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Research Article

Indigenous bacterial endophytic PGPMs of chickpea: Characterization and hidden antagonistic potential against *Rhizoctonia bataticola* causing dry root rot of chickpea

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ABSTRACT: Chickpea (*Cicer arietinum* L.) is grown in more than 50 countries. India is the largest chickpea-producing country accounting for 64% of the global chickpea production. However, the production is contrained by the dry root rot disease caused by *Rhizoctonia bataticola*. Considering this problem, the investigation was carried out to isolate, characterize and the antagonistic potential of indigenous endophytic PGPMs for one of the components in the integrated management of dry root rot of chickpeas in eco-friendly manner. Hence, the isolation of thirty endophytic PGPMs was carried from chickpea by using the spread plate technique. The cultural characters and Gram's staining reaction confirmed that the endophytic PGPMs isolated from chickpea plant tissues were bacteria. Among thirty bacterial strains, eight showed more than 50% of mycelial inhibition of the pathogen. Out of eight strains, five highly superior strains were selected and subjected for 16S rDNA gene sequencing using the universal primers (16Sr DNA F and 16Sr DNA R), which produced amplified products of size 1500 bp. nBLAST results of 16S rDNA gene sequence revealed that all the endophytic bacterial PGPMs showed homology with genus *Bacillus* but with different species. The five potential strains namely, BEPGPM-5, BEPGPM-9, BEPGPM-27, BEPGPM-28, and BEPGPM-30 were identified and confirmed as *B. tropicus*, *B. pacificus*, *B. cereus, B. subtilis,* respectively, based on molecular technique.

KEYWORDS: *Bacillus cereus, B. pacificus*, *B. subtilis, B. tropicus,* chickpea, dry root rot

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the largest produced food legume in South Asia and the third largest produced food legume globally, after the common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). Chickpea is an important source of protein for millions of people and plays a significant role in improving soil fertility by fixing atmospheric nitrogen. However, due to the change in climatic scenario, crop production is majorly threatened by a few diseases including dry root rot. The disease is caused by *Rhizoctonia bataticola.*

Rhizoctonia bataticola is a soil-borne, necrotrophic and polyphagous fungal pathogen. The genus *Rhizoctonia* ("Root killer") was described by the French mycologist Augustin Pyramus de Candolle in 1815 for plant pathogenic fungi that produce both hyphae and sclerotia. The characteristic morphological features of *R. bataticola* are right-angle mycelial branching, multinucleate septate mycelia, cross-wall formation at the beginning of new branching mycelia and partial hyphal fusion. The fungus *R. bataticola* exists in an anamorph (sclerotial) stage in soil and on crop residues (Sharma and Pande, 2013).

At present, disease management strategies rely heavily on the use of chemicals, which are not eco-friendly and economical to many farmers throughout the world and they can cause negative environmental hazards. This in turn creates a major constraint to plant growth and yield, causing low crop productivity and affecting global food security (Lopes *et al.,* 2021). Therefore, to increase global agricultural production in a more economically and environmentally sustainable way, there is a need to use lesser chemicals and increase plant tolerance to biotic stresses. The use of Plant Growth Promoting Microorganisms (PGPMs) is potentially advantageous for improving crop productivity, food quality and security in a more sustainable and eco-friendly manner (Etesami, 2020).

The rhizosphere and endophytic bacterial community can harbour beneficial organisms known as PGPMs. Based on the interaction of roots with plants, PGPMs include organisms present in the soil *i.e*., Plant Growth Promoting Rhizobacteria (PGPR) as well as organisms present inside the plant *i.e*., endophytes (Mitra *et al.,* 2019). PGPMs improve plant growth by enhancing the availability of nutrients as phytostimulators by regulating phytohormones and increasing plant tolerance against biotic stresses (Lopes *et al*., 2021). PGPMs also act as

bio-pesticides or bio-control agents against plant pathogens, through competition for nutrients, antagonism and induce systemic resistance (Khan *et al*., 2013).

However, despite the importance of the PGPMs-plant relationship, the knowledge of the interactions between PGPMs and pathogens under hostile environmental conditions is still rather limited in the case of dry root rot of chickpeas. Hence, there is a need to explore PGPMs now for the purpose of improving plant growth and as well as management of chickpea dry root rot. Keeping this in view, it is essential to collect and isolate the endophytic PGPMs from different geographic regions and to identify the antagonistic endophytic PGPMs against *R. bataticola* so that the PGPMs can be one of the components in the integrated disease management strategy for dry root rot of chickpea.

MATERIALS AND METHODS

Isolation of pathogen

The dry root rot-infected samples were collected from diseased chickpea plants and were washed thoroughly with tap water. A small portion of infected parts containing healthy as well as diseased tissues was cut into 0.5 cm pieces with the help of a sterilized scalpel blade. These pieces were then surface sterilized with 1% sodium hypochlorite solution for 1 minute with 3 subsequent changes in sterilized water to remove traces of the chemical. The pieces were then transferred aseptically to Petri dishes containing sterilized Potato Dextrose Agar (PDA) and incubated at 28 ± 2ºC under a BOD incubator. The Petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces. The pure culture of the pathogen was obtained from hyphal tip culture and such pure culture was used for further studies. Later, the pathogen culture was subjected to Koch's postulates.

Sample collection

Plant parts of healthy chickpea plants were collected during *rabi*, 2020 for isolation of different strains of endophytic PGPMs. The strains were collected from eleven different districts of Northern Karnataka *viz*., Bagalkot, Bellary, Bidar, Dharwad, Gadag, Haveri, Kalaburagi, Koppal, Raichur, Vijayapura and Yadgir wherever chickpea is grown.

Isolation of endophytic bacterial PGPMs

Isolation of thirty bacterial endophytic PGPMs from plant samples was carried out by randomly excising different parts (leaf, shoot and root of 0.5 cm length each) using sterile scissors. The surface sterilization of selected plant tissues was done by dipping in 1% sodium hypochlorite for 1 min and washed thoroughly thrice in sterile distilled water to remove the traces of sodium hpochlorite. After that, the pieces were then transferred by using forceps to 70% alcohol for a few seconds followed by rinsing in sterile double distilled water and later they were dried in laminar air flow before placing it on a nutrient medium.

For isolation, the spread plate technique was used. The sterilized plant tissues were ground using a sterile pestle and mortar. The tissue extract was subsequently incubated at 28ºC for 30 min. to allow the complete release of endophytic microorganisms from the host tissue. Later, the tissue extract was spread on the Nutrient Agar (NA) medium plate by using a sterilized spreader. Then, the plates were incubated for 2-3 days at 28-30 ºC. Bacterial colonies with respect to colour, size and shape were observed after the incubation period and purified in a specific *Bacillus* Agar medium for further studies. The pure bacterial cultures were used for the observations of Gram's staining reaction and cell shape by using a stereo binocular microscope. The source and designation of thirty bacterial endophytic PGPMs are given in Table 1.

Cultural characteristics of endophytic PGPMs

To study the cultural characteristics *viz*., colony colour, colony form and colony elevation, the pure cultures of thirty bacterial endophytes were inoculated on Nutrient Agar medium aseptically and kept for incubation at 28 ± 2°C for two days. The cultural characters of each endophytic PGPM were recorded based on visual observation. Later, the thirty strains were categorized for different colony colours, colony forms, colony elevations and colony margins.

Gram's staining reaction of endophytic bacterial PGPMs

The staining reaction was carried out by the Gram staining technique according to the standard procedure as mentioned in the Laboratory Guide for Identification of Plant Pathogenic Bacteria published by the American Phytopathological Society (Schaad, 1992). A loopful of a colony of 24 h old bacterial cultures was taken and smeared onto the glass slide and passed over the flame for two min to heat fix and then stained with crystal violet (primary stain). The slide was washed with distilled water after one minute and further rinsed with iodine solution for one minute. Thereafter, the slide was washed with Gram's decolourizer and subsequently drained with distilled water. Later, stained with counter-stain safranin for one min and washed with distilled water, dried with tissue paper and observed the bacterial cells under a stereo binocular microscope.

Antagonistic potential of endophytic PGPMs against *R. bataticola*

To identify the potential PGPMs against *R. bataticola,* the efficacy of the antagonistic activity of endophytic PGPMs was tested by dual culture technique (Xu and Kim, 2014) under *in vitro* conditions. In the dual culture technique, test

Table 1. Source and designation of chickpea bacterial endophytic PGPM strains collected during *rabi* 2020

Sr. No.	District	Village	No. of bacterial strains obtained	Strain code
1	Dharwad	Gadag	1	BEPGPM-1
		UAS, Dharwad	1	BEPGPM-2
		Narendra	1	BEPGPM-3
2	Gadag	Gajendrigad	1	BEPGPM-4
		Naregal	1	BEPGPM-5
3	Haveri	Motebennur	2	BEPGPM-6
				BEPGPM-7
		Mugalikatti	1	BEPGPM-8
4	Kalaburagi	ZARS, Kalaburagi	1	BEPGPM-9
5	Yadgiri	Bheemrayanagudi	1	BEPGPM-10
		Saidapur	1	BEPGPM-11
6	Bidar	Dubalgundi	2	BEPGPM-12
				BEPGPM-13
		Hudugi	1	BEPGPM-14
7	Vijayapura	BasavanaBagewadi	1	BEPGPM-15
8	Bagalkot	Bagalkot	1	BEPGPM-16
		Karadi	1	BEPGPM-17
9	Ballari	Ballari	2	BEPGPM-18
				BEPGPM-19
10	Kushtagi	Kushtagi	4	BEPGPM-20
				BEPGPM-21
				BEPGPM-22
				BEPGPM-23
		Ganganal	1	BEPGPM-24
		Hiresindhogi	1	BEPGPM-25
		Yalburga	1	BEPGPM-26
11	Raichur	New area (UAS campus)	1	BEPGPM-27
		Siddanabhavi camp	1	BEPGPM-28
		Askihal	1	BEPGPM-29
		Janakirao camp	1	BEPGPM-30

antagonist bacterial PGPM was streaked at one side and on the opposite side, the mycelial disc of test pathogen measuring 5 mm diameter of five days old culture was placed aseptically on a sterile Petri dish containing NA medium for bacteria. In the control plate, a mycelial disc of pathogen was placed aseptically alone without test endophytic PGPM. The Petri plates were then incubated at 28 ± 2°C. Three replications were maintained for each test PGPM. The observations on the growth of pathogens in the test plate as well as in the control were measured in all the replications. Later, per cent inhibition of mycelial growth of test pathogen was calculated by the formula given by Vincent (1947).

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

Where,

I = Per cent inhibition in growth of test pathogen.

C = Radial growth of pathogen (mm) in control.

T = Radial growth of pathogen (mm) in treatment.

Molecular detection of potential bacterial endophytic PGPMs

The endophytic PGPMs which showed more than 50% mycelial inhibition and higher plant growth-promoting traits were selected for molecular identification. Out of eight bacterial endophytic PGPMs, five bacterial strains were proved to be promising. Hence, they were subjected to molecular characterization by DNA isolation and PCR analysis by using 16S rDNA universal primers (16Sr DNA F and 16Sr DNA R). The amplified products of bacterial PGPMs were sent for sequencing and identification of species to Eurofins, Bangalore.

RESULTS

Endophytic bacterial PGPMs

The bacterial strains were stained on Nutrient Agar medium and incubated at 28 ± 2°C for 48 h to obtain the colonies. After the incubation, the observations on the cultural characters were recorded and the results are presented in Table 2 (Figure 1 and Figure 2).

Cultural characters of endophytic PGPMs

Colony colour

The colony colour of the bacterial colonies were as distinct from each other as dark yellow, maroon, gray, cream, yellow, brown, white, creamy white, creamy yellow, light cream, light gray, and light yellow. The isolates such as BEPGPM-1, BEPGPM-2, BEPGPM-8, BEPGPM-14, BEPGPM-15, BEPGPM-24, BEPGPM-26 and BEPGPM-30 were dark yellow, light cream, creamy, cream, maroon, yellow, white brown and light yellow in colour, respectively. The twelve strains (BEPGPM-3, BEPGPM-4, BEPGPM-5, BEPGPM-9, BEPGPM-11, BEPGPM-13, BEPGPM-17, BEPGPM-18, BEPGPM-19, BEPGPM-22, BEPGPM-25 and BEPGPM-29) were grey, two (BEPGPM-10 and BEPGPM-28) white, three (BEPGPM-6, BEPGPM-12 and BEPGPM-27) creamy yellow and four (BEPGPM-7, BEPGPM-16, BEPGPM-21 and BEPGPM-23) were light gray (Table 2a).

Colony form

The colony form was categorized into circular and irregular forms. The nineteen strains (BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-6, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-28, BEPGPM-29, and BEPGPM-30) were observed circular form. Whereas, eleven strains such as BEPGPM-5, BEPGPM-7, BEPGPM-11, BEPGPM-12, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-25, BEPGPM-26 and BEPGPM-27 were irregular in their form (Table 2b).

Colony elevation

The colony elevation of bacterial endophytic strains was categorized into flat and raised elevation. Twelve strains BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-6, BEPGPM-11, BEPGPM-12, BEPGPM-14, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-26 showed raised elevation and eighteen strains BEPGPM-5, BEPGPM-7, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-15, BEPGPM-16, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-25, BEPGPM-27, BEPGPM-28, BEPGPM-29 and BEPGPM-30 showed flat elevation (Table 2c).

Colony margin

The colony margin of bacterial endophytic strains was categorized into entire and undulated. Eighteen strains (BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-6, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-28, BEPGPM-29, BEPGPM-30) were having entire margin. Twelve strains (BEPGPM-4, BEPGPM-5, BEPGPM-7, BEPGPM-11, BEPGPM-12, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-25, BEPGPM-26, BEPGPM-27) were having undulated margin (Table 2d).

Staining reaction

The staining reaction was tested by using Gram's staining technique. Nineteen strains (BEPGPM-1, BEPGPM-3, BEPGPM-4, BEPGPM-7, BEPGPM-8, BEPGPM-10, BEPGPM-11, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-16, BEPGPM-17, BEPGPM-19, BEPGPM-20, BEPGPM-22, BEPGPM-23, BEPGPM-24, BEPGPM-26 and BEPGPM-28) showed Gram-negative in reaction. Whereas, eleven strains (BEPGPM-5, BEPGPM-2, BEPGPM-6, BEPGPM-9, BEPGPM-12, BEPGPM-18, BEPGPM-21, BEPGPM-25, BEPGPM-27, BEPGPM-29 and BEPGPM-30) were Gram-positive in their reaction (Table 2e, Figure 3).

Cell shape

The twenty-seven strains (BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-10, BEPGPM-11, BEPGPM-12, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-19, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-24, BEPGPM-25, BEPGPM-26 BEPGPM-27, BEPGPM-28, BEPGPM-29 and BEPGPM-30) were rod-shaped cells. On the other hand, only three strains (BEPGPM-7, BEPGPM-8 and BEPGPM-20) were cocci in shape (Table 2f, Figure 3).

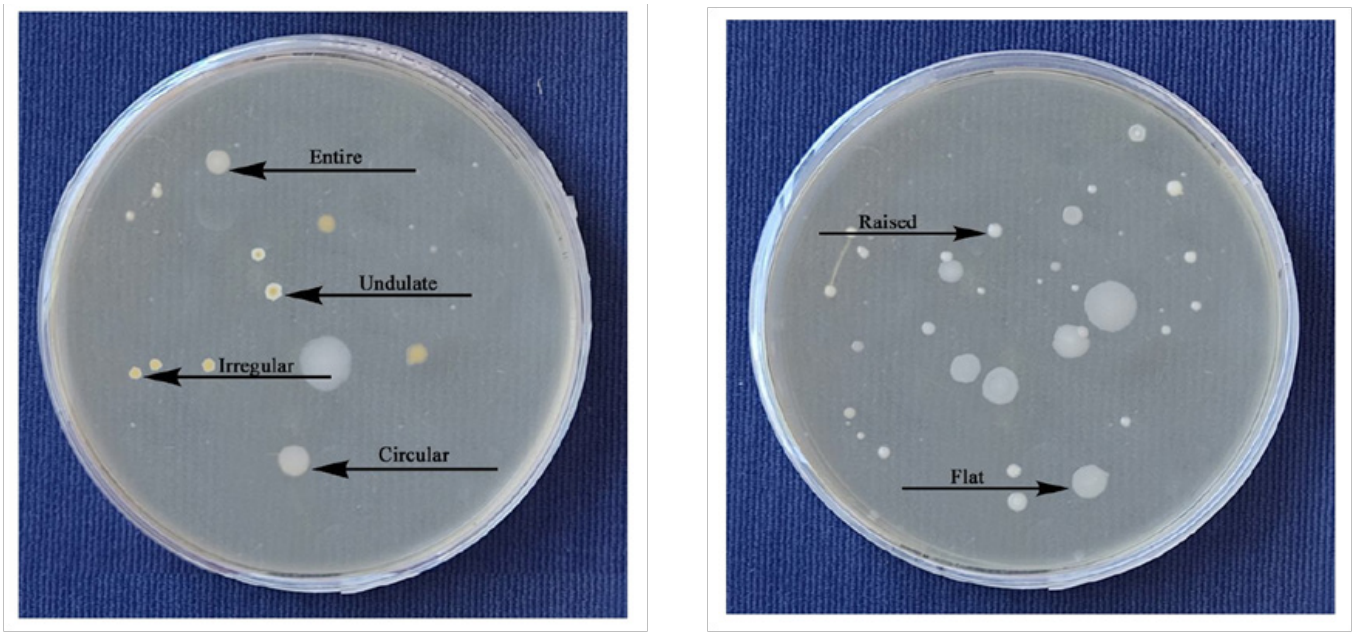

Figure 1. Representative colony characters of bacterial PGPMs.

Table 2. Cultural characters of endophytic bacterial PGPMs on Nutrient Agar

Sl. No.	Strain code	Colony characters				Staining reaction	Cell shape
		Colony colour	Colony form	Colony elevation	Colony margin		
1	BEPGPM-1	Dark yellow	Circular	Raised	Entire	-	Rod
2	BEPGPM-2	Light cream	Circular	Raised	Entire	+	Rod
3	BEPGPM-3	Gray	Circular	Raised	Entire	-	Rod
4	BEPGPM-4	Gray	Circular	Raised	Undulate	-	Rod
5	BEPGPM-5	Gray	Irregular	Flat	Undulate	+	Rod
6	BEPGPM-6	Creamy yellow	Circular	Raised	Entire	+	Rod
7	BEPGPM-7	Light gray	Irregular	Flat	Undulate	-	Cocci
8	BEPGPM-8	Creamy white	Circular	Flat	Entire	-	Cocci
9	BEPGPM-9	Gray	Circular	Flat	Entire	+	Rod
10	BEPGPM-10	White	Circular	Flat	Entire	-	Rod
11	BEPGPM-11	Gray	Irregular	Raised	Undulate	-	Rod
12	BEPGPM-12	Creamy yellow	Irregular	Raised	Undulate	+	Rod
13	BEPGPM-13	Gray	Circular	Flat	Entire	-	Rod
14	BEPGPM-14	Cream	Circular	Raised	Entire	-	Rod
15	BEPGPM-15	Maroon	Circular	Flat	Entire	-	Rod
16	BEPGPM-16	Light gray	Irregular	Flat	Undulate	-	Rod
17	BEPGPM-17	Gray	Irregular	Raised	Undulate	-	Rod
18	BEPGPM-18	Gray	Irregular	Raised	Undulate	+	Rod
19	BEPGPM-19	Gray	Circular	Flat	Entire	-	Rod
20	BEPGPM-20	Cream	Circular	Flat	Entire	-	Cocci
21	BEPGPM-21	Light gray	Circular	Flat	Entire	+	Rod
22	BEPGPM-22	Gray	Circular	Flat	Entire	-	Rod
23	BEPGPM-23	Light gray	Circular	Flat	Entire	-	Rod
24	BEPGPM-24	Yellow	Irregular	Raised	Undulate	-	Rod

Contd.....

Table 2 to be continued…

Sl. No.	Strain code	Colony characters				Staining reaction	Cell shape
		Colour	Colony form	Colony elevation	Colony margin		
25	BEPGPM-25	Gray	Irregular	Flat	Undulate	+	Rod
26	BEPGPM-26	Brown	Irregular	Raised	Undulate	-	Rod
27	BEPGPM-27	Creamy yellow	Irregular	Flat	Undulate	+	Rod
28	BEPGPM-28	White	Circular	Flat	Entire	-	Rod
29	BEPGPM-29	Gray	Circular	Flat	Entire	+	Rod
30	BEPGPM-30	Light yellow	Circular	Flat	Entire	+	Rod

Table 2a. Grouping of bacterial endophytic PGPMs based on colony colour

Sl. No.	Strain code	Colony colour	No. of strains
1	BEPGPM-10, BEPGPM-28	White	2
2	BEPGPM-6, BEPGPM-12, BEPGPM-27	Creamy yellow	3
3	BEPGPM-7, BEPGPM-16, BEPGPM-21, BEPGPM-23	Light grey	4
4	BEPGPM-1	Dark yellow	1
5	BEPGPM-3, BEPGPM-4, BEPGPM-5, BEPGPM-9, BEPGPM-11, BEPGPM-13, BEPGPM-17, BEPGPM-18, BEPGPM-19, BEPGPM-22, BEPGPM-25 and BEPGPM-29	Grey	12
6	BEPGPM-2	Light cream	1
7	BEPGPM-8 and BEPGPM-20	Creamy	2
8	BEPGPM-14	Cream	1
9	BEPGPM-15	Maroon	1
10	BEPGPM-24	Yellow	1
11	BEPGPM-26	White brown	1
12	BEPGPM-30	Light yellow	1

Table 2b. Grouping of bacterial endophytic PGPMs based on colony form

Sl.No.	Strain code	Colony form	No. of strains
1	BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-6, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-28, BEPGPM-29, BEPGPM-30	Circular	19
2	BEPGPM-5, BEPGPM-7, BEPGPM-11, BEPGPM-12, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-25, BEPGPM-26 and BEPGPM-27	Irregular	11

Table 2c. Grouping of bacterial endophytic PGPMs based on colony elevation

Sl. No.	Strain code	Colony elevation	No of strains
1	BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-6, BEPGPM-11, BEPGPM-12, BEPGPM-14, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-26	Raised	12
2	BEPGPM-5, BEPGPM-7, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-15, BEPGPM-16, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-25, BEPGPM-27, BEPGPM-28, BEPGPM-29 and BEPGPM-30	Flat	18

Table 2d. Grouping of bacterial endophytic PGPMs based on colony margin

Sl. No.	Strain code	Colony margin	No of strains
1	BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-6, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-28, BEPGPM-29, BEPGPM-30	Entire	18
2	BEPGPM-4, BEPGPM-5, BEPGPM-7, BEPGPM-11, BEPGPM-12, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-25, BEPGPM-26, BEPGPM-27	Undulated	12

Table 2e. Grouping of bacterial endophytic PGPMs based on staining reaction

Sl. No.	Strain code	Staining reaction	No. of strains
1	BEPGPM-1, BEPGPM-3, BEPGPM-4, BEPGPM-7, BEPGPM-8, BEPGPM-10, BEPGPM-11, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-16, BEPGPM-17, BEPGPM-19, BEPGPM-20, BEPGPM-22, BEPGPM-23, BEPGPM-24, BEPGPM-26 and BEPGPM-28	Gram negative	19
2	BEPGPM-2, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-12, BEPGPM-18, BEPGPM-21, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30	Gram positive	11

Table 2f. Grouping of bacterial endophytic PGPMs based on cell shape

Sl.No.	Strain code	Cell shape	No. of strains
1	BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-10, BEPGPM-11, BEPGPM-12, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-19, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-24, BEPGPM-25, BEPGPM-26 BEPGPM-27, BEPGPM-28, BEPGPM-29 and BEPGPM-30	Rod shaped	27
2	BEPGPM-7, BEPGPM-8, BEPGPM-20	Cocci	3

Antagonistic potential of endophytic bacterial PGPMs

Thirty strains of bacterial endophytic PGPMs were screened against *R. bataticola* for mycelial inhibition by using a dual culture technique. The results indicated that all the strains inhibited the pathogen growth significantly. However, the inhibition per cent varied from 4.26-83.15%. Among them, eight strains (BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-15, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30) showed more than 50% mycelial inhibition of pathogen which were on par with each other, highest being BEPGPM-30 (83.15). The next best efficient antagonist strain was BEPGPM-6 with an inhibition per cent of 62.96. While, the strain BEPGPM-12 recorded minimum per cent mycelial inhibition with per cent inhibition of 4.26 (Table 3, Figure 4).

Molecular detection of potential endophytic bacterial PGPMs

Among thirty bacterial strains, eight strains showed more than 50% mycelial inhibition of the pathogen. Out of eight, five highly superior strains were selected for molecular detection and subjected to 16S rDNA gene sequencing. The universal primers (16Sr DNA F and 16Sr DNA R) were used for PCR amplification, which produced amplified products of size 1500 bp. Further, nBLAST results of the 16S rRNA gene sequence revealed that all the endophytic bacterial PGPMs showed homology with the genus *Bacillus* but with different species. The four strains (BEPGPM-5, BEPGPM-27, BEPGPM-28, BEPGPM-30) showed 100% homology with *Bacillus tropicus* (ON564730), *Bacillus pacificus* (ON564773), *Bacillus cereus* (ON564610) and *Bacillus subtilis* (ON564689), respectively with assigned accession numbers, while, strain BEPGPM-9 showed 99.69% homology with *Bacillus tropicus* (ON564907). As a result, the strains namely, BEPGPM-5, BEPGPM-9, BEPGPM-27, BEPGPM-28, BEPGPM-30 were identified and confirmed as *B. tropicus*, *B. tropicus*, *B. pacificus*, *B. cereus, B. subtilis,* respectively based on molecular technique.

Figure 2. Cultural characters of endophytic bacterial PGPMs on Nutrient Agar medium.

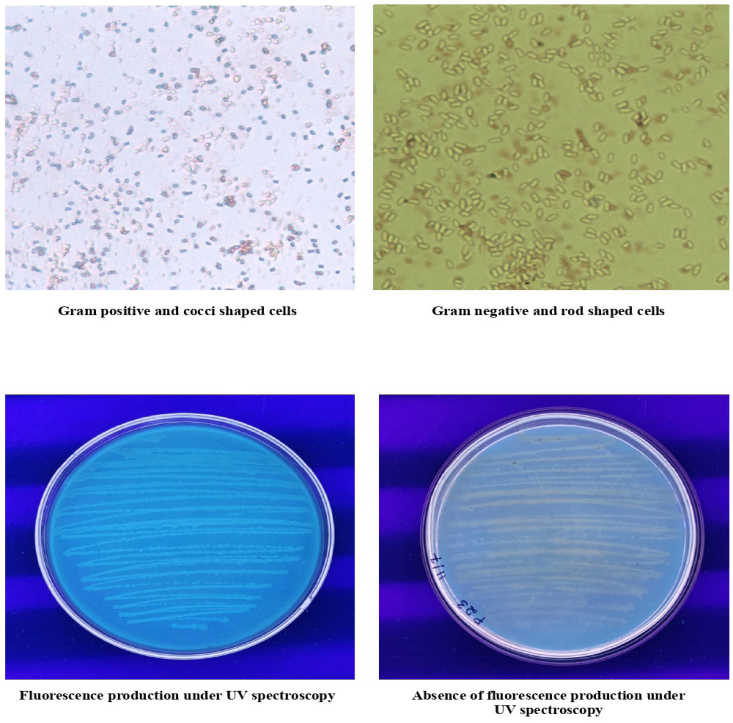

Figure 3. Gram's staining reaction and fluorescence produced by bacterial PGPMs.

Table 3. Antagonistic potential of endophytic bacterial PGPMs against *R. bataticola* in dual culture assay

Sl. No.	Strain code	Colony growth* (mm)	Per cent mycelial inhibition*
1	BEPGPM-1	83.17	7.59 (15.99)
2	BEPGPM-2	59.50	33.89 (35.60)
3	BEPGPM-3	65.87	26.81 (31.18)
4	BEPGPM-4	84.17	6.48 (14.75)
5	BEPGPM-5	43.17	52.04 (46.16)
6	BEPGPM-6	33.33	62.96 (52.51)
7	BEPGPM-7	73.07	18.81 (25.70)
8	BEPGPM-8	82.27	8.59 (17.04)
9	BEPGPM-9	42.47	52.81 (46.61)
10	BEPGPM-10	83.17	7.59 (15.99)
11	BEPGPM-11	83.90	6.78 (15.09)
12	BEPGPM-12	86.17	4.26 (11.91)
13	BEPGPM-13	83.00	7.78 (16.19)
14	BEPGPM-14	64.00	28.89 (32.51)
15	BEPGPM-15	41.50	53.89 (47.32)
16	BEPGPM-16	56.50	37.22 (37.59)
17	BEPGPM-17	53.67	40.37 (39.44)
18	BEPGPM-18	75.40	16.22 (23.75)
19	BEPGPM-19	57.33	36.30 (37.04)
20	BEPGPM-20	58.00	35.56 (36.60)
21	BEPGPM-21	52.93	41.19 (39.92)
22	BEPGPM-22	71.83	20.19 (26.69)
23	BEPGPM-23	64.10	28.78 (32.44)
24	BEPGPM-24	55.87	37.93 (38.01)
25	BEPGPM-25	44.00	51.11 (45.63)
26	BEPGPM-26	57.47	36.15 (36.95)
27	BEPGPM-27	42.03	53.30 (46.88)
28	BEPGPM-28	62.33	30.74 (33.67)
29	BEPGPM-29	42.77	52.48 (46.42)
30	BEPGPM-30	15.17	83.15 (65.75)
	Control	90.00	00.00 (00.00)
S. Em ±		-	0.79
CD at 1%		**-**	**2.98**

*Mean of three replications, Figures in parentheses are arc sine transformed values

Figure 4. Inhibition of *R. bataticola* by endophytic bacterial PGPMs in dual culture assay.

DISCUSSION

The cultural characters of bacterial entophytic PGPMs in the present investigation indicated that the strains varied with respect to colony colour (dark yellow, maroon, gray, cream, yellow, brown, white, creamy white, creamy yellow, light cream, light gray, light yellow), colony form (circular and irregular), colony margin (entire and undulate), cell shape (rod and cocci) and staining reaction. Similar variations in cultural characteristics like colony colour, colony form and colony margin were observed by Hadimani (2018) who isolated bacterial endophytes from tomato plants. Out of eight effective bacterial endophytes, three isolates such as RBDNA-4, SBHKA-6 and SBBSA-11 showed yellowish colour on Nutrient Agar. Whereas, RBDDE-14 and SBDVA-9 showed bright yellowish and dull creamish colonies on both Nutrient Agar and King's B media. In the present investigation also some isolates produced creamish and yellow-coloured colonies. With regard to colony form, RBDNA-4 was irregular on both the media and the remaining isolates showed regular colony. The margin was undulating in RBDNA-4 on both the media and the remaining isolates were of entire margin except SBKHA-2 and SBDOF-6 which showed undulated margin on Nutrient Agar. Similar variations were recorded with respect to colony form and colony margin in the present study.

The morphological characteristics such as staining reaction and cell shape also varied among the thirty bacterial endophytic PGPM strains. Likewise, Salo and Novero (2021) collected five bacterial samples from coconut plumule explants in tissue culture and plated on Nutrient

Agar medium. Macroscopic features of the isolated bacterial colonies were assessed for the colour of colony, elevation, margin, consistency and the surface of the colony. The shape of isolates ranged from cocci to short rods. The isolate CEB1 was Gram-negative and coccobacillus in shape as it was intermediate between coccus and rod shape. Whereas, isolates CEB2, CEB3, CEB4 and CEB5 were Gram-positive and bacilli. In the present investigation also the PGPMs showed a similar kind of variation with respect to cell shape and staining reaction. With respect to cell shape, some isolates were rod-shaped and some were cocci. With respect to staining reaction, PGPMs showed Gram positive and Gram negative reaction as indicated in the present investigation results.

Thirty strains of bacterial endophytic PGPMs were screened against *R. bataticola* for mycelial inhibition. The reason behind the highest inhibition capacity may be due to the antibiosis or competition for the nutrients for their growth and production of HCN. Chiranjeevi *et al.* (2020) assessed the antagonistic effect of endophytic bacterial isolates based on their ability to inhibit pathogen growth in dual culture. A total of 40 endophytic bacterial isolates were evaluated for their antagonistic activity against chickpea dry root rot caused by the pathogen *R. bataticola*. From the data, it is evident that, among the isolates tested CREB 37 showed significant maximum inhibition (74.07%) against *R. bataticola* followed by CREB 15 (71.11%), CREB 21 (71.11%), CREB 36 (64.44%), CREB 16 (60.00%). The least inhibition (0.00%) was recorded in control. In the present study also eight isolates (BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-15, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30) showed more than 50% mycelial inhibition of pathogen which were on par with each other, highest being BEPGPM-30 (83.15%). Similarly, Bhavani *et al.* (2015) tested sixty-three endophytic bacterial isolates for their antagonistic properties against *R. bataticola* causing dry root rot in chickpea by dual culture technique. Out of them, five isolates showed significant inhibition. The isolate B5 was shown the most significant inhibition with 81%, K1 with 77, K2 with 75, C2 and A8 with 74% respectively.

With respect to molecular detection, five highly superior strains were subjected to 16S rDNA gene sequencing with universal primers, which produced amplified products of size 1500 bp. The nBLAST results of the 16S rRNA gene sequence revealed that all the endophytic bacterial PGPMs showed homology with genus *Bacillus* but with different species. Based on the results, the strains namely, BEPGPM-5, BEPGPM-9, BEPGPM-27, BEPGPM-28, BEPGPM-30 were identified and confirmed as *B. tropicus*, *B. tropicus*, *B. pacificus*, *B. cereus, B. subtilis,* respectively during the present investigation.

CONCLUSION

The present investigation inferred that out of thirty bacterial endophytic PGPMs, five strains were identified as *B. tropicus* (2 strains), *B. pacificus*, *B. cereus* and *B. subtilis*. These identified strains are proficient in inhibiting the growth of *R. bataticola*. Therefore, they can be used for growth promotion and one of the components in the integrated management of chickpea dry root rot.

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