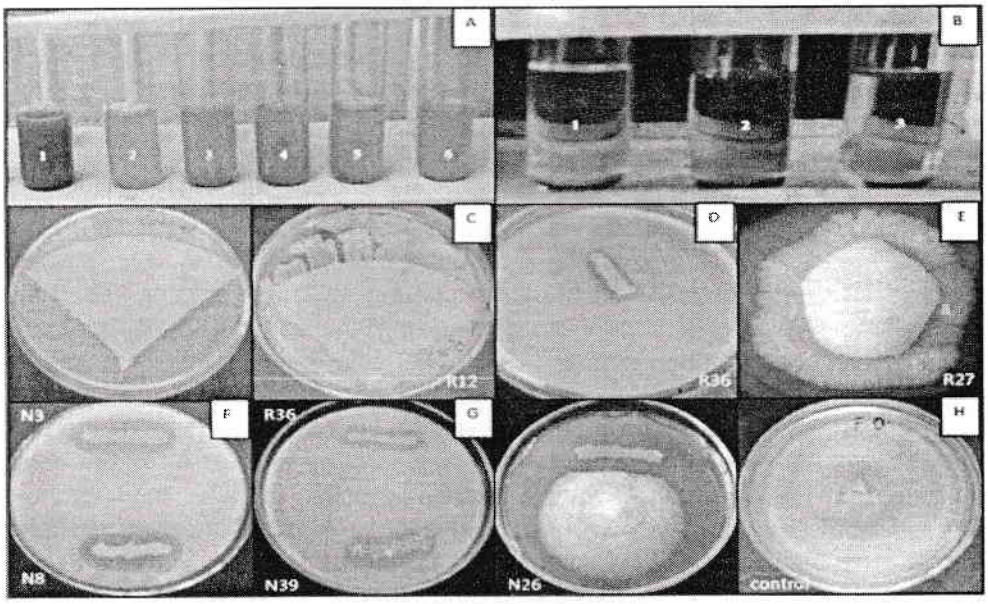

Fig. 1 : A. B. Rajithasri *et al.*, Plant growth promoting rhizobacteria (PGPR) from rhizosphere soils of Bt and Non Bt cotton

Table 1 : Plant growth promoting activities of selected antagonistic bacteria.

Isolate	Antifungal activity zone of inhibition (%)		NH_3 production	IAA production (µg/ml)	HCN production	P-solubilization	Catalase activity	Protease activity	Lipase activity
	Fo.	*C.g.*							
R12	*56*	*65*	+++	83.0	++	++	+	++	++
R13	56	71	+++	83.0	++	++	++	++	++
R27	57	58	++	46.0	_	++	++	++	+
R36	63	80	+++	8.5	-	++	+	++	++
R39	59	77	+++	8.5	-	++	+	++	++
N3	54	42	+++	59.5	+	++	+	++	+
N8	59	68	+++	36.5	+	++	+	++	+
N25	72	65	+	33	-	++	+	++	++
N26	72	65	++	86.0	+	++	+	++	+
N32	65	45	+++	53	-	++	+	++	+
N35	59	74	++	66	-	++	+	++	+

= No production, + = Weak production; ++ = medium producer; +++ = high producer,
F. o. = *Fusarium oxysporum* f. sp. *vasinifectum*,C. g. = *Colletotrichum gossypi*

vasinfectum and *Colletotrichum gossypi* and zone of inhibition was taken as an indicator of antifungal property. Among the 76 isolates only 23 were antagonistic to both pathogens. Pseudomonad isolates R12, R13, R27, R36 and R39 from Bt cotton were effective inhibitors of wilt and anthracnose pathogens. Among antagonistic isolates from non Bt cotton, N3, N8, N25, N26, N32 and N35 also exhibited significant antifungal activity (Table 1). These isolates have shown varied growth promoting traits such as IAA, HCN and ammonia production. High IAA production was associated with N26, R12 and R13 while the isolates R36 and R39 were low producers of IAA. All isolates were capable of solubilizing phosphates and exhibits catalase, protease and lipase activity. On the other hand, a negative response to HCN production was evident in all the antagonistic isolates except R12, R13, N3, N8 and N26 (Fig. 1). Plant rhizosphere is a specialized ecological niche harboring plant growth promoting rhizobacteria and both Bt and non Bt cotton rhizospheres harbored a different isolates of Pseudomonads diverse group of bacteria with more plant growth promoting traits. Our results indicate rather uniform distribution of bacteria suggesting not much impact on rhizosphere microbes by the transgenic cotton. Shen *et al.*, (2006) stated that the richness of the microbial communities in rhizosphere soil did not differ between Bt and non Bt cotton and the present data also supports the above statement.

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