J.Mycopathol.Res. 62(4) : 777-785, 2024, ISSN : 0971-3719 (Print), 2583-6315 (Online) 10.57023/JMycR.62.4.2024.777

Characterization of endophytic bacteria isolated from cotton and pigeon pea for plant health management

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Received : 14.07.2024 Accepted : 21.10.2024 Published : 30.12.2024

Plant growth promotion (PGP) and biocontrol activities of endophytes provide sustainable alternatives to chemical use in agriculture. In this study, bacterial endophytes were isolated from the leaves, shoots and roots of cottonand pigeon peaplants. Using the Biolog system, microbial isolates were identified as members of the *Bacillus* genus in which cotton endophytic bacteria (CEB) isolatesCEB1 (*B. safensis*) and CEB2 (*B. subtilis*)found in the roots andCEB3 (*B. amyloliquefaciens*)were found in the shoots. Pigeon pea endophytic bacteria (PEB), PEB4 and PEB5 both identified as *B. qingdaonensis* which were derived from the roots. Among the tested strains for biocontrol activity, the CEB3 (*B. amyloliquefaciens*) strain exhibited maximum mycelial growth inhibition against various fungal plant pathogens. All analyzed bacterial endophytes possessed PGP activities and could produce indole-3-acetic acid (IAA), hydrogen cyanide (HCN), siderophore, ammonia, and P-solubilizing enzymes. CEB3 endophytic strains recorded maximum production of IAA, HCN, and siderophorescompared to other endophytes. The seed priming dose of CEB3 applied at 6g/kg to finger millet recorded maximum shoot and root length, shoot and root weight, and the highest vigor index. Higher extracellular enzymatic activities were observed in all bacterial endophytes.

Keywords : *Bacillus*,endophytes, microbial enzymes, plant growth promotion, plant stress tolerance, secondary metabolites

INTRODUCTION

The climate change-induced unpredictable abiotic and biotic stress events negatively affect plant growth and production (Shelake *et al*. 2022). Integrated management of pests and diseases using available inputs is one of the main challenges in recent times (Deguine*et al*. 2021). Using chemical fertilizers, pesticides and other agrochemicals has contributed to higher food grain production across the globe over the last 40 years. Still, it is not sustainable for several reasons (Sharma *et al*. 2019). Consequently, chemical alternatives like microbial formulations are in demand to ensure food security in low-input sustainable agriculture (LISA) (Sarkar *et al*. 2020).

Plant growth-promoting rhizobacteria (PGPR) are indigenous to soil and the plant rhizosphere and play a significant role in the biocontrol of phytopathogens.Recently, insights about the PGPR regarding their diversity, colonizing ability, mode of action and formulations allow their development as reliable biocontrol agents against phytopathogens (Verma *et al*. 2020 and Siddiqui, 2006). Each plant species may acquire a unique set of endophytic microbiomes that dwell in different plant parts. Antimicrobial compounds, secondary metabolites and phytohormones produced by endophytes contribute to the host

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plant defense and PGP activities (Fontana *et al*. 2021; Grabka *et al*. 2022). Overall, the mutualistic interaction of PGPR and endophytes contributes significantly to crop growth and yield (Lacava *et al*. 2022). In the current study, we isolated and characterized bacterial endophytes possessing PGP or biocontrol activitiesisolated from the leaves, shoots and roots of cotton and pigeon pea plants. Biochemical analysis and evaluation against phytopathogens were performed in the laboratory and in greenhouse conditions. Comparative studies of endophytes and PGPR for plant growth and stress tolerance were performed to investigate their potential to be developed as biocontrol agents.

MATERIALS AND METHODS

Isolation and identification

The microbes were isolated from the fields of the National Agricultural Research Project, Polytechnic in Agriculture, Baruch and Regional Cotton Research Station, Navsari Agricultural University, Bharuch, Gujarat, India. Specific details about the sampling site, habitat and codes are described in (Table 1).

Endophytic bacteria were isolated from cotton and pigeon pea plants at three growth stages i.e. 14, 21 and 28 days. Leaf and shoot samples were collected from the above-ground parts of the plants while the root portion was obtained from 5 cm below the soil surface. Notably, the bacterial isolates from cotton leaves and pigeon pea shoots and leaves did not thrive when cultivated under laboratory conditions.The PGPR strains, specifically *Pseudomonas fluorescens* strains (PfWN, PfRB, and PfNC) were identified through a combination of morphological, biochemical, and physiochemical characterization methods, as previously detailed (Waghunde and Sabalpara, 2021). Additionally, five bacterial endophytes were identified using physiological and biochemical characterization techniques. The identification of *Bacillus*strains was carried out using an identification kit from Himedia (India) and further confirmed using the Biolog microplate system (Biolog, Hayward, California, US) following the manufacturer's provided protocol.

The Biolog system is an automated platform technology for classifying microbial species based on their capacity to utilize specific carbon sources. Briefly, a single colony of endophytic isolates was suspended in an inoculating fluid and the concentration was adjusted using a turbidimeter. Subsequently, 100 µl of the microbial suspension was introduced into each well of a Biolog microplate which was then incubated at 30°C for 12-24 hrs. The microplates were subsequently analyzed using a Biolog reader which compares the properties of the inoculated sample with the information available in the software's databases to identify matches.

Biocontrol activities of endophytes against phytopathogens

The endophytic bacterial isolates were screened against major phytopathogens commonly found in the South Gujarat cropping system. These phytopathogens included*Pyricularia oryzae* (finger millet blast), *Colletotrichum falcatum* (sugarcane red rot), *Fusarium moniliformae* (sugarcane wilt), *Macrophomina phaseolina* (chickpea root rot), *Sclerotium rolfsii* (groundnut stem rot), *Pythium aphanidermatum* (tobacco damping off), *Pestalotiopsis anacardii* (*Pestolatia*blight of mango) and *Lasiodiplodia theobromae* (stem end rot of mango).These screenings were performed *in vitro* using the dual culture technique (Nawrot-Chorabik *et al*. 2021). Control plates without fungal pathogens were also included.The plates were then incubated at room temperature (27±2°C) for seven days and the radial growth of each fungal pathogen was measured. The study involved four repetitions of each treatment. The percentage of mycelial growth inhibition of fungal pathogens by endophytic isolates was calculated using the following formula :

$$PGI = 100 \times (DC - DT) / DC$$

Where, PGI = percent growth inhibition, DC = average diameter of a mycelial colony (mm) of control, and DT = average diameter of a mycelial colony (mm) of pathogens.

$$[(Ar - As)/Ar]\ 100 = \%\ \text{siderophore units}$$

where, Ar =absorbance of reference (minimal media + CAS assay solution), As=absorbance

of sample (culture supernatant + CAS assay solution).

Estimation of hydrogen cyanide (HCN) production

To estimate the HCN content, TSA medium amended with glycine (4.4g/l) was employed as the medium of choice. The PGPR/bacterial endophytes were streaked on themedia Petri plates.A Whatmanfilter paper soaked with 2 ml sterile picric acid solution (mix of 2% sodium carbonate and 0.5% picric acid) was placed onto each plate. Then, the plates were sealed with parafilm. The color change was monitored for four days at 30°C. Based on the color change from yellow to light brown, brown or reddish-brown was recorded as weak (+), moderate (++), or strong (+++) reaction, respectively(Wei, 1991).

For quantitative analysis of HCN production bacterial endophytic cultures wereinoculated in TSAbroth supplemented with glycine. The bacterial culture was grownfor five days on a rotating shaker at 30°C and sterile paper strips were monitored at the neck of the flask for color change. The colored portion of the paper was immersed in 10 ml of distilled water and used to record absorbance at 625 nm (Waghunde and Sabalpara, 2021).

Ammonia (NH_3) Production

The ability of bacterial isolates to produce NH_3 was evaluated using a peptone water-based assay (ALKahtani *et al*., 2020). The bacterial endophytes were cultured in peptone water for 72 hrs at 32±2°C. In this evaluation, peptone water without endophytic culture was the negative control. To determine NH_3 production,1 mL of Nessler's reagent was added to the peptone water culture. A faint yellow color indicated minimum NH_3 production while a transition from deep yellow to brownish indicated maximum NH_3production.

Phosphate (P)-solubilization assay

Bacterial endophytes and PGPR were assessed for their ability to solubilize insoluble inorganic phosphate by adding the 24-hrs grown culture onto Pikovskaya's agar plates. The phosphate solubilization activity was observed as clearing zones around the colonies, measured in millimeters, as previously described (Waghunde and Sabalpara, 2021).

Pot experiments for PGP analysis

The endophyte isolate CEB3 was chosen to study the seed priming effect on finger millet due to its higher production of phytohormones and effectiveness against various phytopathogens. The roll-towel paper method was employed for this study (ISTA, 1999). Finger millet seeds (GN-4) were treated with a CEB1 cell suspension (10^7 cfu/g) with five seeds per pot. In addition, a pot experiment was comducted a talc-based formulation of CEB3 at concentrations of 2, 4, and 6g/kg of seeds. Plant height and the number of leaves were recorded at 15-day intervals.

Twenty-five seeds were placed on germination paper, moistened with sterile waterand the paper was rolled up. These rolls were then incubated at a temperature of 25±2°C and a relative humidity of 95±3%. After seven days of germination, measured the mean values of root and shoot length and weight. The PGP effect of CEB3 on finger millet seedlings was assessed in terms of the Vigor Index (VI) calculated by multiplying the percent germination by the mean total length of the seedling and the sum of the root length and shoot length (Reza *et al*. 2020).

RESULTS AND DISCUSSION

Isolation and identification of bacterial isolates

The bacterial samples isolated from the leaf, shoot and root of cotton and pigeon pea plants were subjected to identification using the Biolog system. Metabolic fingerprinting of all the bacterial endophytic isolates was conducted using the Biolog microplate system. This system comprised 71 carbon sources and 23 chemical sensitivity assays. The results unveiled a wide range of metabolic fingerprints related to carbon substrate utilization and chemical sensitivities among the isolates (Fig.1).However, these bacterial endophytes were found to be distinct species namely *B. safensis* (CEB1), *B. subtilis*

(CEB2), *B. qingdaonensis* (PEB4, PEB5) and *B. amyloliquefaciens* (CEB3). Specifically, CEB1and CEB2were isolated from the RCRS cotton root samples.CEB3 isolate was derived from cotton root samples. Both the PEB4 and PEB5 isolates were found in the cotton shoot samples.

Various biochemical properties of potent endophytic bacterial isolatesare summarized in (Table 2). Through biochemical screening tests, distinct enzymatic traits were identified in each endophytic bacterial isolate.For instance, the relevant screening tests indicated that all five isolates exhibited positive results for gelatin hydrolysis but tested negative for ornithine decarboxylase, malonate, and ortho-nitrophenyl-â-galactosidetests. Only PEB4 was positive for the lysine hydrolysis test. All isolates demonstrated citrate utilization except for CEB1. CEB1 yielded a positive result in the methyl red test while all other isolates tested negative.Additionally, PEB5 was favorable for hydrogen sulfide (H_2S) production and glucose utilization whereas the other isolates tested negative for these characteristics.

Physiological factors of culturable endophytic isolates were evaluated and then further characterized. All isolates could grow in the temperature range from 15°C to 37°C (Table 3).In addition, PEB4 and PEB5 isolates tolerated lower temperatures (up to 10°C). Growth assay performed at different pH values indicated that all the isolates tolerated pH range between 5 to 9 and CEB1 and CBE2 could growat higher pH up to 11 (Table 3).CEB2 and CEB3 isolates could tolerate higher pH (up to 11). Moreover, all isolates could tolerate salt (NaCl) concentrations up to 6% (Table 3).

Bacterial isolates exhibit antifungal activities

The antifungal activity of all the endophytes was tested against major phytopathogens known to affect crop cultivation in South Gujarat, India. Among the bacterial endophytic isolates, all except PEB5 exhibited more than 50% growth inhibition under the dual culture technique against all the tested phytopathogens.The CEB3 isolate demonstrated the highest mycelial growth inhibition, ranging from 64.94% to 75.94%, against the various tested fungal phytopathogens (Table 4). Mainly, CEB3 showed exceptional performance in inhibiting the growth of*Fusarium moniliformae* (75.94%),the highest among all the pathogens.Conversely, PEB5 exhibited the lowest growth inhibition against *P. anacardii* (44.71%).Endophytic microbes have been shown to influence host gene expression by modulating physiological responses and defense pathways, ultimately safeguarding host plants (Shelake *et al*., 2019). The ability of endophytes to produce volatile organic compounds provides a broad range of antimicrobial activities against phytopathogenic bacteria, fungi, and nematodes (Khare *et al*. 2018).

PGP and enzymatic activities

The bacterial endophytes displayed significant extracellular enzymatic activities across all isolates. Specifically, all the bacterial endophytes exhibited positive activity for protease, amylase, cellulase and pectinase (Fig2).The highest protease and amylase activities were observed in CEB1with values of 15.55+0.54 mm and 21.12+0.58 mm, respectively. On the other hand, the highest cellulase and pectinase activities were recorded in the isolateCEB3 measuring 19.97+1.20 mm and 22.11+0.81 mm, respectively. These findings highlight the diverse and robust extracellular enzymatic capabilities of the examined bacterial endophytes.

Plant growth promotion through growth hormone production and enzymatic activities related to mineral solubilization are critical factors when considering the application of endophytes and PGP bacteria as biofertilizers. In this context, we evaluated the properties of the bacterial endophytes identified in the current study and compared them with the PGPR isolates reported in our previous work(Waghunde and Sabalpara, 2021). The main objective of these analyses was to gain insights into their collective potential for enhancing plant growth and contributing to plant health management. All bacterial endophytes and PGPR demonstrated a favorable capacity for IAA production (Table 5). Notably, the highest phytohormone IAA production (65.69+0.47) was recorded in CEB3while the lowest (36.33±0.36)

was observed in PfWN(Table 6). Most P-solubilizing bacteria also produce a siderophoreand make it available to plants and other microorganisms. CEB1 recordedthe highest siderophore ûuorescence, while the other isolates recorded medium siderophore ûuorescence. The weak siderophore ûuorescence was observed in PfWN endophytes (Table5). The maximum quantitative production of siderophore was observed in CEB1 (22.11±0.81), while the minimum was seen in PfWN (5.38±0.06) PGPR species. Overall, the endophytic isolates exhibited higher levels of IAA and siderophore production than the PGPR isolates (Table6).

All the bacterial endophytes and PGPR strainstested for HCN production displayed varying HCN production capacities (Table5). The highest HCN production was observed in the isolated PfWN (0.052±0.001), followed by PfNC (0.047±0.006) and PfRB (0.032±0.001), the lowest production was observed in the bacterial endophyte CEB1 (0.01+0.003).In general, PGPR isolates exhibited higher HCN production compared to bacterial endophytes. Furthermore, all the bacterial endophytes and PGPR strains

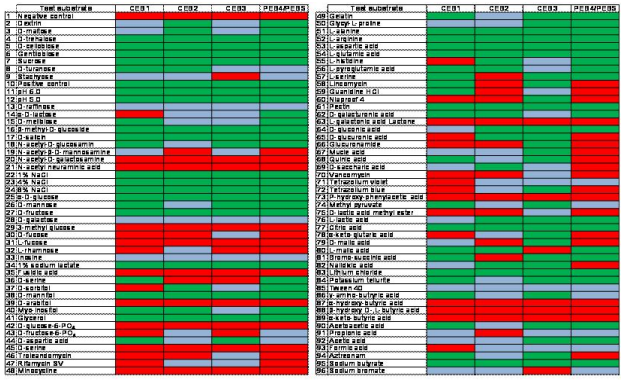

Fig.1: Metabolic profiling of isolated endophytes performed using the Biolog system. Green indicates the positive, red indicates the negative and blue indicates the borderline.

exhibited favorable chitinase production (Table 6). PfNC displayed the highest chitinase enzyme activity (50.06±0.14) followed by PfRB (34.56±0.08). PEB5 endophytic strain exhibited the most increased chitinase activity (16.06±0.24) while the lowest chitinase activity was found in PGPR strain- PfWN (12.36±0.21). Within the endophytic group, CEB1 isolates displayed the lowest chitinase activity (12.45±0.84)(Table 6).Moreover, all bacterial endophytes and PGPR strains demonstrated high ammonia production capabilitiesexcept PEB5 and CEB3. In addition, we observed the formation of P-solubilization zones with PfWN showing the largest zone

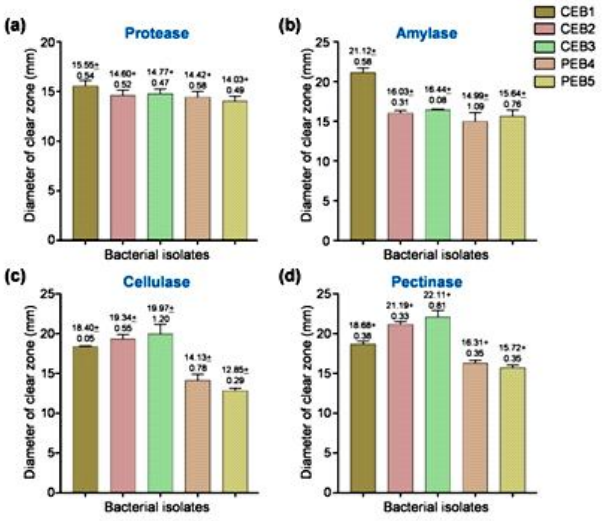

Fig. 2: Hydrolytic enzymatic activities of bacterial isolates from cotton and pigeon pea

The data plotted in the bar graph represents the mean values of five independent replicates. Error bars indicate the standard deviation

(18.00mm) followed by CEB1 (14.22mm).Conversely, PEB5 recorded the most minor P-solubilization zone (10.66mm) (Table5).PGP activities, including IAA production, P-solubilization and NH_3 production were observed in endophytic *B. subtilis* isolated from the *Fagoniamallis*(Foudae*t al*. 2021). In addition, PGP activityi.e., IAA production and nutrient-solubilization, was recorded in different endophytic species belonging to the *Bacillus* genus isolated from the pearl millet (Kushwahae*t al*. 2020). We observed extracellular enzymatic activities exhibited by *B. subtilis*, another specific trait of endophytic members of *Bacillus* strains. For example, *in vitro* activities of amylase, pectinase, CMCase, cellulase, xylanaseand gelatinase were recorded for *Bacillus* spp. isolated from *Fagonia mallis* (Kahtani *et al*. 2020). In other studies, Protease, amylase, lipase, chitinase and pectinase activity of *Bacillus* species were recorded under laboratory and greenhouse conditions (Kushwaha *et al*. 2020 ; Deshmukh *et al*. 2018).

Potential of CEB3 formulations as biofertilizers in finger millet

The seed priming treatment with CEB3 at a 6g/kg dose for finger millet demonstrated superior

Table 1: Sampling site, habitat and code of bacteria and endophytes used during investigation

Sr. No.	Sampling site	Tissue/Region of origin	Habitat	GIS location	Code
	PGPR				
1	Hill Millet Research Station, Navsari Agricultural University, Waghai, Gujarat, India	Rhizosphere	Nagli	20.77' N 73.50' E	PfWN
2	Hill Millet Research Station, Navsari Agricultural University, Rambhas, Gujarat, India	Rhizosphere	Banana	20.80' N 73.62' E	PfRB
3	Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat, India	Rhizosphere	Castor	20.95° N 72.93° E	PfNC
	Endophytes				
4	National Agricultural Research Project, Bharuch, Gujarat, India	Leaf, shoot and root	Pigeon pea	21.71' N 73.01' E	PEB4 PEB5
5	Polytechnic in Agriculture, Bharuch, Gujarat, India	Leaf, shoot and root	Cotton and Pigeon pea	21.68' N 73.01' E	CEB3
6	Regional Cotton Research Station, Bharuch, Gujarat, India	Leaf, shoot and root	Cotton	21.70' N 73.01' E	CEB1 CEB2

Table 2: Biochemical tests performed for identification of endophytic bacteria

Test	Result				
	CEB1	CEB2	CEB3	PEB4	PEB5
Growth on Mac Conkey Agar	-	-	+	+	-
Indole test	-	-	+	-	-
Methyl red test	+	-	-	-	-
Voges Proskauer test	-	-	-	+	+
Citrate Utilization	--	+	+	+	+
Gas production from Glucose	-	-	-	-	+
Casein hydrolysis	+	+	-	-	+
Starch hydrolysis	+	+	-	-	+
Urea hydrolysis	+	-	-	-	+
Nitrate reduction	-	+	+	+	+
Nitrite reduction	-	-	+	-	+
H_2S production	-	-	--	-	+
Catalase test	+	+	+	-	+
Oxidation	-	+	-	-	+
Fermentation	-	-	+	+	-
Gelatin hydrolysis	+	+	+	+	+
Arginine hydrolysis	--	+	+	+	+
Lysine hydrolysis	-	-	-	+	-
Ornithine decarboxylase	-	-	-	-	-
Citrate utilization	+	+	+	+	-
Malonate	-	-	-	-	-
ONPG	-	-	-	-	-

results compared to the control treatment. The higher doses proved the most effective in seed priming and germination studies (Table 7). The control treatment exhibited the lowest germination ratewith only 52.73%.Regarding shoot and root length, the maximum sizes were observed in the 6g/kg seed priming treatment i.e. 6.88 cm and 2.39 cm, respectively. This was followed by the 4g/kg treatment which yielded shoot and root lengths of 4.69 cm and 1.74 cm. In contrast, the control treatment resulted in the shortest shoot and root lengths measuring 1.90 cm and 0.52 cm, respectively. These differences were statistically significant with the 6g/kg treatment consistently outperforming the other doses (Table7). Additionally, the seed priming treatment at 6g/kg demonstrated significantly higher shoot and root weights with values of 4.85 mg and 2.08 mg, respectively compared to the other doses. The 4g/kg treatment also showed

Table 3: Physiological characterization of endophytic bacteria

Test	CEB1	CEB2	CEB3	PEB4	PEB5
Growth at temp. (C^0)					
4	-	-	-	-	+
10	--	-	-	+	+
15	+	+	--	+	+
20	+	+	+	+	+
25	+	+	+	+	+
30	+	+	+	+	+
37	+	+	+	+	+
42	+	+	-	+	+
Growth at pH					
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	-
10	--	+	+	--	-
11	--	+	+	--	-
NaCl (%)					
2	+	+	+	+	+
4	--	+	+	+	+
6	+	+	+	+	+
8	-	+	-	-	+
10	-	--	--	-	+
12	-	-	-	-	-
14	-	-	-	-	-

Table. 4. Mycelial growth inhibition of fungal phytopathogens by bacterial endophytes isolated from cotton and pigeon pea

Bacterial isolates	Phytopathogens							
	P. grisea	*C. falcatum*	*P. aphanidermatum*	*P. anacardii*	*L. theobromae*	*M. phaseolina*	*F moniliformae*	*S. rolfsii*
CEB1	57.83^{b}	59.44^{b}	56.43^{b}	50.78^{c}	59.94^{c}	70.52^{b}	52.60^{e}	71.20^{b}
CEB2	52.14^{d}	46.03^{e}	56.42^{b}	47.24^{d}	56.14^{d}	60.24^{e}	60.44^{d}	57.97^{d}
CEB3	69.53^{a}	68.35^{a}	65.01^{a}	64.94^{a}	72.94^{a}	75.69^{a}	75.94^{a}	75.63^{a}
PEB4	54.81^{c}	52.95^{c}	49.01^{d}	54.73^{b}	66.17^{b}	68.84^{c}	72.53^{b}	70.96^{b}
PEB5	49.77^{e}	49.00^{d}	54.89^{c}	44.71^{e}	60.31^{c}	65.52^{d}	65.46^{c}	66.43^{c}

Data are mean values of four replicates. Means followed by the same letter in a column are not significantly different (p d"0.05) by Duncan's multiple range test.

promising results while the control samples had the lowest shoot and root weights measuring 1.56g and 0.69g, respectively (Table7).Furthermore, the vigor index was substantially higher in all the seed priming treatments with CEB3 thanin the control. Among these treatments, the 6g/kg seed priming displayed exceptional performance with a vigor index of 827.16 followed by the 4g/kg treatment with a vigor index 498.71. In contrast, the control treatment had the lowest vigor index, recording only 127.61 (Table7).The application of shoot-specific endophytes revealed improved shoot length and weight during our experiments. Endophytic bacterial isolates of *B. aryabhattai* isolated from rice recorded plant growth-promoting activity, i.e., IAA production, P-solubilization, and N-fixation were recorded (Safari Motlagh*et al*. 2022). The endophytic bacteria (*Bacillus* spp.) application in wheat and chickpea increased total P content, root and shoot biomass, shoot, root weight, and nitrogen content in the shoot (Giri and Dudeja, 2019). IAA

Table. 5. Phytohormones, siderophore, antimicrobial and enzymatic activities of bacterial endophytes and plant growth-promoting rhizobacteria (PGPR)

Isolate	IAA (µg/ml)	Siderophore	HCN	Chitinase
CEB1	49.03±0.43	22.11±0.81	0.01±0.003	12.45±0.84
CEB2	40.89±0.34	21.19±0.33	0.01±0.001	13.25±0.38
PEB4	38.91±0.47	16.31±0.35	0.01±0.002	12.91±0.51
PEB5	41.32±0.56	15.72±0.35	0.01±0.002	16.06±0.24
CEB3	65.69±0.47	18.68±0.38	0.02±0.003	15.59±0.27
PfWN	36.33±0.36	5.38±0.06	0.052±0.001	12.36±0.21
PfRB	40.56±0.41	7.12±0.06	0.032±0.001	34.56±0.08
PfNC	42.12±0.32	10.06±0.07	0.047±0.006	50.06±0.14

Table 6 : Secondary metabolites and lytic enzymes production

Isolate	IAA (µg/ml)	Siderophore	HCN	Chitinase
CEB1	49.03±0.43	22.11±0.81	0.01±0.003	12.45±0.84
CEB2	40.89±0.34	21.19±0.33	0.01±0.001	13.25±0.38
PEB4	38.91±0.47	16.31±0.35	0.01±0.002	12.91±0.51
PEB5	41.32±0.56	15.72±0.35	0.01±0.002	16.06±0.24
CEB3	65.69±0.47	18.68±0.38	0.02±0.003	15.59±0.27
PfWN	36.33±0.36	5.38±0.06	0.052±0.001	12.36±0.21
PfRB	40.56±0.41	7.12±0.06	0.032±0.001	34.56±0.08
PfNC	42.12±0.32	10.06±0.07	0.047±0.006	50.06±0.14

Table.7: Effect of *Bacillusamylofaciens* seed priming on Finger millet using roll-towel paper method

Sr. No.	Seed Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Vigor Index
1	2g/kg	65.12^{c}	2.90^{c}	1.01^{c}	2.22^{c}	1.09^{c}	254.62^{c}
2	4g/kg	77.56^{b}	4.69^{b}	1.74^{b}	3.63^{b}	1.53^{b}	498.71^{b}
3	6g/kg	89.23^{a}	6.88^{a}	2.39^{a}	4.85^{a}	2.08^{a}	827.16^{a}
4	Control	52.73^{d}	1.90^{d}	0.52^{c}	1.56^{d}	0.69^{d}	127.61^{d}

promotes growth which is linked with lateral and adventitious root elongation. The extended root system with potential P-solubilization increases water uptake hence plant vigor may be increased.

CONCLUSION

Endophytes are potentially antagonistic to suppress, inactivate and kill various phytopathogens through direct and indirect methods. In our study, CEB3 (*B. amylofaciens*) recorded a maximum percent of mycelia growth inhibition compared to other endophytes against several phytopathogensknown to cause various diseases in field crops and fruits. Under laboratory conditions, all the endophytes and PGPR were positive for IAA, HCN, siderophore, ammonia production and P-solubilization. The maximum IAA and siderophore activity were recorded in CEB3 while HCN and chitinase were in PGPR. The production of hydrolytic enzymes, i.e., protease, chitinase, cellulaseand pectinase activitywasfound to be higher in CEB3 compared to other isolates. The PGP study conducted with the endophytic isolateCEB3on finger millet found that 6g/kg seed treatmenteffectively enhanced

growth performance.The variations in PGP activities observed among the isolates are likely attributable to their unique capabilities. We propose that optimizing the dosages of these bacterial endophytes for specific crop varieties could play a significant role in managing plant health and achieving higher agricultural productivity. However, further research is needed to understand the molecular-level interactions between these bacterial isolates and their plant hosts.

ACKNOWLEDGEMENT

The authors (RRW, API, MDK and LM) are grateful to the Principal, College of Agriculture, Bharuch and Dean PG Studies, Navsari Agricultural University, Navsari for providing the necessary facilities during the research work. RMS and JYK is acknowledged to National Research Foundation of Korea (NRF 2021R1I1A3057067, 2021R1A5A8029490) and Rural Development Administration, Korea.

DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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