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A Comparative Study of *Azotobacter Spp.* From Various Localities of Beed District (M.S.)

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ABSTRACT

Present investigation was carried out to study *Azotobacter*spp. Collectedfrom Rhizosphere soil samples of various crops from Beed district (M.S.). Twenty two Rhizosphere soil samples were collected from various localities of the district and soil testing was done to determine soil pH, water holding capacity and moisture content. After isolation of the bacteria from Rhizosphere, biochemical tests were performed to study their characters. Bacterial strains collected from various locations were named as Strain 1 to Strain 22. Out of these strains 3, 7, 9, 11, 19 and 21 did not show characters identical with *Azotobacter;* while other strains resembled *Azotobacter*spp. Cell size of *Azotobacter* ranged in between 1.05 to 2.03 µm. Colonies were creamy white, circular, small to large sized, opaque with smooth margins. Biochemical tests confirmed responses to citrate, starch hydrolysis, catalase, indole production, and urease and nitrate reduction. *Azotobacter*is widely used as bio-inoculants for wide variety of crops, and hence, it's morphological and biochemical study of *Azotobacter*spp.will be useful for further research.

Key Words: *Azotobacter*spp., Biochemical test, Beed district, Rhizosphere.

Introduction

*Azotobacter*is plant growth-promoting rhizobacteria (PGPR) have significant influence on soil physiological and structural properties. It is commonly found in soil and can fix nitrogen directly from the atmosphere. *Azotobacter*is sensitive to acidic pH, high phosphatecontent in the soil and high temperature(Above 35ºC, Islam*et al*., 2008). Thisfree living, non-symbiotic,

heterotrophic bacteria iscapable of fixing, on an average, 20 kg N/ha per year (Jnawali*et al.*, 2015) is Plant Growth Promoting Rhizo-bacteria (PGPR) which can be used as alternative fertilizer to chemical fertilizer. Nitrogen fixation is the process that is oxygen sensitive due to the O_2 liability of the nitrogenase component proteins. However, in *Azotobacter* spp. N_2 fixation can occur under fully aerobic condition in all species (Brenner *et al.*, 2005). *Bageshwaret al.*(2017) observed that *A. chroococcum*inoculationenhanceswheat grain yield by 60% in the absence of any urea application.

Beed district of Maharashtra has rocky and thin layered soils in major part of the district except on the banks of Godavari and Sindphana Rivers. Dark brown to black and clayey loamy to loamy soils are observed in Godavari banks. Wide range of crops arecultivated in the district. During the present investigation *Azotobacter*spp.were collectedfrom Rhizosphere of various cropsand studied.

Material and Methods:

Rhizosphere samples were collected from twenty two different location of Beed districtin sterile zip locked plastic bags from the depth of 10-15 cm. (Table 1)Those samples were brought into the laboratory and kept at 4°C for further experimental work(Sadik*et al.*, 2016). Soil pH was determined on pH meter, using glass electrode, while moisture and water holding capacity was determined as described by Kalra(1995).One gm. soil was suspended in 10 ml distilled water; soil suspension was inoculated on specific AzotobacterManitol Agar (HiMedia) media and

incubated at 25±2ºC for 48 Hrs. Isolation was done by using nitrogen free specific AzotobacterManitol Agar (HiMedia) media which allow only growth of *Azotobacter* spp. Confirmation of the bacteria was done by relevant biochemical tests were performed along with motility and Gram-staining Phalke*et al.,* (2017). Growth of colonies was observed for 3-5 days. Small, glistening colonies were observed. Nature of bacterial colony, its morphology, Gram's staining and biochemical tests were performed as described by Phalke*et al.,* (2017).Cultures were preserved at -20 ^{0}C in 20% of glycerol solution for further studies.

Results and Discussion:

The values of soil pH, water holding capacity and moisture content are presented in Table1. On an average pH of the soil ranged from 5.4 to 6.7, indicating its acidic nature. Water holding capacity fluctuated between 31.27 and 49.75%, while moisture from 29.80 to 46.37, showing wide variability. Maximum soil pH was recordedatChikhali (6.7) while minimum at Songaon

(5.4). Maximum water holding capacity and moisture percentage was recorded in the sample collected from Kesapuri.Thecells of *Azotobacter* were gram negative, rod shaped and found abundant in Rhizosphere. Cell size from1.05 to 2.03 µm. The colonies were creamy white, circular, small to large sized, opaque with smooth margins. Out of the22 strains collected, six were not identical with *Azotobacter*. Biochemical tests have been performed for the detailed study. Similar results oncell morphology and colony characters of*Azotobacter* spp.have been reported by Islam *et al.*,(2008); Rahmi*et al.,* (2015) and; Roy Chowdhury*et al.*,(2017).

References

Brenner, D.J., N.R. Krieg, J.T. Staley and G.M. Garrity**(2005)** Bergry's manual of systematic Bacteriology (Eds. Staley, J.T, D.R. Boone, D.J. Brenner, D.V. Paul, G.M. Garrity, M. Goodfellow, N.R. Krieg, F.A. Rainey and K.H. Schleifer),*Springer Publication, East Lansing*, USA, **2**(2): 384-402.

Bageshwar, U.K., M. Srivastava,P.P.Sardhi, S. Paul, S. Gothepandapani, R.S.Jaat, P. Shankar, R. Yadav, D.R. Biswas, P.A. Kumar,J.C.Padaria, P.K. Mandal, K. Annapurna and H.K. Das**(2017)** An environment friendly engineered Azotobacter can replace substantial amount of urea fertilizer and yet sustain same wheat yield Appl. *Environ. Microbiol*. doi:10.1128/AEM.00590-17

Islam, M.Z., D.I. Sharif and M.A. Hossain **(2008).**A Comparative Study of Azotobacter spp. from Different Soil Samples.*Journal of soil and Nature*, **2**(3): 16.

Jnawali, A.D., R.B. Ojha and S. Marahatta**(2015)**.Role of Azotobacter in Soil Fertility and Sustainability–A Review.*Advances in Plants & Agriculture Research*, **2**(6): 1.

Kalra, Y.P. **(1995)**. Determination of pH of Soils by Different Methods: Collaborative Study. *Journal of AOAC International*, **78**(2): 310.

Phalke, V.S., B.T. Pawar and R.M. Gulve**(2017)**. Halophilic bacteria in salt stressed soils of Aurangabad district (MS) India. *Bioscience Discovery*, **8**(1): 58.

Rahmi, Baharudin, Nasaruddin, and Burhanuddin**(2015)**. Morphological Characteristics and Ability Isolate of Azotobacter sp. To Produce IAA Origin from Cocoa Rhizosphere International *Journal of Current Microbiology and Applied Science*, **4**(7): 593.

Roychowdhury, D., M. Paul and S.K. Banerjee **(2017)**.Isolation Identification and Partial

Characterization of Nitrogen Fixing Bacteria from Soil and Then the Production of Bio fertilizer*International Journal for Research in Applied Science and Engineering Technology*, **5**(11):4021.

Sadik, A.S., N.A. El-Khamash and S.H. Mohamed **(2016)**.Isolation and characterization of free-nitrogen fixer bacterial Strains (*Azotobacter* spp.) And their phages from maize rhizosphere-soil at Taif. *Pak. J. Biotechnology*, **13**(1): 31.

Table 1: Collection of Rhizosphere Soilsamples.

Sr. No.	Location	pH	Water Holding capacity in Percentage	Moisture percentage	Botanical name of the crop (Vernacular Name)
01	Kumbhephal Tq. Ambajogai Dist. Beed	5.9	37.24	32.85	*Gossypiumherbaceum* (Cotton)
02	Anjanpur Tq. Ambajogai Dist. Beed	6.1	36.48	33.52	*Sorghum bicolor* (Sorghum)
03	Chikhali Tq. Ashsti Dist. Beed	6.7	31.99	30.25	*Triticumastivum (*Wheat*)*
04	Dhanora Tq. Ashsti Dist. Beed	6.5	42.18	39.27	*Gossypiumherbaceum* (Cotton)
05	Adas Tq. Kej Dist. Beed	5.9	32.35	34.82	*Gossypiumherbaceum* (Cotton)
06	Kej Tq. Kej Dist. Beed	6.0	34.40	30.70	*Glycine max*(Soybean)

07	Antarwali Tq. Georai Dist. Beed	6.2	41.37	38.44	*Glycine max* (Soybean)
08	Khamgaon Tq. Georai Dist. Beed	6.1	36.55	32.72	*Gossypiumherbaceum* (Cotton)
09	Ambewadgaon Tq. Dharur Dist. Beed	6.0	40.25	34.62	*Sorghum bicolor* (Sorghum))
10	Telgaon Tq. Dharur Dist. Beed	5.9	35.80	32.54	*Gossypiumherbaceum* (Cotton)
11	Bodhegaon Tq. Parali Dist. Beed	6.1	31.27	31.42	*Glycine max* (Soybean)
12	Parali Tq. Parali Dist. Beed	6.4	42.55	38.99	*Gossypiumherbaceum* (Cotton)
13	Antapur Tq. Patoda Dist. Beed	6.3	36.25	30.20	*Carthamustinctorius (*Safflower*)*
14	Karegaon Tq. Patoda Dist. Beed	5.8	40.00	38.50	*Pennisetumglaucum*(Bajara)
15	Songaon Tq. Beed Dist. Beed	5.4	37.05	33.50	*Glycine max* (Soybean)
16	Neknur Tq. Beed Dist. Beed	6.3	39.20	35.60	*Carthamustinctorius (*Safflower*)*
17	Kesapuri Tq. Majalgaon Dist. Beed	6.1	42.90	40.80	*Glycine max* (Soybean)
18	Takarwan Tq. Majalgaon Dist. Bee	6.4	49.75	46.37	*Gossypiumherbaceum* (Cotton)
19	Chinchwadgaon Tq. Wadawni Dist. Beed	5.8	32.80	29.80	*Glycine max* (Soybean)

20	Wadawni Tq. Wadawni Dist. Beed	5.7	33.00	32.20	*Gossypiumherbaceum* (Cotton)
21	Anandgaon Tq. Shirur Dist. Beed	6.0	34.50	30.70	*Carthamustinctorius (*Safflower*)*
22	Khopati Tq. Shirur Dist. Beed	5.9	35.30	31.20	*Glycine max* (Soybean)

Table 2: Morphological characters of Bacterial strains collected from *Rhizosphere.*

Strain	Cell Morphology			Colony Morphology				
	Gram's Staining	Cell Size	Cell Shape	Color	Shape	Size	Appearance	Margins
Strain 1	-ve	1.05 to 2.03µm	Rod	Dull White	Spherical	Medium	Slimy	Smooth
Strain 2	-ve	1.05 to 1.80µm	Large Rod	Creamy White	Oval	Large	Opaque	Smooth
Strain 3	+ve	4µm	Rod	Slightly yellowish	Irregular	Medium	Opaque	Rough
Strain 4	-ve	1.40 to 1.90µm	Large Rod	Dull White	Spherical	Medium to Large	Slimy	Smooth
Strain 5	-ve	1.09 to 1.75µm	Large Rod	White	Oval	Medium	Opaque	Smooth
Strain 6	-ve	1.11 to 1.90µm	Rod	Creamy White	Spherical	Large	Slimy	Smooth
Strain 7	+ve	1.15µm	Rod	Creamy White	Circular	Medium	Opaque	Smooth
Strain 8	-ve	1.07 to 1.80µm	Large Rod	White	Spherical	Medium	Slimy	Smooth
Strain 9	+ve	1.10 to 1.90 µm	Large Rod	Milky White	Spherical	Small	Opaque	Smooth

Strain 10	-ve	1.05 to 2.0µm	Rod	White	Spherical	Medium	Opaque	Smooth
Strain 11	+ ve	4µm	Rod	Cream to pale orange	Circular	Large	Opaque	Rough
Strain 12	-ve	1.10 to 1.90 µm	Rod	White	Spherical	Small	Slimy	Smooth
Strain 13	-ve	1.40 to 1.90µm	Large Rod	Dull White	Spherical	Medium to Large	Slimy	Smooth
Strain 14	-ve	1.10 to 1.90 µm	Rod	White	Spherical	Small	Slimy	Smooth
Strain 15	-ve	1.40 to 1.90µm	Large Rod	Dull White	Spherical	Medium to Large	Slimy	Smooth
Strain 16	-ve	1.07 to 1.80µm	Large Rod	White	Spherical	Medium	Slimy	Smooth
Strain 17	-ve	1.10 to 1.90 µm	Rod	White	Spherical	Small	Slimy	Smooth
Strain 18	-ve	1.40 to 1.90µm	Large Rod	Dull White	Spherical	Medium to Large	Slimy	Smooth
Strain 19	+ ve	4µm	Rod	Cream to pale orange	Circular	Large	Opaque	Rough
Strain 20	-ve	1.07 to 1.80µm	Large Rod	White	Spherical	Medium	Slimy	Smooth
Strain 21	+ve	3.08µm	Rod	Cream to pale orange	Circular	Large	Opaque	Rough
Strain 22	-ve	1.40 to 1.90µm	Large Rod	Dull White	Spherical	Medium to Large	Slimy	Smooth