Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
Impact Factor (RJIF): 6.35
www.phytojournal.com
JPP 2025; 14(6): 234-236
Received: 05-10-2025
Accepted: 08-11-2025

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Screening of *Azotobacter spp* for its plant growth promoting activity from rhizosphere soil of tomato

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DOI: https://www.doi.org/10.22271/phyto.2025.v14.i6c.15663

Abstract
In the present study *Azotobacter* isolates were tested invitro for Indole Acetic Acid production, Phosphate solubilization, Ammonia production, Hydrogen cyanide production, Siderophore production as plant growth promoting activities for the isolates. All the isolates showed positive for IAA production. Isolates AMSRT - 2,4 showed positive for HCN production. isolates AMSRT - 6, 9 showed positive for Siderophore production and all the isolates showed positive for NH_3 formation and P-Solubilization.

Keywords: Azotobacter, Plant growth promotion, IAA production, Phosphate solubilization, Siderophore production

Introduction
Azotobacter is a gram negative, motile, spherical bacterium which produces capsular slime. It is a free-living bacterium in the soil which plays an important role in nitrogen fixation and makes it available to the plants. *Azotobacter* is reported to synthesize Auxins, cytokinin, and Gibberellic Acid like substances that have been found to be directly associated with improved plant growth (Wani *et al*., 2013) [12] phosphate solubilization (Hafez *et al*., 2016) [15], Hydrogen Cyanide production and Siderophore production. (Baars *et al*., 2015) [2]. IAA production will help the plants to produce a greater number of roots, lateral roots and longer roots which help in nutrient uptake also help in cell division and cell elongation. They multiply rapidly and develop a thick population in rhizosphere, when applied as seed treatment or seedling root-dip or as soil application.
Tomato (*Solanum lycopersicum L*.) belonging to family Solanaceae is one of the most important and widely consumed vegetable crops. It is rich in Vitamins, Minerals and Organic acids which are good for health. Hence an attempt was made to isolate *Azotobacter spp* grown in the native soils of Raichur district which are well adapted to native environment conditions grown in the rhizosphere of tomato plants and to evaluate the efficacy of isolates in terms of Plant growth promoting traits.

Materials and Methods
Total 15 *Azotobacter spp* were isolated from 15 rhizosphere soil samples of Tomato crop grown in the native soils of Raichur district. *Azotobacter* was isolated from soil samples using serial dilution technique and spread plate method and incubated at 28+2°C for 48-72 hrs. the bacterial isolates were characterized by their morphological and biochemical characterization by using standard method as described by Cappucino and Sherman, 1992. and all the *Azotobacter* isolates were Screened for invitro for Indole Acetic Acid production, Phosphate solubilization, Hydrogen Cyanide production, Siderophore production as plant growth promoting traits of the isolates.

Indole Acetic Acid Production
IAA production was detected by the modified method as described by Brick *et al*.,1991 [3]. 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 positive test for the IAA production.

Phosphate Solubilization
Pure culture of the *Azotobacter* isolates were spotted on Pikovskaya's agar plates with TCP as P source. The plates were incubated at 28 °C for 7 days. appearance of the solubilisation zone

around the bacterial colonies was taken as positive test for the phosphate solubilization. Phosphate solubilizing index (PSI) = (colony diameter + halozone diameter) - colony diameter. (PSE) Phosphate Solubilization Efficiency = Z/C x 100. Z- Clearance zone including bacterial growth C- Colony diameter.

Hydrogen Cyanide Production

Isolates were screened for the production of hydrogen cyanide by using the method of Lorck (1948). Nutrient broth was amended with 4.4g glycine/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 ±2 ºC for 4 days. Development of orange red colour indicated positive for HCN production.

Siderophore production

Azotobacter culture was grown in Chrome Azurol S agar plates and incubated at 28 °C for 48 - 72 h. Plates were observed for the appearance of an orange halo around the bacterial colony was considered as positive test for siderophore production (Schwyn and Neelands, 1987).

Table 1: Screening of isolates for its plant growth promoting potential activity

Isolate code	IAA (µg/ml)	HCN Production	Siderophore production	NH_3 Production
AMSRT-1	06.29	-	-	+
AMSRT-2	07.12	+	-	+
AMSRT-3	05.14	-	-	+
AMSRT-4	15.24	+	-	+
AMSRT-5	04.43	-	-	+
AMSRT-6	07.23	-	+	+
AMSRT-7	05.40	-	-	+
AMSRT-8	05.35	-	-	+
AMSRT-9	14.35	-	+	+
AMSRT-10	03.16	-	-	+
AMSRT-11	05.37	-	-	+
AMSRT-12	06.13	-	-	+
AMSRT-13	03.25	-	-	+
AMSRT-14	06.37	-	-	+
AMSRT-15	05.62	-	-	+
Reference strain	14.42	-	-	+

Table 2: Phosphate Solubilization by the isolates on Pikovskaya's medium

Isolate code	Zone Diameter (mm)	Colony Diameter (mm)	PSI	PSE%
AMSRT-1	0.92	0.70	2.31	131.42
AMSRT-2	1.52	0.46	4.30	330.43
AMSRT-3	0.73	0.40	2.83	182.50
AMSRT-4	1.56	0.96	2.62	162.50
AMSRT-5	1.12	0.89	2.26	125.84
AMSRT-6	0.76	0.50	2.52	152.00
AMSRT-7	0.81	0.62	2.30	130.64
AMSRT-8	0.78	0.57	2.37	136.84
AMSRT-9	0.95	0.75	2.27	126.67
AMSRT-10	0.82	0.66	2.24	124.24
AMSRT-11	1.62	0.42	4.85	385.71
AMSRT-12	0.83	0.66	2.25	125.75
AMSRT-13	0.75	0.54	2.38	138.88
AMSRT-14	0.62	0.38	2.63	163.15
AMSRT-15	0.76	0.44	2.72	172.72
Reference strain	1.58	0.46	4.43	343.47

Results

A total of 15 *Azotobacter spp* were isolated from 15 rhizosphere soil samples of Tomato crop. Isolated colonies morphologically showing Light brown, Dark brown, pale yellow, milky, creamy white, colorless, transparent, raised, round, smooth and gummy colonies were purified and preserved in nutrient agar slants under refrigerated conditions. Isolate AMSRT-4 showed highest IAA production i.e 15.24 µg/ml and lowest IAA production was by the isolate AMSRT-10 i.e 3.16 µg/ml. isolates AMSRT-2,4 showed positive for HCN production and isolates AMSRT -6, 9 showed positive for Siderophore production. and all isolates showed positive for NH_3 formation (Table no 1**)** and P-Solubilization by forming solubilisation zone dimeter around the bacterial colonies Isolate AMSRT-11showed highest P-Solubilization zone diameter i.e 1.62 mm and lowest P-Solubilization zone diameter i.e 0.62mm was by the isolate AMSRT-14 **(**Table no 2**).**

Results and Discussion

IAA is a phytohormone which plays an important role in plant growth and development. Enhanced root proliferation was attributed to bacterial IAA synthesis. All the isolates showed positive for IAA production through development of pink colour in tubes. HCN help plants in their defense against pathogens. isolates AMSRT- 2, 4 showed positive for HCN production as there was development of orange red color which indicated positive for HCN production. Bacteria secrete Siderophores during low availability and provide plants with Iron enhancing their growth directly by increasing the availability of Iron in the soil surrounding the roots. isolates AMSRT-6, 9 showed positive for Siderophore production. bacteria release various types of organic acids which lower the pH in the rhizosphere and thus make available to plants. Phosphorous helps plants in development of root system and all the isolates showed positive for P-Solubilization by forming solubilisation zone around the bacterial colonies. Results are in confirmation with Mahalaxmi and Reetha, 2009, Ayme *et al*., 2020 [8, 1].

Conclusion

From the present research analysis it is found that isolates AMSRT - 2,4,6,9 and 11 were found to be efficient in terms of plant growth promoting potential activity by producing IAA, Hydrogen Cyanide and Siderophore and Phosphorous solubilization and Ammonia production.

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