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Isolation and evaluation of bacterial endophytes against *Sclerospora graminicola* (Sacc.) Schroet, the causal of pearl millet downy mildew

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Abstract

Background: Pearl millet remains prone to many diseases; among them downy mildew caused by *Sclerospora graminicola* (Sacc.) Schroet is economically more important. The use of endophytic bacteria for management of downy mildew of pearl millet as eco-friendly approach is increasing attention as sustainable alternative to pesticides. The objective of the present study was to isolate endophytic bacteria from roots of pearl millet cultivars and assess for biocontrol activity against *Sclerospora graminicola*.

Results: Thirty pearl millet root bacterial endophytes (PMRBEs) were isolated and screened in vitro for biocontrol activities such as: siderophore production, hydrogen cyanide (HCN) production and 1-amino cyclopropane-1-carboxylate (ACC) deaminase activity. Sixteen isolates possessed siderophore production potential, 3 isolates were found to be HCN producers, and 30% of the bacterial endophytes showed a good growth on ACC supplemented plates. On the basis of biocontrol activities, promising endophyte PMRBE6 was selected for seed treatment as well as a foliar spray to manage downy mildew of pearl millet in screen house experiment. The isolate PMRBE6 was found to be effective in managing downy mildew disease. Grain yield, test weight, plant height and average number of productive tillers were found to be maximum on inoculation of seeds of different pearl millet cultivars with PMRBE6, and the results were statistically significant as compared to control.

Conclusions: On the basis of biochemical characterization and partial 16S rRNA sequencing, the isolate PMRBE6 was identified as *Bacillus subtilis* strain PD4 (Accession no. MN400209). Pearl millet root bacterial endophyte (PMRBE6) exhibiting biocontrol activities could be exploited in friendly, sustainable organic agriculture.

Keywords: Biocontrol, *Sclerospora graminicola*, Downy mildew, Endophyte, Pearl millet, Siderophore

Background

Pearl millet [*Pennisetum glaucum* (L.) R.Br. Syn. *Pennisetum americanum* (L.) Leeke] is a short duration rain fed crop mostly grown in low fertile soil, able to thrive well in the rainfall as low as 250 mm. The crop is cultivated for grain as well as fodder, and the fodder of this crop is

of excellent quality. This is the oldest cultivated crops of Asian and African countries due to its adaptability under very wide range of agro-climatic conditions. Pearl millet remains prone to many diseases; among them downy mildew is economically more important. *Sclerospora graminicola* (Sacc.) Schroet, incitant of downy mildew of pearl millet, is an obligate, pathogenic fungus belonging to family *Peronosporaceae*, order *Peronosporales*.

The use of endophytic bacteria for managing pathogenic fungi and bacteria is receiving increasing attention as sustainable alternative to pesticides, etc. Their

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application can reduce the use of chemical fertilizers, pesticides and effectively alleviate environmental pollution. Endophytes colonize the internal tissues of plants without causing any visible harm to plants. Endophytic bacteria have been found virtually in every plant, where they colonize internal tissues of their host plants and can form different relationships like symbiotic, mutualistic, commensalistic and trophobiotic. They influence plant growth by producing phytohormones such as indole acetic acid, cytokinins and gibberellins (Sandhya et al. 2017). The metabolic activities of endophytes can help in phosphate solubilization (Prakash and Arora 2019), atmospheric nitrogen fixation and reduce ethylene production by 1-amino cyclopropane-1-carboxylate (ACC) deaminase activity (Souza et al. 2015). Some endophytes inhibit pathogenic micro-organisms by producing siderophores (Wang et al. 2013) and antimicrobial metabolites (Liu et al. 2016). Endophytic bacteria also secrete alkaloids, steroids and enzymes which protect plants against pathogenic invasion (Banik et al. 2016). Bacterial endophytes are promising alternative for plant disease control and have potential to be used in environmental restoration and agriculture. Endophytic bacteria are ubiquitously distributed in most plant species either through their active colonization or as latent residents in plant tissues. In contrast to phytopathogenic bacteria, they do not cause any disease symptoms; indeed, they can promote plant growth (Berg 2009). Endophytic microorganisms can vary based on plant source, age, type of tissue, season of sampling and environment. Endophytes have been reported to be isolated from all plant tissues. In most of the plants, roots have the higher endophytic population as compared to above-ground tissues (Rosenblueth and Martínez-Romero 2004); and their population decreases progressively from the stem to the leaves (Quadt-Hallman et al. 1997).

Endophytes influence plant growth after establishment in a plant and provide resistance. Endophytic bacteria on the basis of their effect on host plants can be divided into 3 groups, viz. plant-growth promoting, plant-growth inhibiting and plant-growth neutral (Bai et al. 2002). The plant growth promoting bacteria can be used to manage plant pathogens, insects and nematodes through various mechanisms (Ryan et al. 2008). Siderophore production is one of the traits that make microorganisms successful competitors in various environments and facilitate plant bacterial association as well as colonization of roots, stem and leaves thus, make iron unavailable to pathogenic microorganisms.

The usage of endophytic bacteria for management of downy mildew of pearl millet aimed to be an eco-friendly approach. The objective of the present study was to isolate endophytic bacteria from roots of pearl millet

cultivars and assess for biocontrol activity against *Sclerotinia graminicola* within the framework of integrated plant disease management (IDM).

Methods

Isolation of bacterial endophytes

Roots of pearl millet cultivars, viz. HHB226, HHB67 Imp and 7042 S, were collected after harvesting of the pearl millet crop from infested plots of Plant Pathology experimental area of CCS Haryana Agricultural University Hisar with latitude 29° 14' N and longitude of 75° 70' E. Roots were washed with running tap water and then, surface sterilized sequentially in 75% (v/v) ethanol for 2 min, 2.6% (w/v) sodium hypochlorite solution for 5 min and 75% (v/v) ethanol for 1 min. Finally, roots were thoroughly washed 6 times with sterile distilled water, and the final wash was spread on nutrient agar (NA) plates and incubated at 28 ± 2 °C for 3 days as sterility check. For isolation of bacterial endophytes, 1-g root sample was crushed in pestle and mortar with 10 ml sterile distilled water to get homogenous paste and allowed to settle down for 20 min. The supernatant was diluted, and approximately 10 µl was spread on NA plates and incubated at 28 ± 2 °C for 3 days. Bacterial colonies considered as endophytes were characterized according to different visual observations and finally purified using streak plate technique. The bacterial colonies were maintained at 4 ± 1 °C for further studies.

Screening of endophytic bacteria for biocontrol activity

Bacterial endophytes were tested for biocontrol activities, viz. siderophore production, HCN production, and ACC deaminase activity.

Siderophore production

Pearl millet root bacterial endophytes were evaluated for siderophore production on chrome azurol S assay plates (Schwyn and Neilands, 1987). Five µl inoculant of each log phase grown bacterial culture was spotted on chrome azurol agar plates and incubated at 28 ± 2 °C for 5 days. The presence of siderophore was indicated by decolourization of the blue-coloured ferric dye complex, resulting in yellow halo zones around the colonies.

HCN production

HCN production potential of pearl millet root bacterial endophyte was detected using alkaline picrate filter paper (Alstrom and Burns 1989). The inoculum of different bacterial endophytes was prepared by inoculation of 48-h-old culture from nutrient agar slants into freshly prepared King's B broth. The production HCN was detected after 72 h of incubation at 28 ± 2 °C, using picrate/ Na_2CO_3 paper fixed underside of test tube. A change

Fig. 1 Siderophore production ability of pearl millet root bacterial endophytes

of colour from yellow to brown, brown, reddish brown was recorded as indication of weak, moderate or strong cyanogenic potential.

ACC deaminase activity

1-aminocyclo propane-1-carboxylate (ACC) utilization as indicator of ACC deaminase activity of pearl millet root bacterial endophytes was assayed by inoculating 48-h-old bacterial culture on minimal medium agar plates supplemented with 2 mM ACC/ammonium sulphate (Penrose and Glick 2003). Growth on ACC and ammonium sulphate supplemented medium plates was recorded after 5 days of incubation at 28 ± 2 °C. The bacterial isolates showing good growth on ACC supplemented plates indicated the efficiency of ACC deaminase activity, while growth on ammonium sulphate was used as control. The isolate PMRBE6 showing all growth promoting characters was selected for morphological, biochemical characterization and screen house experiment.

Morphological and Biochemical characterization

Pearl millet root bacterial endophyte PMRBE6 was observed for morphological characters, viz. cell shape, colour, colony morphology and Gram reaction. Biochemical characterization was done as per procedure described in Bergey's manual of Determinative Bacteriology (Holt et al. 1994).

Molecular characterization of pearl millet root bacterial endophyte

Promising bacterial endophyte PMRBE6 retrieved from pearl millet roots was identified on the basis of partial 16S rRNA sequencing. The genomic DNA of endophytic

bacterium was isolated using cetyltrimethyl ammonium bromide (CTAB) method and amplified using forward primer 5' AGA GTT TGA TCC CTC AG 3' and reverse primer 5' AAG GAG GTG ATC CAG CCG CA 3'. The PCR reaction consisted of 1 µl of template DNA, 400 ng of 16S forward primer, 400 ng of 16S reverse primer, 4 µl dNTP (2.5 mM, each), 10 µl 10× Taq DNA polymerase Assay Buffer and 1 µl Taq DNA Polymerase Enzyme (3U/µl). Final volume of reactive mixture was adjusted to 100 µl. PCR was carried out in a thermocycler with an initial denaturation for 5 min at 95 °C, then 35 cycles of 30 s at 94 °C, 15 s of 50 °C, 1.30 min at 72 °C and a final extension for 7 min at 72 °C. The amplified PCR products were analysed by electrophoresis on 1% agarose gel with Tris-acetate-EDTA (TAE) buffer and visualized on a gel documentation system. The partial sequence of 16S rRNA gene of promising bacterial endophyte was obtained after sequencing from Chromous BioTech Pvt. Ltd., Bangalore, and phylogenetic tree was prepared by neighbour joining of BLAST programme. The identified gene sequence was submitted to NCBI GenBank, and accession number was obtained.

Evaluation of pearl millet root bacterial endophyte for plant growth promoting ability under screen house conditions

Bacterial endophyte PMRBE6 was tested for the management of pearl millet downy mildew under screen house conditions during the fall of 2017 and 2018. The experiment was conducted with 5 treatments in a completely randomized design and with 4 replications of each

Table 1 Biocontrol activities of pearl millet root bacterial endophytes

Isolate	Siderophore production	HCN Production	ACC utilization
PMRBE1	+	—	++
PMRBE2	+	—	+
PMRBE3	—	—	+
PMRBE4	+	—	++
PMRBE5	+	—	+
PMRBE6	+	+	+++
PMRBE7	+	—	+++
PMRBE8	—	—	+
PMRBE9	+	—	+++
PMRBE10	+	—	+++
PMRBE11	—	—	++
PMRBE12	—	—	+
PMRBE13	—	—	+
PMRBE14	—	—	+++
PMRBE15	—	—	+
PMRBE16	+	—	++
PMRBE17	+	—	++
PMRBE18	+	—	+
PMRBE19	—	—	+++
PMRBE20	+	—	+
PMRBE21	—	—	++
PMRBE22	—	—	+
PMRBE23	+	—	+
PMRBE24	—	—	+++
PMRBE25	+	+	+++
PMRBE26	+	—	+++
PMRBE27	—	—	+
PMRBE28	+	+	++
PMRBE29	—	—	++
PMRBE30	—	—	—

+++ : Good growth, ++: moderate growth, +: poor growth, —: no growth

treatment. Earthen pots filled with 5 kg of sterilized soil (sand + FYM mix) were mixed with oosporic material.

Seeds of pearl millet cultivars, viz. HHB 226 (Resistant), HHB-67 Imp (Moderately resistant) and 7042S (Highly susceptible) were surface sterilized with 0.2% sodium hypochlorite for 2–3 min, later washed with distilled water several times and dried using sterile blotter paper. Seeds were inoculated by PMRBE6 @5 ml/kg seed, metalaxyl @ 6 g/kg seeds and foliar spray of selected endophyte PMRBE6 @ 10^8 ml⁻¹ till run off and chemical metalaxyl MZ @ 0.25% was given on 2-week-old seedling. Suitable control was maintained as check in which seeds were surface sterilized and kept uninoculated. Five seeds were sown in each pot, and after germination, only 2 plantlets in each pot were maintained. The observations on downy mildew disease incidence at 30 and 60 days after sowing (DAS), plant height, average number of productive tillers/plant, yield as well as test weight were recorded. Statistical analysis was carried out through online software <https://www.hau.ac.in/page/o-p-stat>.

Results

Isolation of pearl millet root bacterial endophytes and their evaluation for biocontrol activities

A total of 30 pearl millet root bacterial endophytes of various morphology (PMRBE1-PMRBE30) were retrieved from roots of pearl millet raised during the fall of 2016 and 2017. All endophytic bacterial isolates were screened for biocontrol activity by assessing their ability for siderophore production, HCN production and ACC utilization.

Siderophore production

Out of 30 pearl millet root bacterial endophytes, 16 isolates, viz. PMRBE1, PMRBE2, PMRBE4, PMRBE5, PMRBE6, PMRBE7, PMRBE9, PMRBE10, PMRBE16, PMRBE17, PMRBE18, PMRBE20, PMRBE23, PMRBE25, PMRBE26 and PMRBE28, showed development of orange halo zone on CAS medium amended with ferric chloride indicating siderophore production ability (Fig. 1).

Hydrogen cyanide production

Among 30 pearl millet root bacterial endophytes screened for HCN production, change in the colour of soaked alkaline picrate filter paper from yellow to brown was observed in isolates PMRBE6, PMRBE25 and PMRBE28.

1-aminocyclopropane-1-carboxylate (ACC) utilization

On ACC supplemented plates, out of thirty endophytic bacterial isolates, nine isolates (30%), viz. PMRBE6,

Fig. 2 Categorization of pearl millet root bacterial endophytes for ACC utilization

Fig. 3 ACC utilization by pearl millet root bacterial endophytes

Fig. 4 PCR products of endophytic bacterial isolate PMRBE6 loaded on 1% agarose gel

PMRBE7, PMRBE9, PMRBE10, PMRBE14, PMRBE19, PMRBE24, PMRBE25 and PMRBE26, showed good growth, eight isolates (27%), viz. PMRBE1, PMRBE4, PMRBE11, PMRBE16, PMRBE17, PMRBE21, PMRBE28 and PMRBE29, showed moderate growth, 12 isolates (40%) showed poor growth and the isolate PMRBE30 (3%) showed no growth (Table 1, Figs. 2, 3).

Morphological and biochemical characterization of promising pearl millet root bacterial endophyte, PMRBE6

Morphological characterization of PMRBE6 revealed that bacterium was fast growing, rod shaped with whitish, circular, smooth and raised colony on nutrient agar medium. Gram reaction revealed that endophyte PMRBE6 was Gram positive.

Molecular characterization of pearl millet root bacterial endophyte PMRBE6

Sequencing of the 16S rRNA gene is an important tool for identification and characterization of bacteria. In the present study, the promising pearl millet root bacterial endophyte PMRBE6 showed 97.6% similarity with *Bacillus subtilis* strain PD4 (Accession no. MN400209) on the basis of 16SrRNA sequencing (Figs. 4, 5).

Effect of pearl millet root bacterial endophyte on downy mildew incidence under screen house conditions

On the basis of biocontrol activities promising endophyte, PMRBE6 was selected for seed treatment as well as foliar spray to manage downy mildew in pearl millet cultivars, HHB 226, HHB-67 Imp and 7042S. Downy mildew incidence at 30 days after sowing (DAS) was recorded as nil on inoculation of HHB 226 seeds with isolate PMRBE6, with metalaxyl MZ, seed treatment+foliar spray with bacterial isolate PMRBE6 and seed treatment+foliar spray with metalaxyl MZ, while it was 50% in control. In HHB67 Imp, no disease was recorded on seed treatment with metalaxyl+foliar spray with metalaxyl MZ. In 7042 S minimum disease incidence (31.3%) was observed on seed treatment with isolate PMRBE6, seed treatment+foliar spray with bacterial isolate PMRBE6, seed treatment with metalaxyl+foliar spray with metalaxyl MZ, while in control disease incidence it was 93.75%.

Downy mildew incidence at 60 days after sowing was recorded nil in HHB226 on inoculation of seeds with PMRBE6 and also was nil on seed treatment+foliar spray with isolate PMRBE6 and seed treatment+foliar spray with metalaxyl MZ. In HHB 67 Imp, it was 6.3% on inoculation with isolate PMRBE6, on seed

treatment+foliar spray with isolate PMRBE6 and on seed treatment+foliar spray with metalaxyl MZ. In 7042 S cultivar, seed treatment with bacterial isolate PMRBE6 and seed treatment+foliar spray with isolate PMRBE6 recorded minimum disease incidence of 50% (Table 2).

Effect of pearl millet root bacterial endophyte PMRBE6 on growth attributes under screen house conditions

PMRBE6 significantly affected average number of productive tillers/plant and height of pearl millet cultivars (Table 3). In HHB 226 and HHB67 Imp, average number of productive tillers was maximum (2.8 tillers/plant and 2.7 tillers/plant) on inoculation of seeds+foliar spray with isolate PMRBE6 and in 7042 S average number of productive tillers/plant was 1.3 on inoculation of seeds of 7042 S with isolate PMRBE6 as compared to control (0.0).

Maximum plant heights of 164.4, 151.0 and 131.7 cm in HHB226, HHB67 Imp and 7042 S, respectively, were recorded on seed treatment with isolate PMRBE6 as compared to 135.0, 127.2 and 121.3 cm, respectively, in control.

Impact of pearl millet root bacterial endophyte PMRBE6 on yield attributes under screen house conditions

Grain yield of HHB226 was maximum (78.7 g/plant) on seed treatment with metalaxyl+foliar spray with metalaxyl MZ @ 0.25%, which was statistically *at par* to 78.3 g/plant on seed treatment+foliar spray with isolate PMRBE6. In HHB67 Imp and 7042 S, maximum yield (78.3 g/plant and 42.7 g/plant) was recorded on seed treatment+foliar spray with isolate PMRBE6, which was significantly higher than control. Test weight of HHB226 seeds was 8.9 g on seed treatment+foliar spray with isolate PMRBE6. Seeds of HHB67 Imp and 7042 S had

Table 2 Effect of bacterial endophyte on downy mildew incidence in pearl millet under screen house conditions

Treatments	Kharif 2017				Kharif 2018				Pooled mean			
	Downy mildew incidence at 30 DAS (%)				Downy mildew incidence at 60 DAS (%)				Downy mildew incidence at 30 DAS (%)			
	HHB 226	HHB67 Imp	7042S	HHB 226 HHB67 Imp	HHB 226 HHB67 Imp	7042S	HHB 226 HHB67 Imp	7042S	HHB 226 HHB67 Imp	HHB 226 HHB67 Imp	7042S	7042S
Seed treatment with PMRBE6	0.0 (0.0)	12.5 (11.2)	37.5 (33.7)	0.0 (0.0)	12.5 (11.2)	50.0 (44.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.3 (5.6)	31.3 (28.1)	50.0 (44.9)
Seed treatment + Foliar spray with PMRBE6	0.0 (0.0)	12.5 (11.2)	37.5 (33.7)	0.0 (0.0)	12.5 (11.2)	50.0 (44.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.3 (5.6)	31.3 (28.1)	50.0 (44.9)
Seed treatment with metalaxyl	0.0 (0.0)	25.0 (22.4)	50.0 (44.9)	12.5 (11.2)	37.5 (33.7)	62.5 (56.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	12.5 (11.2)	50.0 (44.9)	68.6 (61.8)
Seed treatment with metalaxyl + Foliar spray with metalaxyl	0.0 (0.0)	0.0 (0.0)	37.5 (33.7)	0.0 (0.0)	12.5 (11.2)	62.5 (56.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	31.3 (28.1)	68.6 (61.8)
Control	50.0 (44.9)	37.5 (33.7)	87.5 (78.7)	50.0 (44.9)	50.0 (44.9)	100.0 (90.0)	50.0 (44.9)	50.0 (44.9)	50.0 (44.9)	43.75 (39.3)	93.75 (84.4)	100.0 (90.0)
CD at (1%)	0.03	NS	30.60	2.08	NS	21.65	0.03	0.03	0.3	—	—	—

Figures in parenthesis are angular transformed value

Table 3 Effect of bacterial endophyte PMRBE6 on growth attributes of pearl millet under screen house conditions

Treatments	Kharif 2017					Kharif 2018					Pooled mean				
	Average No. of productive tillers/plant		Plant height (cm)			Average No. of productive tillers/plant		Plant height (cm)			Average No. of productive tillers/plant		Plant height (cm)		
	HHB 226	HHB67 Imp	7042S	HHB 226	HHB67 Imp	HHB 226	HHB67 Imp	HHB 226	HHB67 Imp	HHB 226	HHB67 Imp	HHB 226	HHB67 Imp	HHB67 Imp	7042S
Seed treatment with PMRBE6	2.5	2.4	1.5	159.0	149.8	127.0	2.5	169.7	152.1	136.3	2.5	2.5	164.4	151.0	131.7
Seed treatment + Foliar spray with PMRBE6	2.5	2.6	1.3	152.8	146.8	128.1	3.0	154.6	127.7	133.1	2.8	2.7	153.7	137.3	130.6
Seed treatment with metalaxyl	2.5	2.0	0.8	158.4	142.3	122.0	2.0	159.5	128.8	123.3	2.3	1.5	159.0	135.6	122.7
Seed treatment with metal-axyl + Foliar spray with metalaxyl	2.5	2.0	0.9	154.6	145.0	121.7	2.5	153.4	138.9	121.2	2.5	2.3	154.0	141.6	121.5
Control	1.5	1.4	0.0	140.1	131.8	120.0	1.5	129.8	122.6	115.7	1.5	1.4	135.0	127.2	121.3
CD at (1%)	0.5	0.5	0.4	6.5	3.0	2.0	0.2	4.7	2.8	5.2	—	—	—	—	—

Table 4

Treatments	Kharif 2017				Kharif 2018				Pooled mean							
	Yield (g/plant)		Test weight (g)		Yield (g/plant)		Test weight (g)		Yield (g/plant)		Test weight (g)					
	HHB 226	HHB67 Imp	7042S	HHB 226	HHB67 Imp	HHB 226	HHB67 Imp	7042S	HHB 226	HHB67 Imp	7042S	HHB67 Imp				
Seed treatment with PMRBE6	86.3	85.8	37.5	8.6	9.5	8.2	67.5	69.5	46.3	9.0	9.6	7.9	41.9	8.8	9.6	8.1
Seed treatment + Foliar spray with PMRBE6	88.0	86.3	37.3	8.8	8.6	7.8	68.5	70.3	48.0	9.0	9.8	8.0	78.3	8.9	9.2	7.9
Seed treatment with metalaxyl	87.3	68.3	30.3	8.2	8.8	7.0	68.5	69.8	28.3	8.2	8.9	7.7	77.9	8.2	8.9	7.4
Seed treatment with metalaxyl + Foliar spray with metalaxyl	88.3	72.0	29.5	8.4	8.9	7.3	69.0	70.8	26.5	8.1	9.0	7.7	78.7	8.3	9.0	7.5
Control	52.8	53.5	0.0	8.0	8.1	0.0	58.8	56.3	0.0	7.9	8.4	0.0	55.8	8.0	8.3	0.0
CD at (1%)	6.2	6.4	11.3	0.3	0.3	0.2	4.0	3.6	NS	0.3	0.3	0.4	—	—	—	—

test weight of 9.6 g and 8.1 g on inoculation with isolate PMRBE6, respectively (Table 4).

Discussion

Pearl millet is prone to many diseases, and amongst them downy mildew is economically more important. Endophyte influence plant growth after establishment in a plant and provide resistance. Endophyte bacteria can promote plant growth and health by other mechanisms like siderophore production, cyanogenic potential and ACC deaminase activity. Siderophore are low molecular weight compounds with high iron chelating affinity and responsible for solubilization and transport of iron into bacterial cells. Iron is an essential mineral, and its sequestration by specific endophytic bacterial siderophores can make it available to plants under iron limiting conditions and also make it unavailable to pathogenic microbes, thereby affecting the extent of pathogenicity. In the present study, out of 30 pearl millet root bacterial endophytes, 16 isolates showed siderophore production ability as indicated by development of orange halo zone on CAS medium amended with ferric chloride exhibiting the suitability for biocontrol activity. Siderophore production ability in endophytic bacteria isolated from various crops has also been reported by different researchers. Joshi et al. (2018) isolated 10 bacterial cultures from roots, stem and leaves of *Ocimum sanctum* and *Aloe vera* and reported siderophore production ability in 3 isolates. Similarly, Etemani and Harighi (2018) reported that out of 10 isolates retrieved from the leaves and stems of healthy wild Pistachio trees, 5 isolates, viz. Pb1, Pb71, Pb78, Sp15 and Bp108, were able to produce siderophores.

Bacterial endophytes produce hydrocyanic acid which acts as an inducer of resistance and constitutes a mechanism of defence against pathogens in plants. This volatile compound inhibits the electron transports, disrupts the energy supply to cells, thus ultimately leading to death of the pathogens (Aarab et al. 2015). Screening of pearl millet root bacterial endophytes for HCN production revealed that 3 isolates were able to produce HCN along with siderophore production potential. The results were in corroboration to the findings of Padder et al. (2017) that amongst 81 endophytes isolated from root samples of brown sarson (*Brassica rapa* L.), 15 endophytic isolates produced HCN.

Ethylene is overproduced in plants as a result of wide range of biotic and abiotic stresses. In all higher plants, 1-aminocyclo propane-1-carboxylate (ACC) is the

precursor of plant hormone ethylene. Some bacterial endophytes have the capability to produce ACC deaminase enzyme, which catalyses the degradation of ACC into α -ketobutyrate and ammonia, thereby reducing stress induced ethylene level in plants and also reduce negative consequences of ethylene on plant growth and development (Sun et al. 2009). In the present study, out of 30 endophytic bacterial isolates, 9 isolates (30%) showed good ACC deaminase activity. The results were in close agreement with the findings of Hynes et al. (2008) that out of 563 bacteria originating from pea, lentil and chickpea roots, 5% isolates showed ACC deaminase activity. Similarly, Etesami et al. (2014) isolated 200 bacterial isolates from rhizospheric soil and roots, nodules of berseem clover plants and found that only 72 isolates were positive for ACC deaminase production.

The bacterial isolate PMRBE6 found promising for different biocontrol attributes was identified as *B. subtilis* strain PD4 based on morphological, biochemical and molecular characterization (Accession no. MN400209). Different species of *Bacillus* have also been reported as endophytes for biocontrol activity in different crop disease management. The results are in close agreement with findings of Kumar et al. (2016).

Downy mildew incidence at 30 and 60 DAS was recorded minimum on inoculation of HHB 226, HHB67 Imp and 7042 S seeds with isolate PMRBE6 and with seed treatment + foliar spray with isolate PMRBE6. The results were in accordance with findings of Chandrashekhra et al. (2007) that endophytic bacteria showed significant growth promoting effects on pearl millet crop and exhibited resistance against downy mildew caused by *Sclerospora graminicola* under pot house conditions with disease reduction ranging from 15 to 53%.

Seed treatment with endophytic bacteria was reported to improve vegetative growth parameters such as plant height, fresh weight, dry weight and number of basal tillers in pearl millet over control (Chandrashekhra et al. 2007). Similarly, in the present study, inoculation of pearl millet seeds with isolate PMRBE6 showed positive effect on various growth parameters of pearl millet.

Conclusions

The pearl millet bacterial root endophyte PMRBE6, identified as *B. subtilis* strain PD4, exhibiting various plant growth promoting traits in the present study stands out as possible candidate for usages as biocontrol agent against *S. graminicola*, the causal of pearl millet downy mildew.

Abbreviations

CD: Critical difference; MR: Methyl red; VP: Voges–proskauer.

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Authors' contributions

PS contributed to methodology, data collection, statistical analysis, writing—original manuscript. KR contributed to conceptualization, planning, conductance, monitoring, writing—review and editing. LW contributed to endophyte identification, review and editing and AK contributed to conceptualization, planning, supervision, examination of pathogen prevalence. All authors proof-read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed in this study are available in this manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

This study does not contain any individual person's data and authors consent for publication.

Competing interests

The authors declare that they have no competing interests.

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