

Original Research Article

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Characterization of Rhizosphere Soils of Foxtail Millet and Isolation of *Azospirillum* Strains

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ABSTRACT

A Total of 40 different foxtail millet roots samples along with rhizospheric soil were collected from Raichur and Koppal districts of Karnataka. *Azospirillum* strains were isolated and physico-chemical properties of the soil were analyzed. Among them, 20 samples each from Raichur and Koppal districts were collected. The physico-chemical properties of the soil and available nutrients of Raichur district samples ranged from, pH (7.58-8.85), electrical conductivity (0.12-0.99 dS m⁻¹), organic carbon (0.11-0.87%), available nitrogen (110.40 to 358.80 kg ha⁻¹), available phosphorus content (9.35 to 29.92 kg ha⁻¹), available potassium (117.60 to 362.88 kg ha⁻¹). Likely, the physico-chemical properties of the soil and available nutrients of Koppal district samples ranged from, pH (6.30-8.50), electrical conductivity (0.12-0.85 dS m⁻¹), organic carbon (0.12-0.91%), available nitrogen (188.60 to 552.10 kg ha⁻¹), available phosphorus content (8.66 to 35.07 kg ha⁻¹), and available potassium (117.60 to 406.54 kg ha⁻¹). Forty *Azospirillum* isolates were obtained by adopting enrichment culture technique. All the isolates formed subsurface pellicles in NFBTB medium including the reference strain. Pellicle was formed 1-2 mm from the surface of semisolid NFBTB medium. All the *Azospirillum* strains turned olive green colour of Bomothymol Blue (BTB) to brilliant blue.

Keywords

Azospirillum spp,
Physico-chemical
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Introduction

Soil microorganisms, like *Azospirillum* spp., *Azotobacter* Sp. and *Enterobacter* Sp. have shown to encourage plant growth, by promoting the outbreak of secondary roots. *Azospirillum* have been isolated from the rhizosphere and roots of a variety of plants including cereals and grasses. Inoculation with indigenous *Azospirillum* is an important procedure when studying their inherent

capacity to benefit crops. In some cases, indigenous strains can perform better than introduced strains in promoting the growth of crops due to their superior adaptability to the environment.

Azospirillum live in close association with plants in the rhizosphere. The plant stimulatory effect exerted by *Azospirillum* has been attributed to several mechanisms, including biological nitrogen fixation and

production of plant growth promoting substances (Okon and Itzigsohn, 1995; Salomone *et al.*, 1996). Upon *Azospirillum* inoculation change in root morphology was observed, which has been described to the bacterial production of plant growth regulating substances (Umali-Garcia *et al.*, 1980; Tien *et al.*, 1979). An increased number of lateral roots and root hairs enlarge the root surface available for nutrients. This results in higher nutrient uptake by inoculated roots and an improved water status of the plant, which in turn could be the main factor for enhancing plant growth (Fallik and Okon, 1996). *Azospirillum* is not only able to fix atmospheric N (Dobereiner and Day 1976), but also to mineralize nutrients from the soil, to sequester, Fe, to survive to marsh environmental conditions, and can help plants minimize the negative effects of abiotic stresses.

Materials and Methods

Collection of soil and root samples and their characterization

A total of 40 foxtail millet root bit samples were collected from the foxtail millet fields located in the Raichur and Koppal districts of Northern Karnataka for isolation of *Azospirillum* strains. The details on the soil type and locations from where the samples taken are presented in Table 1. Roots of foxtail millet along with rhizosphere soils were collected from 40 different localities of Raichur and Koppal districts. The collected samples were brought in polythene bags and stored in a refrigerator at 4°C to maintain their chemical properties and for further study.

Soil Chemical analyses

The soil samples were analyzed for pH, EC, OC, N, P and K content by following the standard procedure.

Soil pH

Soil pH was determined in 1:2.5 soil water suspension using pH meter (Piper, 1966) with a glass electrode.

Electrical conductivity (EC)

The Electrical conductivity was determined in 1:2.5 soil water extract using conductivity bridge (Jackson, 1973).

Organic carbon

The organic carbon content of the soil samples was determined by using wet oxidation method (Jackson, 1973). For this a known weight of soil was treated with excess volume of potassium dichromate solution in the presence of concentrated H₂SO₄. Organic carbon in the soil was oxidized to CO₂. The excess of potassium dichromate unused is titrated back against ferrous ammonium sulphate in the presence of concentrated phosphoric acid and diphenyl amine indicator.

Available nitrogen of soil samples

For the estimation of available N content in soil, the alkaline potassium permanganate method of Subbaiah and Asija (1956) was followed. A known weight of soil was treated with excess of alkaline (0.32%) potassium permanganate (made alkaline with 25% NaOH solution). The liberated ammonia was trapped in boric acid and determined by titration against standard H₂SO₄. The available N content in kg ha⁻¹ was computed using titer value.

Available phosphorus of soil samples

Available phosphorus content of the soil was extracted by using 0.5 M NaHCO₃. The Phosphorus content was determined by chloromolybdic blue colour method using UV

spectrophotometer. The intensity of blue colour was determined at 660 nm wavelength. The available Phosphorus content was computed and expressed in kg ha⁻¹ (Jackson, 1973).

Available potassium of soil samples

Available potassium content was extracted by using neutral normal NH₄OAc solution as described by Jackson (1973). The concentration of Potassium in the extractant was determined by flame photometer.

Isolation of *Azospirillum* strains from foxtail millet root samples

The *Azospirillum* strains from foxtail millet samples were isolated by following the enrichment culture technique as adopted by (Dobereiner and Day, 1976) and (Baldani and Dobereiner, 1980). The fresh roots of foxtail millet were collected from the farmer's fields at different locations of Raichur and Koppal districts. The plants were uprooted carefully with root system intact and brought to the laboratory in sterile propylene bags. Roots were thoroughly washed in running tap water, cut into small bits of 1 cm length and surface sterilized by dipping in 0.1 per cent HgCl₂ solution for three minutes followed by dipping in 70 per cent alcohol for one minute. The roots were finally washed in six to eight changes of sterile distilled water.

The root bits were aseptically placed in tubes containing sterilized semisolid N free malate medium (Baldani and Dobereiner, 1980). The tubes were incubated at 30°C for a period of one week and observed for growth of *Azospirillum* as subsurface white undulating pellicles.

The repeated sub culturing was done to confirm the *Azospirillum* isolates *i.e.*, a loopful of culture was streaked on malate agar

plates containing 1 per cent NH₄Cl. After a week of incubation, typical small, white dense single colonies were picked and transferred to culture tube containing semisolid N-free malate medium. The isolates that formed characteristic subsurface white undulating pellicle in this medium were tentatively considered as *Azospirillum*.

The isolates were finally purified by streaking on potato infusion agar. Typical pink often wrinkled colonies on potato infusion agar were transferred to semi-solid medium for storage and characterization.

Results and Discussion

Chemical properties of rhizosphere soil

The chemical properties of soil samples collected in foxtail millet rhizosphere from different fields of Raichur and Koppal districts are presented in Table 2.

The results of Raichur district samples indicated that the pH ranges between 7.58 and 8.85, EC ranges between 0.12 and 0.99 dS m⁻¹, OC ranges between 0.11 and 0.87 per cent. In UAS campus Raichur, results of soil samples indicated that the pH ranges between 8.30 and 8.60, EC ranges between 0.16 and 0.99 dS m⁻¹, OC ranges from 0.11 and 0.87 per cent. In Tuntapur village of Raichur, results of soil samples indicated that the pH ranges between 7.58 and 8.85, EC ranges between 0.22 and 0.51 dS m⁻¹, OC ranges between 0.15 and 0.55 per cent and in Yergera village of Raichur, results of soil samples indicated that the pH ranges between 7.95 and 8.20, EC ranged between 0.12 and 0.90 dS m⁻¹, OC ranges between 0.19 and 0.85 per cent.

The results of Koppal district samples indicated that the pH ranges between 6.30 and 8.50, EC ranges between 0.12 and 0.85 dS m⁻¹, OC ranges from 0.12 and 0.91 per cent. In

Basapur village of Koppal, results of soil samples indicated that the pH ranges between 6.90 and 8.30; EC ranges between 0.12 and 0.31 dS m⁻¹, OC ranges from 0.27 and 0.91 per cent. In Kutaganalli village of Koppal, results of soil samples indicated that the pH ranges between 7.31 and 8.50, EC ranges between 0.16 and 0.34 dS m⁻¹, OC ranges between 0.52 and 0.77 per cent and in Halarti village of Koppal, results of soil samples indicated that the pH ranges between 6.65 and 7.80, EC ranges between 0.21 and 0.85 dS m⁻¹, OC ranges from 0.12 and 0.56 per cent.

The overall soil samples pH ranges between 6.30 and 8.85, EC ranges between 0.12 and 0.99 dS m⁻¹, OC ranges between 0.11 and 0.91 per cent. The highest and the lowest value of pH, EC and OC were 8.85 and 6.30, 0.99 and 0.12 dS m⁻¹ and 0.91 and 0.11 per cent respectively.

The major nutrients in rhizosphere soil

The major nutrient status of soil samples collected in foxtail millet rhizosphere from different villages of Raichur and Koppal districts are presented in Table 2.

The result of Raichur district samples indicated that the available nitrogen content ranged from 110.40 to 358.80 kg ha⁻¹, available phosphorus content ranged from 9.35 to 29.92 kg ha⁻¹, and available potassium content ranged from 117.60 to 362.88 kg ha⁻¹. In UAS campus Raichur, results of soil samples indicated that the available nitrogen content ranged from 110.40 to 305.12 kg ha⁻¹, available phosphorus content ranged from 9.35 to 26.78 kg ha⁻¹, and available potassium content ranged from 117.60 to 307.30 kg ha⁻¹. In Yergera village of Raichur, results of soil samples indicated that the available nitrogen content ranged from 171.40 to 190.40 kg ha⁻¹, available phosphorus content ranged from 15.07 to 26.78 kg ha⁻¹, and available

potassium content ranged from 120.69 to 319.20 kg ha⁻¹. In Tuntapur village of Raichur, results of soil samples indicated that the available nitrogen content ranged from 166.30 to 288.50 kg ha⁻¹, available phosphorus content ranged from 9.92 to 35.63 kg ha⁻¹, and available potassium content ranged from 171.36 to 362.88 kg ha⁻¹.

The result of Koppal district samples indicated that the available nitrogen content ranged from 188.60 to 552.10 kg ha⁻¹, available phosphorus content ranged from 8.66 to 35.07 kg ha⁻¹, and available potassium content ranged from 117.60 to 406.54 kg ha⁻¹. In Basapur village of Koppal, results of soil samples indicated that the available nitrogen content ranged from 219.50 to 552.10 kg ha⁻¹, available phosphorus content ranged from 8.66 to 24.64 kg ha⁻¹, and available potassium content ranged from 117.60 to 319.20 kg ha⁻¹. In Kutaganalli village of Koppal, results of soil samples indicated that the available nitrogen content ranged from 188.60 to 298.60 kg ha⁻¹, available phosphorus content ranged from 15.35 to 28.92 kg ha⁻¹, and available potassium content ranged from 117.60 to 406.54 kg ha⁻¹. In Halarti village of Koppal, results of soil samples indicated that the available nitrogen content ranged from 212.30 to 402.60 kg ha⁻¹, available phosphorus content ranged from 10.50 to 29.64 kg ha⁻¹, and available potassium content ranged from 117.60 to 376.35 kg ha⁻¹.

The *Azospirillum* isolates responded the overall available nitrogen content in soil samples ranged from 110.40 to 552.10 kg ha⁻¹, available phosphorus content ranged from 8.66 to 35.63 kg ha⁻¹ and available potassium content ranged from 117.60 to 406.54 kg ha⁻¹. The highest and lowest value of N, P and K were 552.10 and 110.40 kg ha⁻¹, 35.63 and 8.66 kg ha⁻¹ and 406.54 and 117.60 kg ha⁻¹ respectively.

Table.1 Coding of foxtail millet root samples along with rhizosphere soil from different locations

Sl. No.	Name of place	Soil type	Strain code
1	UAS, Raichur	Black	MARV-1
2	UAS, Raichur	Black	MARV-2
3	UAS, Raichur	Black	MARV-3
4	UAS, Raichur	Black	MARV-4
5	UAS, Raichur	Black	MARV-5
6	UAS, Raichur	Black	MARV-6
7	UAS, Raichur	Black	MARV-7
8	UAS, Raichur	Black	MARV-8
9	UAS, Raichur	Black	MARV-9
10	UAS, Raichur	Black	MARV-10
11	Basapur, Koppal	Red	MARV-11
12	Basapur, Koppal	Red	MARV-12
13	Basapur, Koppal	Red	MARV-13
14	Basapur, Koppal	Red	MARV-14
15	Basapur, Koppal	Red	MARV-15
16	Basapur, Koppal	Red	MARV-16
17	Basapur, Koppal	Red	MARV-17
18	Basapur, Koppal	Red	MARV-18
19	Basapur, Koppal	Red	MARV-19
20	Basapur, Koppal	Red	MARV-20
21	Kutaganalli, Koppal	Red	MARV-21
22	Kutaganalli, Koppal	Red	MARV-22
23	Kutaganalli, Koppal	Red	MARV-23
24	Kutaganalli, Koppal	Red	MARV-24
25	Kutaganalli, Koppal	Red	MARV-25
26	Halarthi, Koppal	Red	MARV-26
27	Halarthi, Koppal	Red	MARV-27
28	Halarthi, Koppal	Red	MARV-28
29	Halarthi, Koppal	Red	MARV-29
30	Halarthi, Koppal	Red	MARV-30
31	Tuntapur, Raichur	Red	MARV-31
32	Tuntapur, Raichur	Red	MARV-32
33	Tuntapur, Raichur	Red	MARV-33
34	Tuntapur, Raichur	Red	MARV-34
35	Tuntapur, Raichur	Red	MARV-35
36	Yeragera, Raichur	Red	MARV-36
37	Yeragera, Raichur	Red	MARV-37
38	Yeragera, Raichur	Red	MARV-38
39	Yeragera, Raichur	Red	MARV-39
40	Yeragera, Raichur	Red	MARV-40

Table.2 Chemical properties and status of available N, P and K content in foxtail millet rhizospheric soils of Raichur and Koppal districts, Karnataka

Sl. No.	Isolate code	Soil type	pH	EC (ds/m)	OC (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)
1	MARV-1	Black	8.62	0.22	0.47	185.54	15.64	171.35
2	MARV-2	Black	8.46	0.23	0.34	165.60	9.35	117.60
3	MARV-3	Black	8.30	0.41	0.11	110.40	26.20	120.96
4	MARV-4	Black	8.50	0.99	0.55	305.12	19.64	201.60
5	MARV-5	Black	8.31	0.16	0.87	231.51	26.78	307.30
6	MARV-6	Black	8.60	0.20	0.63	302.10	25.64	289.40
7	MARV-7	Black	8.70	0.24	0.71	179.40	22.64	201.60
8	MARV-8	Black	8.42	0.39	0.11	358.80	15.63	248.64
9	MARV-9	Black	8.31	0.85	0.31	274.84	10.50	127.68
10	MARV-10	Black	8.58	0.77	0.15	289.36	13.55	201.10
11	MARV-11	Red	7.58	0.38	0.27	250.88	16.20	248.64
12	MARV-12	Red	8.10	0.12	0.91	250.88	19.64	319.20
13	MARV-13	Red	6.90	0.28	0.58	361.70	21.92	164.64
14	MARV-14	Red	7.95	0.17	0.71	219.52	15.63	117.60
15	MARV-15	Red	7.73	0.19	0.33	220.90	19.92	120.96
16	MARV-16	Red	7.01	0.31	0.87	219.50	18.07	120.96
17	MARV-17	Red	6.50	0.23	0.27	552.10	8.66	319.20
18	MARV-18	Red	6.30	0.12	0.58	431.20	23.64	164.64
19	MARV-19	Red	7.73	0.18	0.60	282.24	19.88	265.40

Table 2. Contd.....

Sl. No.	Isolate code	Soil type	pH	EC (ds/m)	OC (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)
20	MARV-20	Red	8.30	0.14	0.45	250.88	24.64	189.04
21	MARV-21	Red	8.50	0.16	0.77	219.52	28.92	366.24
22	MARV-22	Red	8.10	0.20	0.86	250.88	19.92	120.24
23	MARV-23	Red	7.95	0.24	0.52	188.16	24.51	127.68
24	MARV-24	Red	7.31	0.34	0.57	188.06	24.20	319.20
25	MARV-25	Red	7.73	0.26	0.73	298.60	15.35	406.54
26	MARV-26	Red	7.80	0.28	0.46	250.88	23.12	376.35
27	MARV-27	Red	7.05	0.85	0.56	248.89	29.64	117.60
28	MARV-28	Red	6.90	0.40	0.32	219.60	35.07	120.96
29	MARV-29	Red	6.65	0.77	0.12	212.30	10.50	280.00
30	MARV-30	Red	7.01	0.21	0.51	402.60	24.64	225.12
31	MARV-31	Red	7.58	0.31	0.15	166.30	9.92	194.88
32	MARV-32	Red	8.10	0.46	0.55	234.60	29.92	171.36
33	MARV-33	Red	8.85	0.51	0.47	288.50	18.92	201.60
34	MARV-34	Red	8.30	0.22	0.51	256.60	28.62	362.88
35	MARV-35	Red	8.15	0.23	0.32	188.16	35.63	248.64
36	MARV-36	Red	7.95	0.41	0.85	190.40	15.07	127.68
37	MARV-37	Red	8.10	0.90	0.79	184.70	20.35	319.20
38	MARV-38	Red	8.20	0.12	0.82	171.40	25.65	261.30
39	MARV-39	Red	8.10	0.31	0.47	181.1	21.92	120.96
40	MARV-40	Red	8.05	0.23	0.19	176.3	26.78	194.88

The foxtail millet rhizospheric soil samples were analyzed for pH, EC, Organic carbon, available N, available P₂O₅, and available K₂O. The soil samples were low in available nitrogen, low to moderate in available phosphorus and moderate to high in available potassium.

Isolation of *Azospirillum* isolates

All the isolates formed subsurface pellicles in NFBTB medium including the reference strain. The formation of pellicle was below 1-2 mm from the surface of semisolid NFBTB medium. All the *Azospirillum* strains turned olive green colour of Bomothymal Blue (BTB) to brilliant blue. All isolates were microscopically observed for their cell shape and gram reaction. The cell shape of all the isolates was spiral; all the isolates were Gram negative and had cork screw movement when observed under microscope.

Rajyalakshmi *et al.*, (2007) isolated *Azospirillum* from rhizosphere of 30, 45, 60 and 75 days old foxtail millet. Peter and Stenberg (1979) isolated five strains of *A. brasilense* from sorghum root segments. Previous investigations were shown that the *Azospirillum* are very common in temperate, tropical and subtropical regions of the world. *Azospirillum* occur in association with different plants *viz.*, oil seeds, cereals, legumes, spices, vegetables, flower plants and grasses (Gadagi *et al.*, 2002; Vasanth Kumar, 2003; Tejera *et al.*, 2005; Akbari *et al.*, 2007; Sangeeth *et al.*, 2008 and Attitalla *et al.*, 2010; Senthil kumar *et al.*, 2013).

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