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Identification and characterization of *Klebsiella variicola* collected from Akole, Ahemednagar

S R Adik, B T Pawar
Department of Botany, Research center of Botany, Shri Muktanand College, Gangapur, Ch. Sambhajinagar, Maharashtra, India

Abstract
Endophytic nitrogen fixing bacteria *Klebsiella variicola* was collected from Akole, taluka of Ahmednagar district (MS). Total 24 samples were collected from various localities of the taluka. Soil type, pH and Water Holding Capacity (WHC) of the collected samples were studied. After isolation of bacteria from sugarcane sample, Cell morphology and colony morphology of the collected bacterial samples was studied. With the help of special media and morphological characters preliminary identification of *Klebsiella variicola* was done. Out of these 24 bacterial strains, morphologically 19 bacterial strains were identical with *Klebsiella variicola* bacterial strains *viz.* AK01, AK02, AK03, AK04, AK06, AK08, AK09, AK10, AK11, AK12, AK13, AK14, AK15, AK16, AK17, AK19, AK20, AK22 and AK24 While 05 bacterial strains AK05, AK07, AK18, AK21 and AK23 were different from one another and they are grouped in Group-II. Colonies were creamy white, circular, small to large sized, opaque with smooth margins. *Klebsiella variicola* is able to increase nutrient supply, soil fertility and crop growth of sugarcane. The study of *Klebsiella variicola* will be useful for further researchers and it will be better alternative for chemical fertilizers.

Keywords: *Klebsiella variicola,* endophytic bacteria, Akole, Ahmednagar

Introduction

Akole tahsil is well known tribal tahsil in Ahmednagar district, which is located extreme western margin of Ahmednagar. It is extending between the 19º15'12" N to 19º45'06" North latitudes and 73°37'00" E to74°07'21" East longitudes. The western part of the tahsil is surrounded by Thane district, southern part covered by Pune district and Northern part delimited by Nasik district. It is well surrounded by the mountains of Sahyadri. River Pravara, Mula, Adhala and Mhalungi are important rivers, rises in different places in the Sahyadri mountains in the study area. The tahsil acquire 1505.08 km² geographical areas, in which 65.59 per cent area under agriculture, 27.7 percent area under forest and 6.71 per cent area utilized for other purpose (Landge, 2020) [9].

Gulve and Gadekar (2022) [5] studied watershed development program 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) [2]. Bacterial endophytes are consistently reported in the root, stem, leaf, fruit, seed and tuber tissues of a wide range of agricultural, horticultural and forest plants but they represent a largely untapped resource for the discovery of novel traits. This microbial community could play an important role in agriculture by conferring advantages to the plant by producing antibiotics (Strobel and Daisy 2003) [13], Endophytic bacteria were found in both the roots and the stems of sugarcane plants, with a significantly higher density in the roots. Many of the cultivated endophytic bacteria were shown to produce the plant growth hormone indole acetic acid, and this trait was more frequently found among bacteria from the stem (Mendes *et al.,* 2007) Interaction between plants and endophytic bacteria has been studied taking into account plant growth promotion (Flores *et al*. 2007). *K. variicola* strains is able to colonize in its sugarcane plant hosts, to fix Nitrogen in association with plants and to promote plant growth (We *et al.,* 2013). Bacteria that surround plant roots and exert beneficial effects on plant growth are known as plant growth-promoting rhizobacteria (PGPR). In addition to the plant growth-promotion, PGPR also imparts resistance against salinity and oxidative stress and needs to be studied. Such PGPR can function as dynamic bio inoculants under salinity conditions (Kusale *et al*., 2021) [8].

K. variicola colonizing the root intercellular spaces and the interior of root epidermal cells. They proposed that *K. variicola* could be distributed from the base of the stem to other organs via stem xylem vessels, since they also detected xylem colonization in the basal region of the stalk in non-inoculated sugarcane plants (Dong *et al.,* 1997) [3]. Hence during the present investigation report of *K. variicola* was collected from sugarcane from Akole taluka of Ahemednagar district

Materials & Methods

a. **Collection of bacterial samples:** Sugarcane samples were collected from 18 different locations of Akole 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 Water Holding Capacity (WHC) was determined as described (Kalra, 1995) [6].

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 *K. variicola* manitol agar media (Hi-Media) and incubated at 25±2^{O}C for 48 Hrs. which allow only the growth of *K. variicola*.

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

Soil samples were collected from 18 different localities of the study area and the value of soil pH and WHC are presented in Table 01. An average pH of soil samples collected from the study area was ranging between 5.6 to 6.6 pH. This indicates acidic nature of the soil in the study area. WHC was observed between 33.30 to 42.60 %; Maximum soil pH was recorded at Sugaon Bk (6.6); while minimum at Ganore (5.6). Maximum WHC was recorded in the sample collected from Kalas (42.60%); while minimum at Rumbhodi (33.30%) Overall average pH of all samples is 6.1 out of 18 localities, 10 localities *viz.* Ambegavhan (6.2), Ambit (6.1), Indori (6.2), Jamgaon (6.3), Kalas Bk (6.1), Ladgaon (6.2), Rajapur (6.1), Pimpalgao khand (6.1), Sugaon Bk (6.6) and Waghapur (6.2) showed high pH than that of the average pH of all samples and 8 localities showed less pH than the average pH *viz* Chas (5.8), Devthan (5.7), Ganore (5.6), Kotul (5.9), Rumbhodi (5.8), Pimpalgaon nipani (5.7), Takli (5.8) and Virgaon (5.8) The average WHC of all samples is 38.24; out of which 11 soil samples showed high WHC than the average and 7 samples showed less WHC than the average.

Table 1: Localities selected for collection of Sugarcane sample

Sample Code No.	Location	Soil Type	pH	Water holding capacity (WHC) in Percentage
AK01	Ambegavhan	Black	6.2	40.80
AK02	Ambit	Laterite soil	6.1	39.90
AK03	Chas	Black	5.8	35.60
AK04	Devthan	Black	5.7	38.48
AK05	Ganore	Lommy soil	5.6	39.20
AK06	Indori	Red and black soil	6.2	41.80
AK07	Jamgaon	Black	6.3	40.60
AK08	Kalas Bk.	Laterite soil	6.1	42.60
AK09	Kotul	Black	5.9	33.80
AK10	Ladgaon	Black	6.2	35.90
AK11	Rajapur	Black	6.1	39.30
AK12	Rumbhodi	Black	5.8	33.30
AK13	Pimoalgaon Khand	Black	6.1	35.70
AK14	Pimoalgaon Nipani	Laterite soil	5.7	33.20
AK15	Sugaon Bk.	Black	6.6	41.80
AK16	Takli	Black	5.8	34.72
AK17	Virgaon	Black	5.8	40.20
AK18	Waghapur	Black	6.2	41.50
Average			6.1	38.24

Morphological details of the bacterial samples are presented in Table 02. Morphologically 13 bacterial strains *viz.* AK01, AK02, AK03, AK04, AK06, AK08, AK09, AK11, AK13, AK14, AK15, AK17 and AK18 were identical showing similar morphological characters. These strains are grouped as Group-I. While 05 bacterial strains AK05, AK07, AK10, AK12 and AK16 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 1.44 µm to 1.89 µm. Group-II cell size was larger than that of Group-I which was varying between 2.48 µm to 2.69 µm. All the bacterial strains of Group-I was rod shaped but in Group-II, AK05, AK07, AK10, AK12 was large rod shaped while bacterial cell of AK16 was coccus in shape.

Table 2: Morphological characters of Nitrogen fixing Endophytic bacterial strains collected from Sugarcane

Strain	Cell Morphology			Colony Morphology				
	Gram Staining	Cell Size (Avg.)	Cell Shape	Color	Shape	Size (Avg.)	Appearance	Margins
AK01	-ve	1.55 µm	Rod	Creamy White	Circular	1.26 mm	Glistering	Entire
AK02	-ve	1.60 µm	Rod	Creamy White	Circular	1.32 mm	Glistering	Entire
AK03	-ve	2.40 µm	Rod	Yellow	Circular	1.34 mm	Opaque	Rough
AK04	-ve	1.65 µm	Rod	Creamy White	Circular	1.27 mm	Glistering	Entire
AK05	+ve	2.56 µm	Large rod	Creamy pale orange	Irregular	1.44 mm	Glistering	Rough
AK06	-ve	1.44 µm	Rod	Creamy White	Circular	1.39 mm	Glistering	Entire
AK07	+ve	2.48 µm	Large rod	Creamy pale orange	Irregular	1.54 mm	Opaque	Rough
AK08	-ve	1.89 µm	Rod	Creamy White	Circular	1.31 mm	Glistering	Entire
AK09	-ve	1.60 µm	Rod	Creamy White	Circular	1.28 mm	Glistering	Entire
AK10	+ve	2.69 µm	Large rod	Yellow	Irregular	1.49 mm	Glistering	Rough
AK11	-ve	1.70 µm	Rod	Creamy White	Circular	1.37 mm	Opaque	Entire
AK12	+ve	2.55 µm	Large rod	Yellow	Irregular	1.46 mm	Opaque	Rough
AK13	-ve	1.72 µm	Rod	Creamy White	Circular	1.29mm	Glistering	Entire

AK14	-ve	1.69 µm	Rod	Creamy White	Circular	1.31mm	Glistering	Entire
AK15	-ve	1.71 µm	Rod	Creamy White	Circular	1.35mm	Glistering	Entire
AK16	+ve	2.62 µm	Coccus	Creamy pale orange	Irregular	1.59mm	Opaque	Rough
AK17	-ve	1.63 µm	Rod	Creamy White	Circular	1.36 mm	Glistering	Entire
AK18	-ve	1.66 µm	Rod	Creamy White	Circular	1.39 mm	Glistering	Entire

All the bacterial colonies of Group-I strains were creamy white colored on the special media. And in Group-II, Strain AK05, AK07, AK16 showed creamy pale orange color while AK10 and AK12 showed yellow color while. 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 1.26 mm to 1.39 mm while colony size of Group-II was ranging between 1.44 mm to 1.59 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 *K. variicola*.

Similar bacterial cell and colony morphology of *K. variicola* was described by various research workers (Mahl *et al.*, 1965; Wen *et al.*, 2021; Wei *et al.*, 2013) [10, 16, 15]. Wei *et al.*, (2013) [15] reported colonization of sugarcane by *K. variicola* inhibited by high N-fertilization. vanhooren *et al.*, (1999) [14] suggested improved methodology for isolation of *K. variicola* and confirmation of its endophytic habitat. Shi *et al.*, in 2020 [12] described role of nitrogen fixing family Enterobacteraceae in agriculture. Similar characterization of *Gluconacetobacter diazotrophicus* is reported by Ahmed *et al.*, (2016) [1] isolated from sugarcane cultivated in Upper Egypt. Kuchekar and Pawar (2019) [7] also studied morphological characterization of *Azotobacter* spp. from various localities of Aurangabad district (MS). Wei *et al.*, (1994) reported presence of *K. variicola* as nitrogen-fixing bacterium in sugarcane.

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