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ASSESSMENT OF PLANT GROWTH PROMOTING ACTIVITIES OF BACTERIAL ISOLATES FROM THE RHIZOPHERE OF TOMATO (*LYCOPERSICON ESCULENTUM* L.)

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Abstract

Plant growth promoting bacteria in the rhizosphere of plants, enhanced plant growth by exerting their beneficial effects through metabolites that directly or indirectly influence the plant growth. In the present study a total of 44 bacterial isolates were isolated from the rhizosphere of tomato grown in Cuddalore and Nagapattinam districts of Tamil Nadu, India. These bacterial isolates were grouped into *Azospirillum* (18 isolates) *Azotobacter* (9) *Pseudomonas* (12) and *Bacillus* (5) based on their morphological and biochemical characteristics. All the isolates were screened for their plant growth promoting activities viz., IAA production, phosphate solubilization, siderophore production, HCN production, ACC deaminase activity and antifungal activity. The results showed that not all the isolates possessed all the PGP activities. The range of percentage of positive isolates of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillums* for each of PGP activities varies greatly. Among the 44 isolates, three isolates of *Azospirillum*, 2 from *Azotobacter*, one from *Bacillus* and four from *Pseudomonas* were selected and the IAA production siderophore production, and antifungal activity against *R. solani* and *Fusarium oxysporum* were determined quantitatively. The maximum 1AA production of 3.6 μ g/ml siderophore production of 0.86 μ g/ml were recorded by TMPS-9 and TMPS-7 respectively. All the tested isolates were active against the two fungal species. The efficiency of the PGPR isolates should be tested under field conditions in the rhizosphere of tomato to obtain a suitable consortium for increasing growth and yield of tomato.

Key Words: PGP; IAA; *Azospirillum; Azotobacter; Bacillus; Pseudomonas.*

Introduction

Numerous plant growth promoting bacteria in the rhizophere soils have been isolated, each containing one or more mechanisms for the enhancement of plant growth(1). When in contact with plants, bacteria may exert a range of activities that singly or in combination can result in the promotion of plant growth. The direct promotion of plant growth may involve one or more of the mechanisms *viz.,* (i) Asymbiotic N_2 fixation (2). (ii) Production of phytohormones such as Indole acetic acid (IAA)(3), Gibberellic acid(4), cytokinins. (iii) Lowering ethylene level (5,6). (iv) Antagonism against phyto pathogenic micro organism by production of siderophore (7), production of β 1, 3, glucanase (8) and (vii) Solubilization of mineral phosphate and other nutrients (9).

In addition to the above mentioned traits, the plant growth promoting bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizospheric soil(10). The good results obtained *in vitro* cannot always be dependably reproduced under field condition (11, 12). The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effect on the plant.

Therefore, it is essential to develop efficient strains in field condition. One possible approach is to explore soil microbial activity for PGPR having plant growth promoting activities and adaptation to particular soil environment. In the present study, an attempt has been made to isolate and screen the bacterial strain possessing multiple PGP activities from the rhizosphere soils of tomato, to develop a consortium for increasing the growth and yield of tomato. We isolated a total of about 44 bacterial isolates from the rhizosphere of

tomato and screened for their PGP activities and some of the traits are estimated quantitatively.

Materials and Methods

Isolation and characterization of bacterial isolates

A total of 44 bacterial isolates were isolated from twenty rhizosphere soil samples of tomato obtained from different places of coastal districts viz., Cuddalore and Nagapattinam of Tamil Nadu.

The bacterial isolates were characterized by their cultural conditions, morphological and chemical characteristics such as hydrolysis of starch, utilization of glucose, sucrose, lactose, using standard methods (13).

(ii) In vitro screening of bacterial isolates for PGP activities

Assay for IAA production

IAA production was detected by the modified method as described by Brick et al., (14). Quantitative analysis of IAA was performed using the method of Loper and Scroth (15) at the 50 μ g/ ml concentration of tryptophan. Fully grown bacterial cultures were centrifuged at 3000 rpm for 30 min. The supernatent (2 ml) was mixed with two drops of ortho phosphoric acid and 4 ml of Salkowski reagent (50ml, of 35% perchloric acid, 1ml of 0.5 μ $FeCl_3$). Development of pink colour indicates the IAA production. Optical density was taken at 530nm with the help of spectrophotometer. Conc. of IAA produced by cultures was measured with the help of standard graph of IAA (Hi-media) obtained in the range of 10-100 μ g/ ml.

Phosphate solubilization by test bacteria

All isolates were first screened on Pikovskaya's agar plates for phosphate solubilization as described by Gaur (16). Quantitative analysis of solubilization of tricalcium phosphate in liquid medium was made as described by King (17). Briefly, the test isolates were inoculated in 25ml Pikovskaya's broth and incubated for 4 days at 28 $\pm$ 2ºC. The bacterial cultures were centrifuged at 15,000 rpm for 30 min. Supernatant (1 ml) was mixed with 10 ml of chloromolibidic acid and the volume was made up to 45ml with distilled water. The absorbance of the developing blue colour was read at 600nm. The amount of soluble phosphorous was detected from the standard cuve of KH_2PO_4.

Siderophore Production

Bacterial isolates were assayed for siderophore production on the chrome azurol S agar medium described by Schwyn and Neilands, (18).

Chrome azurol S agar plates were prepared and divided in two equal sectors and spot inoculated with test organism (10µ1 of 10^6 CFU/ ml) and incubated at 28±2ºC for 48-72h. Development of yellow –orange halo around the growth was considered as positive for siderophore production. It was determined in the solution described by Schwajn and Neilands [18); except the medium was 0.1 x tripticase soy broth.

HCN Production

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lock (19). Nutrient broth was amended with 4.4g gly cine/l and bacteria were streaked on modified agar plate. A whatman filter paper No.1. soaked in 2% sodium carbonate in 0.5%. Picric acid solution placed in the top of the plate. Plates were sealed with parafin and incubated at 28 ±2ºC for 4 days. Development of orange red colour indicated HCN production.

ACC deaminase Production

Production of ACC deaminase was determined as described by Glick *et al.,* (6) except the C sources were Sucrose (5.0g L^{-1}), mannitol (5.0gL^{-1}) and the N source was ACC (5.0 g L^{-1}).

Antifungal assay

The agar well diffusion method as adopted earlier (20) was used. The bacterial isolates tested for their antifungal activity were fully grown in the respective broth media. Test fungi were grown on Sabaroud dextrose agar (SDA). The spores were scraped and suspended in 10ml of sterile normal saline solution (NSS).

Diluted spore suspension (0.1ml, 105 CFU/ml) of the fungi was spread on Muller Hinton agar. Wells of 8mm diameter were punched into the agar medium and filled with 200 µl (2 x10^7 CFU/ ml) of bacterial culture. Nutrient broth was taken as negative control and 100µg/ml antifungal antibiotic nistatin was made as positive control. The plates were incubated for 5-6 days at 28±2ºC. The antifungal activity was evaluated by measuring the growth of inhibition zone against test fungi.

Results and Discussion

In the present study a total of 44 isolates of plant growth promoting rhizhobacteria were isolated from the rhizosphere soils of tomato grown in Cuddalore and Nagapattinam districts of Tamil Nadu, India. The bacterial isolates were identified and grouped into *Azospirillum* (18 isolates), *Azotobacter* (9 isolates), *Pseudomonas* (12 isolates) and *Bacillus* (5 isolates), based on the colony morphology, Cell morphology, Pigmentation, Polysacharade production and biochemical characteristics such as starch hydrolysis, and carbohydrate utilization. These isolates were designated as TMAZS 1-18 (tomato *Azospirillum*). TMAZ01-9 (Tomato *Azotobacter*) TMPS 1-12 (Tomato *Pseudomonas*) and TMB 1-5 (Tomato *Bacillus*). The results are presented in the table 1.

Table 1.Characterization of PGPR isolates

Characteristics	*Azospirillum*	*Azotoabacter*	*Pseudomonas*	*Bacillus*
No. of isolates	18	9	12	5
Colony morphology	Small, wrinkled colonies, entire margins	Watery, mucillaginous, serrated margin	Button shaped	Serrated margin
Pigmentation	Pink pigmentation on BMS Agar	White, on aging brown, blackish	Fluorescent green	No pigmentation
Polysaccharide production	+	+	-	-
Cell morphology	Small, vibrio/ curved rods	Coccal	Rod	Rod
Gram reaction	-Ve	-Ve	Gram – Ve	Grame + Ve
Growth on N free medium	+	+	-	-
Hydrolysis of				
a) Starch	+	+	+	+
Carbohydrate utilization				
a) Glucose	++	++	+	+++
b) Sucrose	+	++	+	+++
c) Lactose	-	+	-	+++
Designation of the isolates	TMAZSI- 18	TM AZO-1-9	TM PS 1-12	TMB 1-5

\- - No. utilization
\+ - Poor utilization
++ - Moderate utilization
+++ - Good utilization

TM AZS - Tomato *Azospirillum*
TMAZO - Tomato *Azotobacter*
TMPS - Tomato *Pseudomonas*
TMB - Tomato *Bacillus*

The identified isolates of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* were screened for their plant growth promoting activities such as IAA production, phosphate soulbilization, siderophore production, HCN production, ACC deaminase activity and antifungal activity against *Fusarium sp*. and *Rhizoctonia sp.* The number of positive and number of negative bacterial isolates for each PGP activity were observed and given in Table -2. The results showed that none of bacterial isolate was positive for all the tested activities.

Table 2. Screening of the PGPR activities of the bacterial isolates

Isolates	IAA production		Phosphate solubilization		Siderophore production		HCN production		ACC deaminase production		Antifungal activity	
	+ Ve	-Ve	+ Ve	-Ve	+ Ve	-Ve	+ Ve	-Ve	+ Ve	-Ve	+ Ve	-Ve
Azospirillum	16 (87.8)	2 (12.2)	13 (72.2)	5 (27.9)	14 (77.7)	4 (22.3)	13 (72.2)	5 (278)	- -	18 (100)	12 (66.6)	6 (33.3)
Azotobacter	7 (77.7)	2 (22.3)	6 (66.6)	3 (33.3)	2 (22.2)	7 (77.7)	1 (12.2)	8 (88.8)	3 (33.3)	6 (66.6)	2 (22.3)	7 (77.7)
Pseudomonas	12 (100)	- 0	10 (83.3)	2 (16.7)	4 (33.3)	8 (66.6)	12 (100)	- -	10 (83.3)	2 (16.7)	4 (33.3)	8 (66.6)
Bacillus	3 (60)	2 (40)	4 (80)	1 (20)	2 (40)	3 (60)	- -	5 (100)	- -	5 (100)	1 (20)	4 (80)

Figures in parenthesis represent the percentage of +Ve, -Ve strains

Table-3 Assessment of the IAA, siderophore production and antifungal activities of selected PGPR isolates

Isolates of PGPR	IAA production µg/ml	Siderephore production (µg /ml)	Antifungal activity	
			F. oxysporum	*R. solani*
TM AZS –1	2.0	6.54	15.67	16.0
TM AZS – 4	3.3	0.78	18.67	23.5
TM AZS – 7	2.3	0.63	25.67	19.0
TM AZO – 2	1.50	0.42	13.33	21.00
TM AZO–4	1.23	0.53	12.67	16.50
TM B – 3	1.90	0.47	20.19	14.25
TMPS –2	2.60	0.80	15.33	14.83
TMPS – 4	3.00	0.11	11.50	13.33
TMPS –7	2.8	0.86	16.50	13.83
TMPS – 9	3.6	0.60	14.83	14.25
S.E	**0.02**	**0.005**	**0.08**	**0.05**
CD	**0.039**	**0.010**	**0.15**	**0.10**

Among the 18 *Azospirillum* isolates, 87.8% of isolates were positive for IAA production, followed by Siderophore production (77.7%) phosphate soulbilization (72.2%), Antifungal activity (66.6%) and none of the Azospirillum isolate was positive for ACC deaminase activity. Among the nine *Azotobacter* isolates, the no of positive isolates for 1AA production, phosphate soulbilization, siderophore production, HCN production ACC deaminase activity and antifungal activity were 7, 6, 2, 1, 3, and 2 respectively. Among the 12 *Pseudomonas* isolates, all (100%) the isolates produced IAA, 83.3% solubilized phosphorous, 100% produced HCN, 83.3% produced ACC deaminase and 33.3% produced Siderophore and positive for antifungal activity. The results also showed that 100% of *Bacillus* were negative for HCN and ACC deaminase production. Among the 44 isolates, three *Azospirillum* isolates (TM-A2S 1, 4 &7), two *Azotobacter* isolates (TMAZ0, 2 and4) four

Pseudomonas isolates (TMPS- 2, 4, 7, 9) and one *Bacillus* isolate (TMB -3) were selected and their efficiency of IAA production, Siderophore production and antifungal activity against *Fusarium oxysporum* and *Rhizoctonia solani isolate* were determined quantitatively and given in table -3.

The results showed that, IAA production by the selected isolates ranged from 1.23 μ g/ml to 3.6 μ g/ml. The maximum of 3.6 μ g/ml of IAA was recorded by the *Pseudomonas* sp (TMPS -9). The Siderophore production was ranged from 0.11 to 0.86 μ g/ml. It was also observed that, all the bacterial isolates were effective in inhibiting the growth of fungal pathogens significantly. The PGP activities of the bacterial isolates should he tested under *in vivo* conditions in the rhizosphere of tomato, in the further studies.

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