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Report of *Acetobacter diazotrophicus* in sugarcane collected from Shrirampur, Ahemednagar

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Abstract
Ahmednagar district is largest district in Maharashtra occupying 17048 sq. km area. Shrirampur taluka is located in the northern part of Ahmednagar district covering 579.87 sq. km. Nitrogen fixation is an important process in plants providing nitrogen as the most valuable macronutrient required by the plants. *Acetobacter diazotrophicus* as an endophytic nitrogen fixing bacteria is collected from Shrirampur taluka of Ahmednagar district (M.S). Total 12 rhizospheric samples were collected from various localities of the taluka and soil testing was done to determine pH, Water holding capacity and moisture percentage. After isolation of bacteria from soil sample, cell morphology and colony morphology was studied. With the help of special media and morphological characters preliminary identification of *A. diazotrophicus* was done. Out of these 12 bacterial strains 4 and 7 did not show characters identical with *A. diazotrophicus;* while other strains resembled *A. diazotrophicus.* Cell size of *A. diazotrophicus* was ranging between 1.04 to 2.00 µm. Colonies were creamy white, cicular, small to large sized, opaque with smooth margins. *A. diazotrophicus* is able to increase nutrient supply, soil fertility and crop growth of sugarcane. The study of *A. diazotrophicus* will be useful for further researchers and it will be better alternative for chemical fertilizers.

Keywords: *Acetobacter diazotrophicus,* sugarcane, shrirampur, Ahmedangar

Introduction

Ahmednagar district is largest district in Maharashtra state occupying 17048 sq. km area. Shrirampur tahsil is located in the northern part of Ahmednagar district covering 579.87 sq. km extended between 19° 45 to 20° 30 N latitudes and 74°00 to 74°30 E longitudes. The tahsil is having sub-tropical monsoon climate. The temperature recorded in the tahsil ranges between 44°C to 8°C (Cholke, 2015) [4]. Gulve and Gadekar (2022) [6] studied watershed development programme in Ahmednagar district. Nitrogen fixation is an important process in plants providing Nitrogen as the most valuable macronutrient required by the plant. Crop rotation with legumes has been recognized to increase soil fertility and agricultural productivity (Cheng, 2008) [3]. Santi1 *et al.,* (2013) [15] studied biological nitrogen fixation in non-legume plants. Endophytic bacteria can also influence plant growth & productivity through Nitrogen fixation. Conceptually, plant growth promoting endophytic bacteria may affect plant growth either directly or indirectly (Sansanwal *et al.,* 2017) [14]. Large and diverse populations of N2-fixing bacteria are associated with sugarcane.

Endophytic bacteria establish in between and within the spaces of all plant parts and not causing any plant disease. They create array of relationship include mutualism, cannibalistic, commensalistic and trophobiotic in nature. Endophytic bacteria play a major role in developing plant growth enhancement, phytoremediation, phosphate solubilization, nitrogen fixation, modulation of plant metabolism and phytohormone signaling. There is an increased interest in the use of endophytes for their agricultural applications that promote plant growth under cold, drought or contaminated soil structure conditions or induce disease resistance in plants (Muthukumar *et al.,* 2018) [10]. Endophytic bacteria are alternative to agrochemicals (fertilizers and pesticides) in developing environment friendly agriculture (Adeleke and Babolola, 2021) [1]. Therefore, endophytic bacteria play an important role in microbial ecology, associating environmental factors, and their roles that contribute to their effectiveness in promoting plant growth for maximum agricultural crop productivity was highlighted (Adeleke and Babolola 2021) [1].

A. diazotrophicus is a nitrogen-fixing endophytic bacterium, originally isolated from sugarcane. Its colonizing ability was evaluated in field of agriculture to promote the growth and development of crop plant. A preliminary study regarding contributions of the bacterial endophyte *A. diazotrophicus* to sugarcane nutrition was reported by Sevilla *et al.,* (1998) [16]. *A. diazotrophicus* was found mainly inside cortical cells of stems and inside xylem vessels. No L-glucuronidase activity was observed in non-inoculated plants. *A. diazotrophicus* is able to increase nutrient supply, soil fertility and crop growth of sugarcane. The study of *A. diazotrophicus* will be useful for further researchers and it will be better alternative for chemical fertilizers. Hence during the present investigation Report of *Acetobacter diazotrophicus* was collected from Sugarcane from Shrirampur taluka of Ahemednagar district.

Materials & methods

a. Collection of bacterial samples

Rhizosphere samples were collected from 12 different locations of Shrirampur taluka of Ahmednagar district in sterile zipped locked polythene bags. Those samples were brought to the laboratory and kept at $4^{O}C$ for further investigations. Soil pH was calculated using pH meter, while moisture percentage and Water Holding Capacity (WHC) was determined as described (Kalra, 1995) [8].

b. Isolation of bacterial samples

One gram of soil was suspended in 10 ml distilled water to prepare soil suspension. It was inoculated on specific *Acetobater* manitol agar media (HiMedia) and incubated at 25±2^{O}C for 48 Hrs. which allow only the growth of *Acetobacter diazotrophicus*.

c. Morphological characterization

Confirmation of the bacteria was done by relevant morphological characterization (Phalke *et al*, 2017) [11]. Growth of colonies was observed after 48 Hrs. Morphology characterization of bacterial cell was studied in respect to cell size, shape and gram staining. While Colony morphology was studied in respect to color, shape, size, appearance and colony margins on the special culture media as described by Phalke *et al*, (2017) [11]. Cultures were preserved at 20^{O}C for further studies.

Results & discussion

The value of soil pH, WHC & moisture percentage are presented in Table 01. An average pH of soil samples collected from the study area was ranging between 5.8 to 6.6 indicating acidic nature of the soil, WHC was observed between 32.70 to 42.15%; while moisture percentage from 31.66 to 47.40%. Maximum soil pH was recorded at Bhokar village (6.6) while minimum at Undirgaon (5.8). Maximum water holding capacity and moisture percentage was recorded in the sample collected from Wadala-Mahadeo (42.15%).

Overall average pH of all samples is 6.2. Out of 12 localities, 8 localities *viz.* Malwadgaon (6.2), Malewadi (6.2), Wadala-Mahadeo (6.2), Kamalpur (6.2), Bhamathan (6.3), Bramhangaon-Vetal (6.4), Khanapur (6.5) and Bhokar (6.6) showed high pH than that of the average pH of all samples and 4 localities showed less pH than the average pH. The average WHC of all samples is 36.23; out of which 5 soil samples showed high WHC than the average WHC and 7 samples showed less WHC than the average WHC. Average moisture percentage of the soil samples is 38.85%; out of which 5 samples showed high moisture percentage and 7 samples showed less.

Table 1: Localities selected for collection of sugarcane rhizosphere sample.

Sample Code No.	Location	Soil Type	pH	Water holding capacity (WHC) in Percentage	Moisture percentage
SH01	Khanapur	Black	6.5	37.40	47.40
SH02	Malwadgaon	Black	6.2	36.50	38.50
SH03	Bhamathan	Black	6.3	34.60	37.80
SH04	Bhokar	Black	6.6	35.48	39.70
SH05	Taklibhan	Black	6.1	38.70	41.50
SH06	Wadala-mahadeo	Black	6.2	40.20	44.20
SH07	Bramhangaon-Vetal	Black	6.4	42.15	36.50
SH08	Kamalpur	Black	6.2	39.50	34.54
SH09	Muthewadgaon	Black	5.9	32.70	36.42
SH10	Malewadi	Black	6.2	31.20	38.63
SH11	Undirgaon	Black	5.8	33.50	31.66
SH12	Shirasgaon	Black	6.1	32.80	39.37
Average			6.2	36.23	38.85

Morphological details of the bacterial samples are presented in Table 02. Morphologically bacterial strains *viz.* Strain 01, Strain 02, Strain 04, Strain 05, Strain 06, Strain 08, Strain 09, Strain 10 and Strain 12 were identical showing similar morphological characters. These strains are grouped as Group-I. While Strain 03, Strain 07 and Strain 11 were different from one another and they are grouped in Group-II. Bacterial cells of Group-I were gram negative. While Bacterial cell of Group-II were Gram positive in staining. The cell size of Group-I varies from 1.55 µm to 1.83 µm. Group-II cell size was larger than that of Group-I which was varying between 2.27 µm to 2.38 µm. All the bacterial strains of Group-I was rod shaped but in Group-II, Strain 3 and Strain 7 was large rod shaped while bacterial cell of Strain 11 was coccus in shape.

Table 2: Morphological characters of nitrogen fixing endophytic bacterial strains collected from rhizosphere

Strain	Cell Morphology			Colony Morphology				
	Gram Staining	Cell Size (Avg.)	Cell Shape	Color	Shape	Size (Avg.)	Appearance	Margins
Strain 01	-ve	1.65 µm	Rod	Creamy White	Circular	1.25 mm	Glistering	Entire
Strain 02	-ve	1.70 µm	Rod	Creamy White	Circular	1.32 mm	Glistering	Entire
Strain 03	+ve	2.38 µm	Large rod	Yellow	Irregular	1.54 mm	Opaque	Rough
Strain 04	-ve	1.75 µm	Rod	Creamy White	Circular	1.27 mm	Glistering	Entire
Strain 05	-ve	1.83 µm	Rod	Creamy White	Circular	1.24 mm	Glistering	Entire
Strain 06	-ve	1.55 µm	Rod	Creamy White	Circular	1.34 mm	Glistering	Entire
Strain 07	+ve	2.34 µm	Large rod	Creamy pale orange	Irregular	1.45 mm	Opaque	Rough
Strain 08	-ve	1.78 µm	Rod	Creamy White	Circular	1.31 mm	Glistering	Entire
Strain 09	-ve	1.70 µm	Rod	Creamy White	Circular	1.28 mm	Glistering	Entire
Strain 10	-ve	1.68 µm	Rod	Creamy White	Circular	1.29 mm	Glistering	Entire
Strain 11	+ve	2.27 µm	Coccus	Creamy pale orange	Irregular	1.47 mm	Opaque	Entire
Strain 12	-ve	1.64 µm	Rod	Creamy White	Circular	1.34 mm	Glistering	Entire

All the bacterial colonies of Group-I strains were creamy white colored on the special media. And in Group-II, Strain 3 showed yellow color while Strain 7 and Strain 11 showed creamy orange color. The bacterial colonies of Group-I Strains were circular in shape while Group-II showed
Irregular shape. Colony size of the Group-I was ranging between 1.24 mm to 1.34 mm while colony size of Group-II was ranging between 1.45 mm to 1.54 mm. Appearance of the Group-I bacterial strain is glistering while Group-II showed opaque colonies. The bacterial strain margins of Group-I showed smooth margins while Group-II showed rough and smooth margin. These morphological characters resembled *A. diazotrophicus*.
Similar bacterial cell and colony morphology of *A. diazotrophicus* was described by various research workers. Fuentes-Ramirez *et al.,* (1998) [5] reported colonization of sugarcane by *A. diazotrophicus* inhibited by high N-fertilization. Reis *et al.,* (1994) [7, 13] suggested improved methodology for isolation of *A. diazotrophicus* and confirmation of its endophytic habitat. Reis *et al.,* in 2015 [12] described role of nitrogen fixing family Acetobacteraceae in agriculture. Similar characterization of *Gluconacetobacter diazotrophicus* isolated from Sugarcane Cultivated in Upper Egypt is reported by Ahmed *et al.,* (2016) [2]. Kuchekar and Pawar (2019) [9] also studied morphological characterization of *Azotobacter* spp. from various localities of Aurangabad district (MS). James et al., (1994) [7] reported infection of sugarcane by the nitrogen-fixing bacterium *A. diazotrophicus.*

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