J. Soils and Crops 24 (1) 148-153, June, 2014 ISSN 0971-2836

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF *Azotobacter chroococcum* FROM SOILS OF DIFFERENT LOCATIONS OF NAGPUR DISTRICT

V.R.Patil[1], S.R. Potdukhe[2], D.D. Guldekar[3] and A.M. Ghate[4]

ABSTRACT

An investigation entitled "Morphological and Biochemical characterizations of *Azotobacter chroococcum* from soils of different locations of Nagpur district" was under-taken at Plant Pathology Section. College of Agriculture, Nagpur during the year 2012-13. Out of twenty two soil samples of different locations and collected from the rhizosphere region of cultivated field crops only 12 samples could form the colonies of *Azotobacter chroococcum* on the Jensen's medium. Morphological characters viz., gram reaction, cell shape and biochemical characters viz., H2S production, gelatin liquefaction, starch hydrolysis, catalase test, KOH test, phosphate solubilization and IAA production test were carried out. All bacteria were gram -ve and rod shaped. All the tests were +ve except KOH reaction. The study revealed that 1% sucrose salt found better for carbohydrate utilization. As regards antagonist studies, AZ 8 isolate was found significantly superior for control of *Rhizoctonia solani*, AZ 21 isolate for *Sclerotium rolfsil* and AZ 1 for *Fusarium oxysporium* f. sp. ciceri.

(Key words ; A*zotobacter chroococcum*, *Rhizoctonia solani, Sclerotium rolfsil, Fusarium oxysporium* f. sp. ciceri)

INTRODUCTION

Azotobacter spp. are gram negative, aerobic, asymbiotic, free living nitrogen fixing bacteria belongs to family Azotobacteriaceae. Nitrogen fixing bacteria *Azotobacter* was discovered by Beijerinck (1901). It is a pleomorphic often motile (Polar or peritrichus flagella) non spore forming relatively large rods or even yeast like appearance, mesophilic (optimum growth temperature 30^0C), obligate aerobes macrocyst forming, capable of fixing atmospheric nitrogen asymbiotically and widely distributed in soil (Dobereiner, 1961).

Azotobacter chroococcum and *Azotobacter agilis* are studied by Beijerinck (1901). *Azotobacter* release ammonia into the soil. The first period of the research on the genus was marked by studies on its morphological cytological and biochemical characteristics. *Azotobacter chroococcum* happens to be the dominant inhabitant of the rhizosphere.

Azotobacter chroococcum happens to be the dominant inhabitant of the rhizosphere. It benefits plants in multiple ways which includes a) production of indole acetic acid and other auxins such as gibberllins and cytokinins which enhance root growth and aid in nutrient absorption, b) inhibition of phytopathogenic fungi through antifungal substances and c) production of siderophores which solubilize Fe*** and suppress plant pathogens through iron deprivation. (Mali and Bodhankar, 2009). In the local soils, *Azotobacter* fixes annually about 60 - 90 kg N ha^{-1} and it may be used in crop production as a substitute for absorption of mineral nitrogen fertilizers. Inoculation with *Azotobacter* increased the yield by 5-28% of wheat crops. (Milosevic *et al*., 2012). Considering the importance of *Azotobacter chroococcum* in atmospheric nitrogen fixation, a study was undertaken to derive its morphological and biochemical characters from the soils of different locations of Nagpur district.

MATERIALS AND METHODS

Collection of soil samples for isolation of *Azotobacter chroococcum*

Collection of soil samples :

In the present study, twenty two soil samples were collected from rhizosphere of cultivated field of different locations of Nagpur district (Table 1) and were processed in the laboratory for isolation. These soil samples include five each from maize and jowar, nine from cotton, two from bajra and one from rice respectively. For each soil sample collection code have been given as AZ 1 to AZ 22.

Isolation of *Azotobacter chroococcum* :

The *Azotobacter chroococcum* was isolated from soil sample collected from rhizosphere of

1 and 4. P.G. Students, Plant Pathology section college of Agriculture, Nagpur
2. Assoc. Professor, Plant Pathology Section, College of Agriculture, Nagpur
3. Asstt. Professor, Plant Pathology Section, College of Agriculture, Nagpur

Table 1. Collection of *Azotobacter chroococcum* from different locations

Sr. No.	Name of cultivator(s)	Location	Crop name	Isolation code
1.	Manoj Chalakh	Kanolibara	Maize	AZ 1
2.	Pravin Shelote	Kolar	Cotton	AZ 2
3.	Vinod Pise	Vyahad	Bajra	AZ 3
4.	Vasantrao Bahurupe	Devali	Maize	AZ 4
5.	Ramkrishna Lende	Sindhkheda	Cotton	AZ 5
6.	Shard Dofe	Ghanikhapari	Jowar	AZ 6
7.	Lomeshwar Balpande	Ladgaon	Maize	AZ 7
8.	Shripatrao Balpande	Ladgaon	Cotton	AZ 8
9.	Prabhakar Marotkar	Mohpa	Jowar	AZ 9
10.	Manohar Marotkar	Mohpa	Cotton	AZ 10
11.	Kishor Dakre	Khapari	Jowar	AZ 11
12.	Kiran Nimbalkar	Burijwada	Cotton	AZ 12
13.	College of Agriculture farm Nagpur	Nagpur (Urban)	Maize	AZ 13
14.	CICR,Nagpur	Nagpur (urban)	Cotton	AZ 14
15.	Divalu Durugvar	Sonegaon	Cotton	AZ 15
16.	Hemraj Nakhede	Gundri	Jowar	AZ 16
17.	Krishna Badule	Mushewadi	Cotton	AZ 17
18.	Subhash Parteti	Mauda	Rice	AZ 18
19.	Sudhakar Patiye	Mauda	Jowar	AZ 19
20.	Harish Pawnikar	Kalmna	Maize	AZ 20
21.	Sanjay Bhutmange	Dongarmauda	Cotton	AZ 21
22.	Jagan Bhutmange	Dongarmauda	Bajra	AZ 22

cultivated different fields by serial dilution method (Mostara *et al*., 1988). For isolation of *Azotobacter chroococcum* Jensen's nitrogen free media (Sucrose 20g, $K_2HPO_4$1.0g. $MgSO_4.7H_2O$ 0.5g, CaCO 2.0g, NaCI 0.5g, $FeSO_47H_2O$ 0.1g, Agar 15g. Distilled water 1 liter, pH7) was used. The inoculated plates were incubated at 28±2^{0}C for 3 days. The Colonies were developed and transferred in Jensen's medium slants and the pure culture so obtained was stored in BOD incubator at 4^{0}C for further investigation.

Morphological and biochemical characters of *Azotobacter chroococcum* :

Isolated from rhizopheric soils were identified based on morphological and biochemical test. All the isolates were tested for their shape, gram reaction, H_2S production, gelatin liquefaction, starch hydrolysis, catalase activity, KOH test, phosphate solubilisation and IAA production test was carried out as per standard methods (Salle, 1967).

Screening :

The isolate of *Azotobacter chroococcum* was subjected to different sugar concentrations which influence the 'N' content (Kalaygandhi *et al*., 2010). Hence, the strains were grown in varying sugar (sucrose) concentrations like 1,2,3 and 4 per cent and influence of sugar was recorded with Burk's broth for estimating nitrogen in 1 per cent sugar of Burk's broth by using kjelhdahi method (Bermner, 1960).

In Vitro antibiosis :

After screening high nitrogen fixing *Azotobacter chroococcum*, isolates were selected and studied for their antagonistic ability against three soil borne plant pathogens i.e. *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsil*. The bacterial isolates were screened by dual culture test as followed by Morton and Stroube, 1955. In petriplates containing 20 ml PDA medium (without antibiotics) and loopful of fresh bacterial culture was streaked at sides and fungal mycelial disk at center towards the edge of petriplates (Kapoor and Kar, 1989) and petriplates were incubated at 28±2^{0}C for 7 days. The per cent inhibition of test fungi with each bacterial isolate was calculated. The per cent growth inhibition was calculated using following formula (Vincent, 1947).

$$I = \frac{C - T}{C} \times 100$$

Where
I= Per cent inhibition
C= Growth of fungus in control (mm)
T = Growth of fungus in treatment (mm)

RESULTS AND DISCUSSION

Isolation of *Azotobacter chroococcum* :

After three days of incubation, milky whitish to brown or black colonies were obtained on Jensen's agar medium which were later picked and streaked on fresh Jensen's agar medium for pur culture and used for investigation. Out of twenty two, twelve samples were used for isolation only, because twelve samples could produce *Azotobacter chroococcum* colonies and designated as AZ1, AZ3, AZ 4, AZ 5, AZ 6, AZ 8, AZ 9, AZ 12, AZ 13, AZ 18, AZ 21 and AZ 22 respectively.

Morphological and biochemical test of *Azotobacter chroococcum* isolates :

It can be seen from table 2 that all the isolates were rod shaped and gram negative in reaction. The entire isolates exhibited positive test for H_2S production, gelatin liquefaction, starch hydrolysis, catalase test, phosphate solubilization and Indole acetic acid prodction while all the isolates showed negative test for KOH test. The isolates responded to the gram negative and were rods that produced smooth circular colonies and with brownish to blackish pigment production. This confirmed isolates as *Azotobacter chroococcum*. The biochemical tests i.e. test and indole acetic acid production test further confirmed to be *Azotobacter chroococcum*. These morphological and biochemical characters are similar with the earlier report of Ahamad *et al*. (2005) who reported that *Azotobacter* had +ve test namely gram raction, IAA production, H_2S production, catalase test and starch utilization test. Dhamangaonkar (2009) reported the potential of *Azotobacter* produce IAA and also noted +ve effect of phosphate solubilisation activity.

Screening of *Azotobacter chroococcum* isolates :

The per cent nitrogen fixed by all these isolates was determined by Micro-kjeldhal method.

These isolates were categorized into the following segment and the results are interpreted in table 3.

1. **Highly efficient :** Four isolates (AZ1, AZ 8, AZ 21 and AZ 22) having more than 0.190 mg nitrogen fixed 10 g^{-1} (1%) of sucrose consumed.
2. **Moderately efficient :** Four isolates (AZ3, AZ 12, AZ 13 and AZ 18) having 0.120 mg to 0.190 mg nitrogen fixed 10 g^{-1} (1%) of sucrose consumed.
3. **Less efficient :** Four isolates (AZ 4, AZ 5, AZ 6 and AZ 9) having less than 0.120 mg nitrogen fixed 10 g^{-1} (1%) sucrose consumed.

The data presented in table 3 indicate that there were significant differences due to various isolates on 1% sucrose solution over uninoculated control. The results were ranging from 0.046% to 0.224% N fixed mg 1% sucrose^{-1}. Out of 12 isolates, isolate no AZ 21 fixe the highest amount of N fixed 0.224 mg sucrose^{-1} concentration and was found significantly superior as compared to all other isolates except AZ 8 (0.218 N fixed mg 10 g^{-1} sucrose). However, in control it was 0.037 N fixed mg 10 g^{-1} sucrose. Thus they were categorized into highly efficient isolates 0.190 N fixed mg 10 g^{-1} sucrose (four isolates) and less efficient isolate fixed N less than 0.120 N fixed mg 10 g^{-1} sucrose (four isolates).

Effect of *Azotobacter chroococcum* on growth of *Fusarium oxysporum* f. sp. *ciceri, Sclerotium rolfsii* and *Rhizoctonia solani* at different duration *in Vitro* :

Observations on average colony diameter at 7th DAI and per cent growth inhibition were recorded. All isolates under the test showed their potentiality to check the mycelial growth of all three pathogens i.e. *Fusarium oxysporum, Sclerotium rolfsii* and *Rhizoctonia solani*.

The data presented in table 4 indicate that there were significant differences in radial mycelial growth due to various isolates over uninoculated control. Minimum radial mycelial growth was recorded by the isolate AZ 8 (68.60 mm) and it was at par with all the isolates followed by AZ 22 (70.00 mm) with per cent inhibition of 23.77 and 22.22 % on 7th DAI respectively. All the four isolates produced a

Table 2. Morphological and biochemical test of Azotobacter chroococcum isolates

Characters	Reaction of isolates											
	AZ1	AZ3	AZ4	AZ5	AZ6	AZ8	AZ9	AZ12	AZ13	AZ18	AZ21	AZ22
Morphological properties												
Gram reaction cell shape	-ve Rod	-ve Rod	-ve Rod	-ve Rod	-ve Rod	-ve Rod	-ve Rod	-ve Rod	-ve Rod	-ve Rod	-ve Rod	-ve Rod
Biochemical properties												
H_2S Production	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+
KOH test	-	-	-	-	-	-	-	-	-	-	-	-
Indole acetic acid production	+	+	+	+	+	+	+	+	+	+	+	+
Phosphate solubilization	+	+	+	+	+	+	+	+	+	+	+	+

'+' indicate positive test. '-' indicate negative test.

Table 3. Nitrogen fixing capacity by *Azotobacter chroococcum* isolate

Sr. No.	Number of strain isolate	N fixed (mg $sucrose^{-1}$ conc.)
1	AZ 1	0.196
2	AZ 3	0.158
3	AZ 4	0.084
4	AZ 5	0.112
5	AZ 6	0.046
6	AZ 8	0.218
7	AZ 9	0.065
8	AZ 12	0.130
9	AZ 13	0.121
10	AZ 18	0.121
11	AZ 21	0.224
12	AZ 22	0.212
13	Control	0.037
	F Test	Sig.
	SE±(m)	0.015
	CD(P=0.05)	0.043

Table 4. Antifungal activity of *Azotobacter chrococcum* against *Fusarium oxysporum*, *Sclerotium rolfsii* and *Rhizoctonia solani*.

Sr. No.	*Azotobacter chrococcum* isolates	Radial mycelial growth (mm) DAI			Growth inhibition (%) DAI		
		Fusarium	*Sclerotium*	*Rhizoctonia*	*Fusarium*	*Sclerotium*	*Rhizoctonia*
1	AZ 1	56.0	66.6	74.6	37.77	26.00	17.11
2	AZ 8	57.6	67.0	68.6	36.00	25.55	23.77
3	AZ 21	57.3	65.3	71.3	36.33	27.44	20.77
4	AZ 22	56.3	66.3	70.0	37.44	26.33	22.22
5	Control	90.0	90.0	90.0	-	-	-
	F Test	Sig.	Sig.	Sig.			
	SE±(m)	0.578	0.495	0.60			
	CD (P=0.05)	1.174	1.490	1.820			

antifungai compounds which might have inhibited the growth of *R. solani*. These results are in line with the report of Maiyappan *et al.* (2010), who reported 84.94% inhibition with *Azotobacter* strain 4. Then *Azotobacter* 1 and *Azotobacter* 3 were the next best strains showing 65,72% and 54.45% inhibition, respectively.

Similarly the antifungal activity of *Azotobacter chrococcum* was tested against *Sclerotium rolfsii* and the data are presented in table 4. The observations were recorded at various interval i.e. 3,5 and 7th DAI. It was revealed from the data that there were significant differences at all the intervals. Minimum radial mycelial growth was recorded by the isolate AZ 21 (65.30 mm) followed by AZ 22 (66.30 mm) with higher per cent inhibition of 27.44% and 26.33% respectively. All the isolates were at par with each other. AZ 8 showed less per cent growth inhibition (25.55%) at 7th DAI. These observations are in agreement with the findings of earlier workers Maareg *et al*. (2003), who reported that the substaintial control of *Sclerotium rolfsii* by microbin was observed on clay loam soils (74%) and also Maiyappan *et al*. (2010) found that the *Azotobacter* 7 strain recorded maximum inhibition against (54.04%) followed by *Azotobacter* strain 6 with (43.18%).

Also data in table 4 indicate significant differences at 3, 5 and 7th DAI on radial mycelia growth of *F. Oxysporum* f. sp. *ciceri* due to *Azotobacter chrococcum* isolates. Minimum radial mycelial growth was recorded by AZ 1 isolates (56.00 mm) followed by AZ 22 isolate (56.30 mm) with maximum per cent inhibition 37.77 and 37.44 % respectively at 7th DAI. This may be due to the antibiotic production of *Azotobacter chroococcum* in the medium. The observations recorded in the present investigation are in conformity with the findings of Maiyappan *et al*. (2010), who found that the *F. oxysporium* growth was effectively minimized by *Azotobacter* strain 5 (49.84%) followed by *Azotobacter* strain 9 (49.04%). Kapoor and Kar (1989) reported the antagonistic activity of *Azotobacter chroococcum* against *Fusarium oxysporium*. The *Azotobacter chroococcum* strains showing inhibition by 11.7 to 24.7 per cent after 7 days. The results showed that the *Azotobacter chrococcum* can play an important role in biocontrol of soil borne diseases of rhizosphere.

REFERENCES

Ahamad, F.I. Ahamad and M.S. Khan, 2005. Indole acetic acid production by the indiginious isolate of *Azotobacter* and *Fluorescent Pseudomonas* in the presence and absence of tryptophan. Turk. J. Biol. **29** : 29-34.

Beijerinck, M.W. 1901. Uberologo nitrophilemikroben. Zentralbl, Bakteriol, Parasitenkd. Infektionskr. II Abt. pp. 561-582.

Bremner, J.M. 1960. Determination of nitrogen in soil by the Kjeldahi method. J. agric. Sci. **55**: 11-13.

Dhamangaonkar, S.N. 2009. Effect of *Azotobacter chroococcum* (PGPR) on the growth of Bamboo (Bambusa bamboo) and Maize (*Zea mays*) plants. Biofrontiers. **1**: 24-31.

Dobereiner, J. 1961. Nitogen fixing of bacteria of the genus Beijerinckia Derx. Inrhizosphere of sugarcane. Plant and Soil. **15** (3): 211-216.

Kalaigandhi V.E., Kannapiran, Harimuraleedharan, A. Michael, T. Shivkumar and Thirumalai, 2010. *Azotobacter* population in rhizosphere and non rhizosphere sediment of Tondi coast. Int. J. Biol. Tech. **1** (1): 63-65.

Kapoor, I. J. and B. Kar, 1889. Of *Azotobacter*, Bacillus and *Fusarium oxysporium* f. sp. lycoperisici. Indian Phytopath. **42** (3): 400-409.

Maareg, M.F., M.A. Hassanein and MY Hussein, 2003. Bacterial fertilizer as biological control agent for damping off disease for sugarbeet. Ann Agric. Sci. Moshtohor. **41** (1): 95-101.

Maiyappan, S., E. Leo D. Amalraj, A. Santosh and A. John Peter, 2010. Isolation, evaluation and formulation of selected microbial consortia for sustainable agriculture. Biofertilizer and Biopesticide. **2** (2): 109-115.

Mali, G.V. and M.G. Bodhankar, 2009. Antifungal and phytoharmone production potential of *Azotobacter chroococcum* isolate from groundnut (*Arachis hypogea* L.) rhizosphere. Asian J. Exp. Sci. **23** (1): 293-297.

Milosevic, N.B., R.P. Tintor, G. Civjanovic and T. Dimitrijevic, 2012. Effect of inoculation with *Azotobacter chroococcum* of wheat yield and seed quality. Romanian biotech, letters. **17** (3): 7321-7357.

Morton, D.J. and W.H. Stroube, 1955. Antagonistic and stimulatory effect of soil microorganism upon *Sclerotium.* Phytopath. **45** : 417-420.

Mostara, M.R. 1988. Biofertilizer technology. Marketing and uses, ICAR, New Delhi. pp. 40-60.

Salle, A.J. 1967. Laboratory manual of fundamental principles of Bacteriology McGraw Hill Book Co. New York.

Vincent, J.M. 1947. Distortion of fungal hyphae in the presence of certain inhibiters. Nature pp. 150 : 850.

Rec. on 01.06.2013 & Acc. on 05.09.2013