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Biological Activities of Plant Growth Promoting *Azotobacter* sp. Isolated from Vegetable Crops Rhizosphere Soils

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A total of one hundred and fifty rhizospheric soil samples were collected from the different vegetable (tomato, egg plant, chilli) growing fields in and around Namakkal district, Tamil Nadu, India. Ten isolates of *Azotobacter* sp. were isolated from the rhizospheric soils of vegetable plants using Jenson's and Ashby's mannitol selective media. The isolated *Azotobacter* strains were characterized and identified based on morphological and biochemical characteristics. Multiple plant growth promoting activities of *Azotobacter* isolates were determined in terms of IAA production, NH_3 release, PO_4 solubilization, HCN and siderophore production. IAA production of the *Azotobacter* isolates were tryptophan concentration dependent. Most of the *Azotobacter* isolates were tolerant to zinc and mercury concentration of 100-200 µg/ml. Antifungal activity of the *Azotobacter* was determined against *Aspergillus flavus*, *Cercospora* sp. and *Fusarium oxysporum* and found higher zone of inhibition (18-26 mm) at higher concentration of culture suspension.

Key words: Rhizospheric soil, Plant growth promoting activities, Heavy metal tolerance, Antiphytopathogenic fungal activity.

Actually, agricultural praxis does not sufficiently take into account the biological potentials of soils due to lake of knowledge. In future this will be essential, considering the decrease of some resources and the high costs for synthetic fertilizers and pesticides. To minimize chemical application in agriculture, integrated approach of nutrient management is the option using biofertilizers and plant growth promoting rhizobacteria (PGPR) which not only ensure higher productivity but also good health of our soil and environment[1].

PGPR are a heterogeneous group of bacteria that can be found in the rhizosphere, which can improve the quality of the plant growth directly and or indirectly[2, 3] as (i) their ability to produce plant growth regulators like indoleacetic acid, gibberellic acid and cytokinins[4, 5], (ii) asymbiotic nitrogen fixation[6], (iii) antagonism against phytopathogenic microorganisms by production of siderophores[7, 8, 9], antibiotics[10, 11] and cyanide[12, 13], (iv) solubilization of mineral phosphates and other nutrients[14, 15, 16] and (v) active removal and bioaccumulation of heavy metals and their capacity to assist the root growth[17, 18, 19]. In last decades a

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large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance plant growth[4].

In addition, PGPR isolates must be rhizospheric competent, able to survive and colonize in the rhizospheric soil[20]. The variability in the performance of PGPR may be due to climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil that may affect their growth and exert their effect on the plant[21]. Therefore the results obtained *in vitro* cannot always be reproduced under field conditions[2, 10] and one possible approach is to explore and develop efficient strains in field conditions which are having multiple plant growth promoting activities and are well adapted to particular rhizospheric soil environment. The present study designed to screen certain rhizospheric *Azotobacter* isolates from vegetable growing in soils of Namakkal district, Tamil Nadu, India for their multiple plant growth promoting activities in relation to indole acetic acid, ammonia, hydrogen cyanide, and siderophore production, phosphate solubilization, heavy metal tolerance to zinc and mercury and antifungal activity against three phytopathogenic organisms as *Aspergillus flavus*, *Cercospora* sp. and *Fusarium oxysporum*.

MATERIALS AND METHODS

Site Description

Soil sampling sites were located in subtropical dryland of Namakkal (latitude – 11° 28' 0" N; longitude – 78° 10' 0" E) and in the neighborhood paddy fields in the Namakkal district in the north western agro climatic zone of Tamil Nadu, South India, India. The average annual rainfall - 785 mm, mean maximum and minimum temperature is 31.5 °C and 20 °C respectively. Agriculture is the main occupation of the people of Namakkal district, who are engaged in the cultivation of rice, millets, cereals, pulses, sugarcane, groundnut, gingely, and Cotton. In addition major vegetable crops are Tapiaco, Onion, Tomato, Brinjal, Potato, Lablab, Drumstick, etc. Out of about 1.90 lakhs ha. of Horticulture Crops in Namakkal district, Vegetable crops are grown in 0.24 lakhs ha. This area belongs to subtropical monsoon climate and climate is warm and moisture. The N fertilizers are applied to horticulture fields with the approximate ratio of base-tiller-ear being 30–15–5% during the vegetables growing seasons and the total amount of N, P_2O_5 and K_2O fertilizers is about 200, 50 and 50 kg ha–1 each year.

Collection of rhizospheric soils from different vegetable crops

One hundred and fifty rhizospheric soil samples of different vegetable crops (tomato, egg plant and chilli) were randomly collected from different vegetable crop fields of Kanavaipatti village in the Namakkal district (latitude – 11° 28' 0" N; longitude – 78° 10' 0" E; average annual rainfall - 785 mm; mean temperature – max. 31.5°C; min. 20 °C; climate - subtropical), Tamil Nadu, India. Plants were uprooted along with a good amount of non-rhizosphere soil, brought immediately to the laboratory in polythene bags (2 kg size: 23 · 32 cm) and were air-dried within 2 h. The non-rhizosphere soil was removed by gentle shaking leaving behind the rhizosphere soil only (strongly adhering to the roots). The root adhering soil was collected from vegetable plants by dipping and gentle shaking in sterilized water under aseptic conditions. The soil suspension obtained was used for further isolation and characterization[22].

Isolation and characterization

Azotobacter spp. were isolated initially from inoculation of soil suspension on nitrogen free Jensen's medium (HiMedia, Mumbai) containing grams per liter of distilled water: 20 sucrose, 1 K_2HPO_4, 0.5 $MgSO_4.7H_2O$, 0.5 NaCl, 0.1 K_2SO_4, 0.005 Na_2MoO_4, pH 6.9 and incubated for one week. After a week period of incubation, it was cultured in Ashby's mannitol agar medium (HiMedia, Mumbai) containing grams per liter of distilled water: 20 mannitol, 0.2 K_2HPO_4, 0.2 $MgSO_4$. $7H_2O$, 0.2 NaCl, 0.1 K_2SO_4, 5.0 $CaCO_3$, 20 agar, pH 6.8–7.0. *Azotobacter* isolates were sub cultured and maintained on the respective slants. The *Azotobacter* isolates were characterized by their cultural conditions, morphological and biochemical characteristics (indole, methyl red, voges proskauer, citrate, catalase, glucose, lactose, mannitol, sucrose, nitrate reduction, starch hydrolysis) using standard methods[23].

In vitro screening of *Azotobacter* isolates for their multiple plant growth promoting (PGP) activities

Assay for indole acetic acid (IAA) production

Estimation of indole acetic acid (IAA) was done by inoculation of 200 µl of bacterial suspension (3 x10^8 CFU/ml) in 10 ml Luria–Bertani (LB) broth amended with different L-tryptophan concentration (10 - 100 µg/ml) and incubating it for a period of 48 h. The IAA content in the culture suspension was estimated by the standard procedure[24]. All the studies were repeated on three independent dates to confirm the results.

Ammonia (NH_3) production

Azotobacter isolates were tested for the production of ammonia in peptone water. The 0.1 ml (2.0x10^6 CFU/ml) of freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48–72 h at 28 ± 2 °C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production[23].

Hydrogen cyanide (HCN) production

All the *Azotobacter* isolates were screened for the production of hydrogen cyanide by adapting the method of Lorck[25]. Briefly, nutrient broth (Peptone 5.0, Yeast Extract 5.0, Beef Extract 3.0, NaCl 5.0, gram per litre and pH 7.2±0.2) was amended with 4.4 g glycine per litre 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 was placed in the top of the plate. Plates were sealed with parafilm and incubated at 28 ± 2 °C for 4 days. Development of orange to red colour indicated HCN production.

Siderophore production

Azotobacter isolates were assayed for siderophores production on the Chrome azurol S agar medium (Sigma, Ltd.) described by Schwyn and Neilands[26]. Chrome azurol S agar plates were prepared and divided into equal sectors and spot inoculated with test organism (10 µl of 10^6 CFU/ml) and incubated at 28± 2 °C for 48–72 h. Development of yellow–orange halo around the growth was considered as positive for siderophore production.

Phosphate solubilization

All isolates were screened on Pikovskaya's agar plates for phosphate solubilization as described by Gaur[16]. Pikovskaya's agar medium containing calcium phosphate as the inorganic form of phosphate was used in this assay. A 0.1 ml (2.0x10^6 CFU/ml) of bacterial culture was inoculated on the plates and maintained for incubation at 28 °C for 7 days. The presence of clear zone around the bacterial colonies indicates the solubilization of phosphate.

Antifungal assay

The agar well diffusion method as adopted earlier by Mehmood et al.[27] was used. The *Azotobacter* isolates tested for their antifungal activity were fully grown in the respective broth media. Test fungi *A. flavus*, *Cercospora* sp. and *F. oxysporum* were grown on sabaroud dextrose agar (SDA), (per liter of distilled water: 40 g dextrose, 10 g peptone, 20 g agar) slants. The spores were scraped and suspended in 10 ml of sterile normal saline solution (NSS). Diluted spore suspension (0.1 ml, 10^5 CFU/ml) of the fungi was spread on Muller Hinton agar (per liter of distilled water: 300 g beef infusion, 17.5 g casein acid hydrolysate, 1.5 g starch, 20 g agar, pH 7.2), nutrient agar (NA) and SDA plates. Wells of 8 mm diameter were punched into the agar medium and filled with different concentrations of *Azotobacter* sp. suspension (50, 100, 150 and 200 µl - 2x10^4 – 10^7 CFU/ml). Nutrient broth was taken as negative control and 100 µg/ml antifungal antibiotics; nystatin was used as positive control. The plates were incubated for 4–5 days at 28 ± 2 °C. The antifungal activity was evaluated by measuring the growth inhibition zone against test fungi.

Heavy metal tolerance

The *Azotobacter* isolates were tested for their resistance to heavy metals by agar dilution method[28]. Freshly prepared agar plates were amended with soluble heavy metal salts namely Hg and Zn, at the concentrations of 50 to 200 ìg/ml were inoculated with 2x10^6 CFU/ml cultures. Heavy metal tolerance was determined by the appearance of *Azotobacter* growth after incubating the plates at 28 ± 2 °C for 72 h.

Statistical analysis

The results are reported as mean ± SD. The statistical variation in IAA production by *Azotobacter* isolates at different tryptophan concentration and antifungal activity against the test fungi were analyzed using an analysis of variance (ANOVA), following a mean separation according to the Tukey multiple range test. In all statistical analysis, $P < 0.05$ was considered significant[29, 30].

RESULTS

On the basis of cultural, morphological and biochemical characteristics a total of 50 *Azotobacter* isolates (AZT- 14 - tomato, AZB - 13- egg plant, AZC - 23 - chilli) were identified from 150 rhizospheric soil samples as described in Bergey's Manual of Determinative Bacteriology[31]. Among the 50 isolates, 10 (AZT-1, 2, 3, AZB – 4, 5, AZC – 6, 7, 8, 9, 10) were selected for further studies based on the efficiency of multiple plant growth promoting activities exhibited in preliminary studies (data not shown). General features of the test isolates are illustrated in Table 1. The isolates were observed as transparent, watery, mucoid, slimy colonies in Ashby's mannitol agar medium; gram negative rods; positive for catalase, indole, MR, VP, citrate, nitrate reduction tests. Moreover, they ferment glucose, lactose, mannitol, and sucrose producing both acid and gas; are able to hydrolyze the starch; and display prototrophy for biotin.

Screening results of multiple PGP traits of ten *Azotobacter* are depicted in Table 2. Out of ten isolates, four *Azotobacter* isolates (AZT1, AZT3, AZB4 and AZC6) were showing multiple PGP activities in relation to indole acetic acid (IAA), ammonia, hydrogen cyanide and siderophore production and phosphate solubilization (Table 2). AZC8, AZT2 and AZC9 isolate neither producing ammonia nor solubilizing the phosphates. All the ten *Azotobacter* isolates were tolerant to mercury and zinc. Higher growth was noticed in AZT1, AZT3, AZB4 and AZC6 in zinc and mercury up to the concentration of 200 µg/ml (Table 3).

A total of 10 isolates of *Azotobacter* were tested for the quantitative estimation of IAA in the presence of different concentrations of tryptophan. With no addition of tryptophan, production of IAA was not observed. However the concentration of tryptophan increased the production of IAA was increased significantly ($p < 0.05$) from a minimum of 0.23 µg/ml in 10 µg/ml of tryptophan to a maximum of 2.56 µg/ml in 100 µg/ml of tryptophan respectively. The production of IAA was highest in the isolate of AZB4 followed by AZC6, AZT1 and AZT3. In AZC7 and AZC10, IAA production was detected only at higher concentration of 60 and 90 µg/ml of tryptophan respectively (Table 4).

Antiphytopathogenic fungal activity of the ten *Azotobacter* isolates was checked against *A. flavus*, *Cercospora* sp. and *F. oxysporum* in SDA (Table 5). The antifungal activity of the *Azotobacter* isolates tested varied with inhibition zones in

Table 1. Morphological and cultural characteristics of *Azotobacter* isolates from the rhizospheric soil of vegetables

Morphological and cultural characterization	*Azotobacter* sp.
Number of isolates	10
Colony morphology onAsbhy's mannitol agar	Watery, mucilaginous shrink, serrated margins
Pigmentation	Transparent, milky
Gram reaction, cell shape	Gram negative rods
Growth on nitrogen free medium	Positive
Biotin prototrophy	Positive (90 %)
Motility	Motile
Indole	Positive (100 %)
Methyl red	Positive (80 %)
Voges proskaeur	Positive (70 %)
Citrate	Positive (100 %)
Catalase	Positive (100 %)
Glucose	Acid and gas production (100 %)
Lactose	Acid and gas production (80 %)
Mannitol	Acid and gas production (40 %)
Sucrose	Acid and gas production (90 %)
Nitrate reduction	Positive (100 %)
Starch hydrolysis	Positive (100 %)

diameter from 1.00 to 34.23 mm. Isolates AZT1, AZT3, AZB4 and AZC6 induced larger inhibition zones showing their high antifungal activity and exhibiting broad-spectrum activities against test fungi compared to the other *Azotobacter* isolates. Neither AZT5 nor AZC10 showed antifungal activity against the three test fungi. No antifungal activity was observed in isolates AZC7 and AZC9 at lower concentrations 50 and 100 µl/ml against test fungi (Table 5). Antiphytopathogenic fungal activity of the ten *Azotobacter* isolates was increased significantly with the concentration of the culture suspension against *A. flavus* (DF – 36, F value – 0.7548 and CD value 1.628), *Cercospora* sp. (DF – 36, F value - 0.5612 and CD value 1.147) and *F. oxysporum* (DF – 36, F value – 0.6481 and CD value 1.398).

Table 2. Multiple plant growth promoting activities of *Azotobacter* isolates from the rhizospheric soil of vegetables

Azotobacter isolates	Indole acetic acid production	Ammonia production	Hydrogen cyanide production	Phosphate solubilization	Siderophore production
AZT1	+++	+++	+++	+++	+++
AZT2	++	+	-	-	+
AZT3	+++	+++	+++	+++	+++
AZB4	+++	+++	+++	+++	+++
AZB5	+	+	-	++	-
AZC6	+++	+++	+++	+++	+++
AZC7	-	+	++	+	+
AZC8	+	-	++	+	+
AZC9	+	++	-	-	+
AZC10	-	+	-	++	-

AZT – *Azotobacter* isolates from tomato rhizospheric soil; AZB – *Azotobacter* isolates from egg plant rhizospheric soil; AZC - *Azotobacter* isolates from chilli rhizospheric soil
+ low colour intensity / zone formation ; ++ medium colour intensity / zone formation; +++ high colour intensity / zone formation; - no colour change / no zone formation

Table 3. Heavy metal tolerance among *Azotobacter* isolates from rhizospheric soil of vegetables grown on Ashby's mannitol agar

Azotobacter	Zinc concentration (µg/ml)				Mercury concentration (µg/ml)			
isolates	50	100	150	200	50	100	150	200
AZT1	+++	+++	+++	+++	+++	+++	+++	+++
AZT2	+	+	+	+	+	+	+	+
AZT3	+++	+++	+++	+++	+++	+++	+++	+++
AZB4	+++	+++	+++	+++	+++	+++	+++	+++
AZB5	+	+	+	+	++	+	+	+
AZC6	+++	+++	+++	+++	+++	+++	+++	+++
AZC7	+	+	+	-	+	+	-	-
AZC8	++	+	+	+	++	+	+	-
AZC9	++	+	+	+	+	+	+	-
AZC10	+	+	+	+	++	+	+	+

AZT – *Azotobacter* isolates from tomato rhizospheric soil; AZB – *Azotobacter* isolates from egg plant rhizospheric soil; AZC - *Azotobacter* isolates from chilli rhizospheric soil
+ low growth; ++ medium growth; +++ high growth; - no growth

Table 4. Production of indole acetic acid at different tryptophan concentration by *Azotobacter* isolates grown on Jensen's medium.

Azotobacter isolates	Indole acetic acid production (µg/ml ± SD) at different tryptophan concentrations (µg/ml)										
	0	10	20	30	40	50	60	70	80	90	100
AZT1	ND	1.72 ± 0.25	1.86 ± 0.30	1.89 ± 0.15	1.92 ± 0.32	1.96 ± 0.15	1.99 ± 0.31	2.06 ± 0.30	2.31 ± 0.15	2.42 ± 0.42	2.56 ± 0.21
AZT2	ND	1.52 ± 0.31	1.59 ± 0.21	1.62 ± 0.20	1.68 ± 0.20	1.73 ± 0.25	1.80 ± 0.20	1.85 ± 0.15	1.89 ± 0.10	1.92 ± 0.35	1.96 ± 0.15
AZT3	ND	1.70 ± 0.20	1.76 ± 0.40	1.78 ± 0.25	1.75 ± 0.25	1.82 ± 0.20	1.88 ± 0.25	1.91 ± 0.20	1.96 ± 0.12	1.98 ± 0.36	2.02 ± 0.26
AZB4	ND	1.76 ± 0.40	1.79 ± 0.25	1.75 ± 0.47	1.79 ± 0.25	1.81 ± 0.35	1.86 ± 0.15	1.90 ± 0.25	1.97 ± 0.50	2.13 ± 0.45	2.18 ± 0.43
AZB5	ND	0.66 ± 0.12	0.69 ± 0.15	0.73 ± 0.45	0.77 ± 0.20	0.81 ± 0.30	0.84 ± 0.17	0.88 ± 0.47	0.91 ± 0.48	0.96 ± 0.15	0.99 ± 0.42
AZC6	ND	1.76 ± 0.15	1.79 ± 0.20	1.82 ± 0.20	1.86 ± 0.32	1.89 ± 0.30	1.96 ± 0.47	2.19 ± 0.45	2.23 ± 0.31	2.37 ± 0.20	2.50 ± 0.35
AZC7	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.47 ± 0.25	0.61 ± 0.41
AZC8	ND	0.72 ± 0.30	0.76 ± 0.50	0.82 ± 0.42	0.87 ± 0.20	0.96 ± 0.15	0.98 ± 0.29	1.22 ± 0.31	1.31 ± 0.32	1.39 ± 0.30	1.40 ± 0.42
AZC9	ND	0.23 ± 0.15	0.29 ± 0.25	0.32 ± 0.28	0.37 ± 0.47	0.41 ± 0.25	0.44 ± 0.50	0.49 ± 0.33	0.51 ± 0.51	0.56 ± 0.25	0.59 ± 0.36
AZC10	ND	ND	ND	ND	ND	ND	0.19 ± 0.18	0.20 ± 0.40	0.29 ± 0.42	0.30 ± 0.16	0.36 ± 0.30

ND – not detectable; AZT – *Azotobacter* isolates from tomato rhizospheric soil; AZB – *Azotobacter* isolates from egg plant rhizospheric soil
AZC - *Azotobacter* isolates from chilli rhizospheric soil

DISCUSSION

Understanding the dynamics of root colonization by specific microbial components of the rhizoplane and rhizosphere is necessary for the development of biological control of soil borne pathogens and the effective use of beneficial microorganisms to enhance plant growth[32]. Plant rhizosphere is known to be the preferred ecological niche for various types of PGPR (*Rhizobium*, *Azotobacter* and *Azospirillium*) due to rich nutrient availability. The three main intrinsic characteristics of PGPR must be ability to: (i) colonize roots, (ii) survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant promotion/protection activities, and (iii) promote plant growth[2, 18, 33, 34]. Further exploration and evaluation of the isolates exhibiting multiple PGP traits on soil-plant system is needed to uncover their efficacy as effective PGPR[2, 33]. In the present investigation multiple PGP activities were found in four *Azotobacter* isolates (AZT1, AZT3, AZB4 and AZC6) out of 10 isolates screened from vegetable growing rhizospheric soil.

Based on earlier reports[35, 36, 37, 38, 39] 80% of microorganisms isolated (*Azospirillum, Pseudomonas, Xanthomonas,* and *Rhizobium* as well as *Alcaligenes faecalis, Enterobacter cloacae, Acetobacter diazotrophicus*, *A. chroococcum* and *Bradyrhizobium japonicum*) from the rhizosphere of various crops have the ability to produce IAA which help in stimulating plant growth. IAA production was detected in all the test isolates of *Azotobacter*. The *Azotobacter* isolates AZB4, AZC6, AZT1 and AZT3 produced higher amount of IAA, but in AZC7 and AZC10 the production was detected at higher concentration of tryptophan. Furthermore, there was an increase in the level of IAA with the increasing concentration of tryptophan (10-100µg/ml) as evidenced by Ahmed et al.[2].

Other important traits of PGPR, that may indirectly influence the plant growth, are the production of ammonia and solubilization of phosphate. However, ammonia production and phosphate solubilization are observed frequently in *Azotobacter* sp. about 60-70%[40]. A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere soil

Table 5. Antiphytopathogenic fungal activity of *Azotobacter* isolates against *Aspergillus flavus*, *Cercospora* sp. and *Fusarium oxysporum* [a].

AZI	Concentration of Azotobacter sp. suspension (µl ml^{-1})											
	50			100			150			200		
	Zone of inhibition (mm) of fungal pathogen											
	Af	Cs	Fo	Af	Cs	Fo	Af	Cs	Fo	Af	Cs	Fo
AZT1	15.61± 0.21a	18.42 ± 1.40 b	16.62 ± 0.58 a	17.33 ± 0.29 b	20.00 ± 1.00 b	19.50 ± 0.71 b	21.33 ± 0.58 b	25.25 ± 0.80 c	23.50 ± 0.71 a	26.65 ± 0.55 b	32.62 ± 1.66 b	29.00 ± 1.00 b
AZT2	1.10 ± 0.42 d	ND	ND	3.00 ± 0.50 d	2.25 ± 0.10 c	2.15 ± 0.15 d	3.45 ± 0.22 d	2.60 ± 0.33 f	2.75 ± 0.66 c	3.51 ± 0.69 g	2.66 ± 0.55 f	2.80 ± 0.15 c
AZT3	12.25 ± 0.70 c	15.10 ± 0.89 d	13.10 ± 0.55 b	16.45 ± 0.78 b	22.68 ± 1.20 a	19.00 ± 1.00 c	23.45 ± 0.69 a	28.45 ± 0.65 b	25.00 ± 0.78 a	29.62 ± 0.56 a	34.23 ± 0.42 a	31.00 ± 1.00 a
AZB4	14.50 ± 0.26 b	19.50 ± 0.50 a	17.00 ± 0.60 a	18.65 ± 0.48 a	23.65 ± 0.75 a	20.65 ± 0.25 a	21.35 ± 0.55 b	29.26 ± 0.67 a	24.33 ± 0.58 a	23.26 ± 1.67 d	32.35 ± 0.95 c	28.65 ± 0.54 b
AZB5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
AZC6	12.32 ± 0.42 c	17.65 ± 0.55 c	15.52 ± 0.66 a	14.55 ± 0.72 c	21.00 ± 1.00 b	18.83 ± 0.76 bc	18.17 ± 0.29 c	24.33 ± 1.15 d	20.67 ± 0.68 b	24.17 ± 0.65 c	30.35 ± 0.62 d	28.75 ± 0.95 b
AZC7	ND	ND	ND	1.00 ± 0.15 e	ND	ND	2.23 ± 0.50 f	1..00 ± 0.05 g	1.89 ± 0.56 c	2.65 ± 0.75 h	2.45 ± 0.95 fg	3.45 ± 0.62 c
AZC8	1.00 ± 0.35 d	1.23 ± 0.66 e	1.15 ± 0.05 c	1.45 ± 0.45 e	2.23 ± 0.75 c	1.00 ± 0.05 d	2.65 ± 0.75 e	3.67 ± 0.85 e	2.88 ± 0.92 c	4.85 ± 0.78 e	3.62 ± 0.86 e	2.65 ± 0.48 c
AZC9	1.23 ±0.56 d	ND	ND	2.62 ±0.52 e	1.88 ±0.55 c	1.75 ±0.25 d	3.50 ±0.55 d	2.25 ±0.75 f	2.63 ±0.58 c	3.83 ±0.76 f	2.26 ±0.58 g	2.62 ±0.45c
AZC10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

AZI – *Azotobacter* isolates; AZT – *Azotobacter* isolates from tomato rhizospheric soil; AZB – *Azotobacter* isolates from egg plant rhizospheric soil; AZC - *Azotobacter* isolates from chilli rhizospheric soil
ND – Not detected; Af – *Aspergillus flavus*; Cs – *Cecospora* spp.; Fo – *Fusarium oxysporum*
Means within a column followed by the same letter are not significantly different
($P > 0.05$, Tukey multiple range test)
[a] Numbers in columns represent mean ± SE (five observations)

[14, 35, 41, 42]. Here, we found that all the isolates of *Azotobacter* obtained from vegetable soils are ammonia producers as well as solubilizing the phosphates, (except AZC8, AZT2 and AZC9) and therefore have good prospects to improve plant growth especially in soil with large amount of precipitated phosphate.

As per earlier reports[14, 43, 44] *Azotobacter* protect several plants from root disease caused by soil borne fungi through HCN and siderophore production. Hydrogen cyanide production by *Azotobacter* isolates accounts for about 60% of the inhibition on phytopathogens observed in soil[2, 35]. In our study, most of the *Azotobacter* isolated (AZT1, AZT3, AZB4, AZC6, AZC7 and AZC8) are producing HCN as well as siderophore and act as potent antifungal agent. Synergistic interaction of these two with other metabolites may further function as stress factors including local and systematic host resistance[8] that led for the suppression of the root pathogens[45]. Out of ten *Azotobacter* isolates, four isolates (AZT1, AZT3, AZB4 and AZC6) were exhibiting high antifungal activity against *A. flavus*, *Cercospora* and *F. oxysporum* The antifungal activity of the screened *Azotobacter* isolates was concentration dependent and statistically significant ($p< 0.05$).

Azotobacter had developed the mechanisms to cope with a variety of heavy metals for their survival in the rhizosphere of vegetable ecosystem[35] and can be used to reduce the toxicity of the metal or increase its bioavailability[17]. It was observed that few *Azotobacter* rhizobacteria tolerate Zn and Hg metal concentrations up to the level of 100-200 µg/ml and exhibit a multiple PGPR activities like IAA, HCN, NH_3 production and siderophores. This was evidenced in our study with *Azotobacter* isolates AZT1, AZT3, AZB4 and AZC6. It was also apparent that most of the cultures of PGPR isolated from vegetable rhizosphere were tolerant to elevated levels of heavy metals that may decrease heavy metal toxicity and increase the PGP activities[46].

The isolation of PGPR from different sources opens new doors to design strategies for improving the efficacy of biocontrol agents. Identification of key antimicrobials produced by superior agents can be exploited for streamlining strain discovery by targeting selection of new isolates that carry relevant biosynthetic genes. Determination of the role of edaphic parameters favorable for disease suppression, particularly those that stimulate antibiotic production and activity, can be exploited by targeting inoculants for soils that are more likely to support biocontrol. Biocontrol with plant growth promotion helps increasing the vegetative yield and thereby increasing crop yield.

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