International Journal of Agriculture and Plant Science
www.agriculturejournal.in
Online ISSN: 2664-7664, Print ISSN: 2664-7656
Received: 26-07-2024, Accepted: 25-08-2024, Published: 09-09-2024
Volume 6, Issue 3, 2024, Page No. 49-52

Identification and characterization of *Burkholderia tropica* collected from Kopergaon, Ahemednagar

S R Adik, B T Pawar
Department of Botany, Research Centre in Botany, Shri Muktanand College, Gangapur, Chhatrapati Sambhajinagar, Maharashtra, India

Abstract
Endophytic nitrogen fixing bacteria *Burkholderia tropica* was collected from Kopergaon taluka of Ahmednagar district (MS). Total 18 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 *Burkholderia tropica* was done. Out of these 18 bacterial strains, morphologically 12 bacterial strains were identical with *Burkholderia tropica* bacterial strains *viz.* KP01, KP02, KP03, KP04, KP06, KP08, KP09, KP11, KP14, KP16, KP18 and KP20 While 06 bacterial strains KP05, KP07, KP10, KP12, KP13, KP15, KP17 and KP19 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. *Burkholderia tropica* is able to increase nutrient supply, soil fertility and crop growth of sugarcane. The study of *Burkholderia tropica* will be useful for further researchers and it will be better alternative for chemical fertilizers.

Keywords: *Burkholderia tropica*, endophytic bacteria, kopergaon, Ahmedangar

Introduction

The Kopergaon tahsil is located in the southern drought prone zone of Ahmednagar district. The tahsil situated partly Godavari River and Nandur madhyameshwar canol basin. In the tahsil length of 60 Km. from East to West and 51 Km. from North to South. The height of tahsil is recorded 600 Mtr. Above the sea level. Generally slope of tahsil is north to South. The latitudinal extend is 180 27' 18" to 180 51' 54" North and longitudinal extend is 740 23' 24" to 740 52' East. It is surrounded by Rahata and Shrirampur tahsil to the northern part, Nashik district to the west and nort - east Yeola tahsil. It's an area of 1630 Sq. Km. is the third rank of tahsil in Ahmednagar district. It is historical and religious which is situated on the bank of river Saraswati (Dhawle, 2017) [6]. Gulve and Gadekar (2022) [8] 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) [4]. Santi1 *et al.*, (2013) [17] studied biological nitrogen fixation in non-legume plants. Endophytic bacteria can 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) [16]. 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 (Lonhienne *et al.*, 2014). 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. *B. tropica* 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 *B. tropica* to sugarcane nutrition was reported by Omarjee *et al.*, (2007). *H. seropediceae* was found mainly inside cortical cells of stems and inside xylem vessels. No L-glucuronidase activity was observed in non-inoculated plants. *B. tropica* is able to increase nutrient supply, soil fertility and crop growth of sugarcane. The study of *B. tropica* will be useful for further researchers and it will be better alternative for chemical fertilizers. *B.tropica* colonizing the root intercellular spaces and the interior of root epidermal cells. They proposed that *B. tropica* 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 (Aroumougame *et al.*, 2020). Hence during the present investigation report of *B. tropica* was collected from sugarcane from Kopergaon taluka of Ahemednagar district

Materials & methods

a. Collection of bacterial samples: Sugarcane samples were collected from 20 different locations of Kopergaon 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) [10].

b. Isolation of bacterial samples: 1 ml of Sugarcane juice was inoculated on specific *Burkholderia* manitol agar media by strick plate method (Reis *et al.,* 1994).
(Hi-Media) and incubated at 25±2^{O}C for 48 Hrs. which allow only the growth of *B. tropica*.

c. Morphological Characterization: Confirmation of the bacteria was done by relevant morphological characterization (Phalke *et al.,* 2017) [13]. 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) [13]. Cultures were preserved at 20^{O}C for further studies.

Results & discussion

Soil samples were collected from 18 different localities of the study area. The observations regarding soil type, soil pH and Water Holding Capacity (WHC) are presented in Table 01. Various types of soil were recorded in study area like Black soil, Regur soil and loamy soil. An average pH of soil samples collected from the study area was ranging between 5.7 to 6.5 pH. Maximum soil pH was recorded at Samvastsar East side of Godavari River (6.5); while minimum at tail tank side of Kanhegaon beside Tukai Mata Mandir side (5.7 pH). Overall average pH of all samples collected from 18 localities was 6.1 Whereas 12 localities showed high pH than that of the average pH of all samples *viz.* Bramhangaon (6.1), Kakdi (6.1), Bhojade (6.3), Dhotre (6.2), Ghodegaon (6.2), Karwadi (6.2), Padhegaon (6.4), Sade (6.3), Samvatsar (6.5), Suregaon (6.3), Wari (6.2) and Yesgaon (6.3) While 06 localities showed less pH than that of the average pH *viz*. Kanhegaon (5.7), Kumbhari (5.8), Kokamthan (5.9), Pohrgaon (6.0) and Shirasgaon (6.0) This indicates acidic nature of the soil in the study area.

WHC in the study area was ranging between 33.20 to 41.90 %; maximum WHC was recorded in the sample collected from Suregaom (41.90%); while minimum at Pohegaon (33.20%). The average WHC of all the samples is 37.37%; out of which 07 soil samples showed high WHC than the average; while 11 samples showed less WHC than the average. Total 5 types Varieties of sugarcane in study area are observed like CoM-0265, Phule- MS10001, and CoM-12085, CoM- 09057, CoM- 86032 (Nira).

Morphological details of the bacterial samples are presented in Table 02. Morphologically 13 bacterial strains *viz.* KP01, KP02, KP03, KP04, KP06, KP08, KP09, KP11, KP13, KP14, KP15 and KP18 were identical showing similar morphological characters. These strains are grouped as Group-I. While 05 bacterial strains KP05, KP07, KP10, KP12 and KP16 were different from one another and they are grouped in Group-II. Bacterial samples of Group-I were gram negative. While bacterial samples of Group-II were Gram positive in staining. The cell size of Group-I varies from 1.51 µm to 1.86 µm. Group-II cell size was larger than that of Group-I which was varying between 2.49 µm to 2.68 µm. All the bacterial strains of Group-I were rod shaped while in Group-II, KP05, KP07, KP10, KP12 Showed large rod and KP16 were coccus shaped.

All the bacterial colonies of Group-I strains were white colored on the special media and in Group-II, KP05, KP07, KP10, KP12, KP16 Showed Creamy white 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 1.26 mm to 1.59 mm while colony size of Group-II was ranging between 2.17 mm to 2.48 mm. Appearance of the Group-I bacterial strain is glistering while Group-II showed opaque colonies. The bacterial strain margins of Group-I showed entire margins while Group-II showed opaque margin. Group-I morphological characters resembled with *B. tropica.*

Biochemical details of the bacterial samples are presented in Table 03. Biochemically 18 bacterial strains are grouped as Group-I *viz.* KP01, KP02, KP04, KP05, KP06, KP07, KP08, KP10, KP11, KP12, KP13, KP15, KP16, KP17 showed positive (+) response for Starch Hydrolysis, Catalase, Urease (Urea Hydrolysis), Citrate, Indole production and Nitrate reduction. Only Gelatin hydrolysis biochemical test showed negative (-) results. These Biochemical characters resembled with *B. tropica.* While KP03, KP09, KP14 and KP18 strains are grouped as Group-II, these showed variation in biochemical test such as KP03 sample showed Starch Hydrolysis, Catalase, Gelatine hydrolysis,) Indole production, Citrate, Nitrate reduction biochemical test was positive (+) only Urease (Urea Hydrolysis test) show negative (-) test, SG09 sample show Starch Hydrolysis, Catalase, Gelatine hydrolysis, Citrate, Urease (Urea Hydrolysis), Nitrate reduction biochemical test was positive (+) only Indole production test show negative (-) test, KP14 sample show Starch Hydrolysis, Catalase, Gelatine hydrolysis, Urease (Urea Hydrolysis) Indole production, Nitrate reduction biochemical test was positive (+) only Citrate test show negative (-) test, KP18 sample show, Indole production, Catalase, Gelatine hydrolysis, Citrate, Urease (Urea Hydrolysis), Nitrate reduction biochemical test was positive (+) only Starch Hydrolysis test show negative (-) test, Hence, this it was concluded that group I Showed do not similarity to *B. tropica.*

Varieties of sugarcane were described by various standard protocols such as Manual of directorate of sugarcane development, Government of India (Jan-2013), Manual of Improved /Hybrid Varieties of sugarcane. Arthee and Marimuthu 2017 [2] was described average production of sugarcane in Maharashtra. Malavath, R.N., 2018 [11] was described various soil types for sugarcane cultivation. Wakgari, T., 2021 [19] was described physical and hydraulic properties of soil for sugarcane cultivation. Indian institute of sugarcane research, Lukhnow was described detail protocol of sugarcane production and management. Hase, C.P., 2019 [9] was described sustainable sugarcane cultivation under monoculturing in Maharashtra. Kalra, Y.P., 1995 [10] was described determination of soil pH by standard methods. Biswas and Mohapatra 2023 [3] was described by biochemical characteristics of *B. Tropica* Similar bacterial cell and colony morphology of *B. tropica* was described by various research workers (Dahash *et al.,* 2013; Donald e *et al.,* 2012 and Silva *et al.,* 2016) [5, 18]. Reis *et al.,* (2004) [14] reported colonization of sugarcane by *B. tropica* inhibited by high N-fertilization.

Sandanakirouchenane *et al.,* (2017) suggested improved methodology for isolation of *B. tropica* and confirmation of its endophytic habitat. Similar characterization of *Gluconacetobacter diazotrophicus* is reported by Ahmed *et*

al., (2016) isolated from sugarcane cultivated in Upper Egypt. Njoloma *et al.,* (2006) [12] also studied morphological characterization of *Azotobacter* spp. from various localities of Aurangabad district (MS). Arthee and Marimuthu, (2017) [2] reported presence of *B. tropica* as nitrogen-fixing bacterium in sugarcane.

Table 1: Sample Collection from Kopergaon Taluka Dist. Ahmednagar

Sr. No.	Sample Code	Location	Site	Varieties of sugarcane	Physical Properties of Soil		
					Soil type	pH	WHC
1	KP01	Bhojade	Samruddhi Highway East side	Co- 86032 (Nira)	Black soil	6.3	38.90
2	KP02	Bramhangaon	Canol north side	CoM- 265	Black soil	6.1	39.30
3	KP03	Derde-korhale	Canol west side	Co- 86032 (Nira)	Black soil	5.9	35.70
4	KP04	Dhotre	Godavari river south side	Co- 86032 (Nira)	Loamy soil	6.2	35.90
5	KP05	Godhegaon	Canol East side	CoM-265	Black soil	6.2	33.80
6	KP06	Kakadi	Airport South Side	CoM-265	Grey soil	6.1	37.40
7	KP07	Kanhegaon	Godavari river east side	Phule MS 10001	Loamy soil	5.7	35.60
8	KP08	Karwadi	Godavari river north side	Co- 86032 (Nira)	Black soil	6.2	33.80
9	KP09	Kokamthan	Godavari river West Side	Phule MS 10001	Laterite soil	5.9	36.48
10	KP10	Kumbhari	Tail Tank Side	CoM-265	Black soil	5.8	33.70
11	KP11	Padhegaon	Godavari river north side	Co- 86032 (Nira)	Black soil	6.4	40.60
12	KP12	Pohegaon	Canol south side	Phule MS 10001	Regur soil	6.0	33.20
13	KP13	Sade	Godavari river east side	CoM-265	Grey soil	6.3	36.80
14	KP14	Samvastsar	Godavari river west side	Co- 86032 (Nira)	Black soil	6.5	39.20
15	KP15	Shirasgaon	Regur soil	Phule MS 10001	Regur soil	6.0	41.55
16	KP16	Suregaon	Canol north side	CoM-265	Grey soil	6.3	41.90
17	KP17	Wari	Godavari river upper north side	Phule MS 10001	Black soil	6.2	37.40
18	KP18	Yesgaon	Canol south side	Co- 86032 (Nira)	Black soil	6.3	41.60
Range						**5.7 To 6.5**	**33.20 To 41.90**
Average						**6.13**	**37.37**

Table 2: Morphological Characters of Nitrogen Fixing Endophytic Bacterial Strains Collected from Various Locations of Kopergaon Taluka

Sr.no	Sample code	Cell Morphology			Colony Morphology				
		Gram Staining	Cell Size (Avg.)	Cell Shape	Color	Shape	Size (Avg.)	Appearance	Margins
1	KP01	-ve	1.51 µm	Rod	Green	Circular	3.37 mm	Glistering	Entire
2	KP02	+ve	1.62µm	Rod	Creamy orange	Irregular	1.32 mm	Opaque	Rough
3	KP03	-ve	1.78 µm	Rod	White	Circular	2.34 mm	Glistering	Entire
4	KP04	-ve	1.81 µm	Rod	Yellow	Circular	2.76 mm	Glistering	Entire
5	KP05	+ve	2.56 µm	Large rod	Pink	Irregular	2.44 mm	Opaque	Lobate
6	KP06	-ve	1.69 µm	Rod	Yellow	Circular	2.83 mm	Glistering	Entire
7	KP07	-ve	2.48 µm	Rod	Yellow	Circular	2.98 mm	Glistering	Entire
8	KP08	-ve	1.99 µm	Rod	White	Circular	3.31 mm	Glistering	Entire
9	KP09	-ve	1.84µm	Rod	White	Circular	2.28 mm	Glistering	Entire
10	KP10	-ve	2.11µm	Rod	White	Circular	3.49 mm	Glistering	Entire
11	KP11	-ve	1.92 µm	Rod	Green	Circular	3.93 mm	Glistering	Entire
12	KP12	-ve	2.24 µm	Rod	White	Circular	3.37 mm	Glistering	Entire
13	KP13	-ve	2.72 µm	Rod	Yellow	Circular	2.89 mm	Glistering	Entire
14	KP14	-ve	2.66 µm	Rod	Yellow	Circular	2.31 mm	Glistering	Entire
15	KP15	-ve	2.23 µm	Rod	Yellow	Circular	2.88 mm	Glistering	Entire
16	KP16	-ve	2.62 µm	Coccus	Grayish	Irregular	2.48 mm	Opaque	Undulate
17	KP17	-ve	2.28 µm	Rod	White	Circular	3.58 mm	Glistering	Entire
18	KP18	-ve	2.19 µm	Rod	White	Circular	2.98 mm	Glistering	Entire

Table 3: Biochemical Characterization of Endophytic Bacterial Strains Collected from Kopergaom Taluka

Sr. No.	Sample Code	Catalase	Citrate	Gelatin Hydrolysis	Indole production	Nitrate Reduction	Starch Hydrolysis	Urea Hydrolysis
1	KP01	+ve	+ve	-ve	+ve	+ve	+ve	+ve
2	KP03	+ve	+ve	-ve	+ve	+ve	-ve	+ve
3	KP04	+ve	+ve	-ve	+ve	+ve	-ve	+ve
4	KP06	+ve	+ve	-ve	+ve	+ve	+ve	+ve
5	KP07	+ve	+ve	-ve	+ve	+ve	+ve	+ve
6	KP08	+ve	+ve	-ve	+ve	+ve	-ve	+ve
7	KP09	+ve	+ve	-ve	+ve	+ve	-ve	+ve
8	KP10	+ve	+ve	-ve	+ve	+ve	-ve	+ve
9	KP11	+ve	+ve	-ve	+ve	+ve	+ve	+ve
10	KP12	+ve	+ve	-ve	+ve	+ve	-ve	+ve
11	KP13	+ve	+ve	-ve	+ve	+ve	+ve	+ve
12	KP14	+ve	+ve	-ve	+ve	+ve	+ve	+ve
13	KP15	+ve	+ve	-ve	+ve	+ve	-ve	+ve
14	KP17	+ve	+ve	-ve	+ve	+ve	-ve	+ve
15	KP18	+ve	+ve	-ve	+ve	+ve	-ve	+ve

References

1. Adeleke BS, OO Babalola. The endosphere microbial communities, a great promise in agriculture. Journal of Plant Growth Regulation,2021:(2):2-19.
2. Arthee R, P Marimuthu. Studies on Endophytic *Burkholderia* Sp. From Sugarcane and It's screening For Plant Growth Promoting Potential. Journal of Experimental Biology and Agricultural Sciences,2017:5(2):242-259.
3. Biswas I, PK Das Mohapatra. *Paraburkholderia tropica* PK17 and *Kosakonia arachibidis* PK18; two newly reported tannase producing bacteria isolated from forest soil and study of their tannase producing potentiality. Notulae Scientia Biologicae,2023:15(1):1-11.
4. Cheng Q. Perspectives in biological nitrogen fixation research. Journal of Integrative Plant Biology,2008:50(7):784-796.
5. Dahash HF, AH Charakh, JK Khafaji. Charrization of *Bulkholderia cepacia* isolated from clinical samples, in Hilla city, Iraq. Medical journal of Babylon,2013:10(2):343-348.
6. Dhawle GM. Assessment of Cropping Pattern and Water Resources Utilization: A Case Study of Shrigonda Tahsil, Ahmednagar. Peer Reviewed International Research Journal of Geography,2017:34(2):9-14.
7. Donald LJ, OV Krokhins, HW Duckworth, B Wiseman, T Deemagarn, R Singh, *et al*. Characterization of the Catalase-Peroxidase KatG from *Burkholderia pseudomallei* by Mass Spectrometry. The Journal Of Biological Chemistry,2003:278(37):35687-35692.
8. Gulve SD, DJ Gadekar. Geographical Study Of Watershed Development Programme In Ahmednagar District. International Research Journal of Modernization in Engineering Technology and Science,2022:4(2):958-990.
9. Hase CP. Sustainable sugarcane cultivation under monoculturing in Maharashtra. International journal of emerging technologies and innovative research,2019:6(6):716-723.
10. Kalra YP. Determination of pH of soils by different methods: collaborative study. Journal of AOAC International,1995:78(2):311-324.
11. Malavath RN, C Mahesh, D Balaguruvaiah, G Vidyasagar. Land use options and site suitability for sugarcane growing red soils, red laterite soils and black soils of Medak district of Telangana. Journal of Pharmacognosy and Phytochemistry,2018:7(2):409-416.
12. Njoloma JP, M Oota, K Taroura, Y Sakki, S Akao. Colonization ability of *Herbaspirillum* spp. B501gfp1 in sugarcane, a non-host plant in the presence of indigenous diazotrophic endophytes. African Journal of Biotechnology,2006:5(10):836-841.
13. Phalke VS, BT Pawar, RM Gulve. Halophilic bacteria in salt stressed soils of Aurangabad district (MS) India. Bioscience Discovery,2017:8(1):55-60.
14. Reis VM, P Estrada-de los Santos, S Tenorio-Salgado, J Vogel, M Stoffels, S Guyon, *et al*. *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant-associated bacterium. International Journal of Systematic and Evolutionary Microbiology,2004:54:2155-2162.
15. Sandanakirouchenane A, Ekramul Haque, T Geetha. Recent Studies on N2 Fixing *Burkholderia* Isolates as a Bio fertilizer for the Sustainable Agriculture. International Journal of Current Microbiology and Applied Sciences,2014:6(11):2780-2796.
16. Sansanwal R, U Ahlawat, Priyanka, L Wati. Role of endophytes in agriculture.Chemical Science Review and Letters,2017:6(24):2397-2407.
17. Santi1 C, D Bogusz, C Franche. Biological nitrogen fixation in non-legume plants. Annals of Botany,2013:111:743-767.
18. Silva MO, FJ Freire, J Kuklinsky-Sobral, E Cantídio A. de Oliveira, MB Galvão, *et al*. Bacteria associated with sugarcane in Northeastern Brazil, African Journal of Microbiology Research,2016:10(37):1586-1594.
19. Wakgari T. Long Term Sugarcane Cultivation Effect on Selected Physical and Hydraulic Properties of Soils at Three Ethiopian Sugarcane Estates. Advances in Crop Science and Technology,2021:9(8):1-7.