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Weed-Derived Endophytic Bacteria As Bioinoculants: Functional Characterization And Growth Enhancement In Vigna Unguiculata

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Abstract:
Endophytic bacteria from invasive weeds and non-crop plants are an untapped source of stress-tolerant, plant growth-promoting microbes. These typically non-pathogenic microbes inhabit healthy plant tissues and enhance plant development. The present study explores the plant growth-promoting potential of endophytic bacteria isolated from common weed plants—Eichhornia crassipes and Wedelia urticifolia—with a focus on their application as bioinoculants in cowpea (Vigna unguiculata) specifically the Pant Grain Cowpea-14 (PGCP-14) variety. A total of eleven bacterial isolates were screened under glasshouse conditions for their ability to enhance seedling vigor. Among them, three strains—ECR3, ECR4, and WUR5—exhibited significantly higher Seedling Vigor Index I and Seedling Vigor Index II compared to the untreated control and standard inputs like chemical fertilizers and farmyard manure. These strains were further subjected to molecular identification via 16S rRNA gene sequencing, revealing their identities as Paracoccus aestuarii (ECR3), Stenotrophomonas maltophilia (ECR4), and Bacillus paramycoides (WUR5). The selected isolates demonstrated various plant growth-promoting (PGP) traits including nitrogen fixation, solubilization of phosphate, potassium, and zinc, siderophore and phytohormone production, antifungal activity, and ACC deaminase activity. Notably, Paracoccus aestuarii ECR3 was identified as the most effective strain based on its multifaceted PGP capabilities. This study highlights the untapped potential of weed-associated endophytes for sustainable agriculture, and suggests future exploration through advanced genomic tools such as Whole Genome Sequencing (WGS) for strain improvement and broader application.
Keywords:*Endophytic bacteria, Eichhornia crassipes, Wedelia urticifolia, Plant growth-promotion (PGP), Bioinoculants, 16S rRNA sequencing.*

INTRODUCTION

Modern agriculture faces the dual challenge of enhancing crop productivity while minimizing environmental damage caused by excessive chemical fertilizer use. This has driven interest in microbial solutions, particularly beneficial plant-associated bacteria, as sustainable alternatives. Among these, endophytic bacteria—those residing inside plant tissues without causing harm—have attracted attention for their ability to enhance plant growth, stress tolerance, and nutrient availability (Tilman, D et al., 2002).

Endophytic bacteria offer several advantages over free-living rhizospheric microbes due to their close association with plant internal tissues. These bacteria can promote plant growth through multiple mechanisms such as nitrogen fixation, phosphate and potassium solubilization, siderophore production, phytohormone synthesis, and biocontrol against pathogens. Given their systemic presence and adaptability, endophytes are promising candidates for the development of next-generation bioinoculants (Glick, B. R. 2014, Hardoim et al., 2008). Interestingly, weed plants that grow vigorously in diverse and often nutrient-poor environments may harbour unique and stress-tolerant endophytic microbial communities. However, weeds are largely overlooked in microbial prospecting studies. In this study, two common and resilient weed species—Eichhornia crassipes and Wedelia urticifolia—were selected as hosts for endophyte isolation. Their ecological success in waterlogged, polluted (E. crassipes) and dry, degraded soils (W. urticifolia) suggests they may harbour unique plant growth-promoting bacteria (PGPB) with potential applications in sustainable agriculture, especially under stress-prone conditions. (Bhattacharyya & Jha 2012).

The present study focuses on the isolation and characterization of root-associated endophytic bacteria from E. crassipes, and W. urticifolia. A total of 11 isolates were obtained and initially screened through pot assays by forming bioinoculants for their potential to enhance crop growth. Three promising strains were selected for further evaluation. These strains were subjected to molecular identification through 16S rRNA gene

sequencing and were characterized in vitro for key plant growth-promoting traits including nutrient solubilization (N, P, K, Ca, Zn), phytohormone production (IAA, GA), siderophore and HCN production, antifungal activity, and ACC deaminase activity.

This study explores endophytic bacteria from underutilized weed species as a novel source of microbial candidates for sustainable agriculture. By identifying multifunctional endophytes with plant growth-promoting traits and nutrient-mobilizing abilities, the research aims to develop eco-friendly bioinoculants that can enhance crop productivity while reducing reliance on chemical fertilizers.

MATERIAL METHODS

Collection of Sample Plants:

Three different weed plant species were collected from various locations across Surat, Gujarat. The specific geographic coordinates and site details are provided in Table 1. Healthy and disease-free plants were carefully uprooted, placed in labelled collection bags, and transported to the laboratory at Bhagwan Mahavir College of Science and Technology, Surat. In the laboratory, plant samples were thoroughly washed under running tap water to remove soil particles, dust, and other surface contaminants prior to further processing (Sangeeta et al., 2018 , Vitagliano et al., 2018). Its identity was authenticated by eminent taxonomist Dr. M H Parabia (Ex HOD, Dept. of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat)

Table 1: Selected Weed Plant Species with Geographic Locations and Representative Images

SR NO.	PLANT NAME	LOCATION	IMAGE
1.	Eichhornia crassipes	Lat:21.217818 Long: 72.801979	
2.	Wedelia urticifolia	Lat: 21.137939 Long: 72.793596	

Isolation of Endophytes:

Healthy root samples were surface sterilized by sequentially immersing them in 0.25% mercuric chloride ($HgCl_2$) for 1 minute, followed by 95% ethanol for 30 seconds, and then rinsing 5–6 times with sterile distilled water to remove surface contaminants. The sterilized tissues were then macerated and inoculated onto nutrient agar (NA) plates for the isolation of endophytic bacteria. Bacterial colonies exhibiting distinct morphological characteristics were selected, purified through repeated streaking, and maintained on NA slants at 4 °C for further analysis (Vitagliano et al., 2018).

Bioinoculant Preparation:

All eleven bacterial isolates were cultured individually in nutrient broth at 28 ± 2 °C for 24–48 hours under shaking conditions (150 rpm). The cell density of each culture was adjusted to approximately 10^8 colony-forming units (CFU/mL) by comparing turbidity with the 0.5 McFarland standard. For greater accuracy, the optical density (OD) was measured at 600 nm (OD_{600}), and the cultures were diluted with sterile distilled water to achieve an OD_{600} value of 0.1, corresponding to ~10^8 CFU/mL, prior to application in pot assays and bioinoculant formulation (Egamberdieva et al., 2008). The prepared bioinoculants were designated based on their plant source and assigned isolate codes accordingly. Isolates from Eichhornia crassipes were labelled ECR1 to ECR5, and those from Wedelia urticifolia as WUR1 to WUR6.

Bioassay-Based Plant Growth Promotion Ability in Cowpea (Vigna unguiculata):

A bioassay was conducted to evaluate the plant growth-promoting potential of the bacterial isolate using Vigna unguiculata (L.) Walp. (Cowpea), specifically the Pant Grain 25 Cowpea-14 (PGCP-14) variety taken for seedlings under glasshouse conditions. The methodology was adapted with slight modifications from the procedure described by Vessey (2003).

A total of treatments (T1 to T15) were applied in the study, with each treatment conducted in four replicates. T1: non-treated (control), T2: Seeds treated with the recommended dose of chemical fertilizer (CF), T3: Seeds treated with the recommended dose of farmyard manure (FYM), T4 to T8: Seeds treated with bioinoculants from strains ECR1 to ECR5, T9 to T14: Seeds treated with bioinoculants from strains WUR1 to WUR6 respectively.

Seed Germination and Pot Experiment

Cowpea seeds were surface sterilized by immersion in 70% ethanol for 2 minutes, followed by 2% sodium hypochlorite for another 2 minutes. The seeds were then rinsed ten times with sterile distilled water to eliminate residual sterilants (Khalid et al., 2004). Germinated seeds were counted. After germination, uniform seedlings were selected and treated with the prepared bioinoculants (Egamberdieva et al., 2017). The treated seedlings were maintained for 15 days under observation. These seeds were sown in plastic pots (15 cm in diameter), each filled with 1 kg of sterile soil. The soil had the following characteristics: pH 7.2, organic carbon 2.6%, available phosphorus 537.5 kg ha^{-1}, available potassium 448 kg ha^{-1}, and iron 40 mg kg^{-1}. The pots were maintained in a temperature-controlled growth chamber at 28 ± 2 °C. Seven days after sowing, seedlings were thinned to maintain two per pot.Throughout the experiment, soil moisture was maintained at 50% of its water-holding capacity. Fifteen days after seedling emergence, plants were harvested, washed, and their morphological traits were measured. The parameters recorded included plant height, root length, shoot length, number of leaves, fresh and dry weights of shoots and roots, and the number of lateral and total roots. For dry weight measurement, shoots and roots were separated and oven-dried at 65 °C overnight (Bharti et al., 2015, Bhutani et al., 2018).Germinated seeds were counted and vital parameters including germination percentage, Seedling Vigor Index I (SVI-I), and Seedling Vigor Index II (SVI-II) were evaluated and calculated to assess plant growth performance (Abdul-Baki et al., 1973, Siva et al., 2024).
Germination percentage = Seeds germinated / Total seeds * 100
SVI-I = Germination Percentage (%) * Seedling length (cm) [Seedling length = Shoot length + Root length]
SVI-II= Germination Percentage (%) * Seedling dry weight (gm) [Seedling dry weight = Shoot dry weight + Root dry weight]. The germinated seeds were transplanted into a germination tray (5 * 5 cm diameter) filled with sterile soil. The trays were placed in a temperature-controlled growth chamber maintained at 28°C ± 2°C. Fifteen days after sowing (DAS), the plantlets were harvested, and various growth parameters were measured, including shoot length, root length, total height, shoot and root fresh weight, shoot and root dry weight, and the number of lateral roots (Siva et al., 2024). Observations from all treatments were compared with one another and with the control.

Molecular characterization of selected isolate:

Quality of DNA was evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA was observed. Fragment of 16S rRNA gene was amplified by PCR. A single discrete PCR amplicon band was observed when resolved on Agarose. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out with 357F & 1391R primers using BDT v3.1 Cycle Sequencing Kit on ABI 3500xl Genetic Analyzer. The 16S rRNA sequence was used to carry out BLAST with the database of NCBI GenBank. Based on maximum identity score first ten sequences were selected and aligned using multiple sequence alignment software programs (Saitou et al., 1987).

Screening for Plant Growth-Promoting Traits via Qualitative Assays:

The endophytic bacterial isolates were qualitatively evaluated for various plant growth-promoting (PGP) traits using standard microbiological techniques. Nitrogen fixation ability was assessed by observing bacterial growth on Jensen's medium, which lacks nitrogen, indicating the isolate's ability to fix atmospheric nitrogen (Jensen, 1954). Phosphate solubilization was evaluated on Pikovskaya's agar medium, where the formation of a clear halo around the colonies indicated phosphate solubilizing activity (Pikovskaya, 1948). Potassium solubilization was tested using Aleksandrov agar medium, and solubilization was determined by the appearance of a halo zone around the bacterial colonies (Hu et al., 2006). Zinc solubilization was performed on mineral salt agar supplemented with insoluble zinc oxide, and solubilization was indicated by a clear zone formation (Saravanan et al., 2007). Calcium solubilization was tested using Devenze-Bruni (DB) agar medium, and halo formation was taken as a positive result for Ca solubilization (Deubel & Merbach, 2005). Siderophore production was screened using nutrient agar plates supplemented with chrome azurol S (CAS)

dye. A color change around colonies from blue to orange indicated siderophore production (Schwyn & Neilands, 1987).

Screening for Plant Growth-Promoting Traits via Quantitative Assays:

Quantitative evaluation of key plant growth-promoting traits was carried out using standardized protocols: Phosphate solubilization was quantified by inoculating bacterial isolates in Pikovskaya's broth medium and incubating under controlled conditions. After incubation, the culture supernatant was analysed using a spectrophotometer at 430 nm to measure solubilized phosphate. Simultaneously, changes in pH were monitored. The concentration of solubilized phosphorus was determined using a standard curve prepared with known concentrations of dipotassium hydrogen phosphate (K_2HPO_4) (Murphy & Riley, 1962). Potassium solubilization was assessed in Aleksandrov broth medium. The concentration of solubilized potassium in the supernatant was quantified using a spectrophotometric method developed by Hu et al. (2006), involving the formation of a colored complex. Siderophore production was measured using the Chrome Azurol S (CAS) liquid assay. The amount of siderophore produced was quantified based on the degree of color change from blue to orange, which was detected spectrophotometrically (Schwyn & Neilands, 1987).

Screening for Plant Growth-Promoting Traits: Hormone Production

Indole Acetic Acid (IAA) Production:

The production of indole-3-acetic acid (IAA) by bacterial isolates was assessed by inoculating them into Luria Bertani (LB) broth supplemented with L-tryptophan at a concentration of 100 µg/mL. After incubation, the culture supernatant was mixed with Salkowski's reagent (comprising 120 mL of 95% sulfuric acid, 2 mL of 0.5 M $FeCl_3$, and 200 mL distilled water) to detect IAA production. The development of a pink color indicated IAA presence, which was quantified spectrophotometrically at 530 nm. The IAA concentration was determined by comparing with a standard calibration curve prepared using pure IAA (1 mg/mL) (Gordon & Weber, 1951).

Gibberellic Acid (GA_3) Production:

To estimate gibberellic acid production, bacterial isolates were inoculated into sterile nutrient broth and incubated at 30 °C for 10 days under shaking conditions (100 rpm). After incubation, the culture was centrifuged, and the cell-free supernatant was used for GA_3 quantification. The amount of GA_3 was estimated using the 2, 4 dinitrophenylhydrazine (DNPH) method, wherein the reaction intensity was measured at 430 nm using a UV-VIS spectrophotometer. A standard curve of gibberellic acid (1 mg/mL) was used to determine GA_3 concentrations in the samples (Graham & Thomas, 1961).

Screening for Plant Growth-Promoting Traits: Biocontrol Activity

Hydrogen Cyanide (HCN) Production:

The ability of endophytic bacterial isolates to produce hydrogen cyanide (HCN) was assessed using the method described by Miller and Higgins (1970). Bacterial cultures were grown on Luria Bertani (LB) agar medium supplemented with 4.4 g/L glycine. A strip of filter paper soaked in alkaline picrate solution was placed in the lid of the Petri dish, and the plate was sealed and incubated. The development of a reddish-brown coloration on the filter paper indicated the production of HCN by the bacterial isolate.

Antagonistic Activity against Fusarium oxysporum:

The antagonistic potential of endophytic bacteria against the fungal phytopathogen Fusarium oxysporum was evaluated using a dual culture technique on potato dextrose agar (PDA) medium. The method was performed according to Dennis and Webster (1971), where the fungal pathogen and the bacterial isolate were co-inoculated on the same plate, and inhibition of fungal growth was observed to assess biocontrol efficacy.

Screening ACC Deaminase Activity:

Qualitative Assay:

The qualitative estimation of ACC (1-aminocyclopropane-1-carboxylate) deaminase activity was carried out using the method described by Penrose and Glick (2003), which is a modified version of the protocol originally developed by Honma and Shimomura (1978). This assay detects the presence of ACC deaminase-producing bacteria by observing growth and zone formation on minimal medium plates containing ACC as the sole nitrogen source, indicating the release of α-ketobutyrate due to ACC cleavage.

Quantitative Assay:

After initial confirmation of the isolate's ability to utilize ACC, quantitative analysis was performed using late log-phase bacterial cultures. The bacterial pellet was obtained by centrifugation and washed with 0.1 M Tris-HCl buffer. The pellet was then resuspended in DF (Dworkin and Foster) minimal medium supplemented with 3 mM ACC as the nitrogen source and incubated for 72 hours. Post-incubation, the induced cultures were analyzed for α-ketobutyrate concentration as a measure of ACC deaminase activity (Penrose & Glick, 2003).

ACC Consumption Assay Using Colorimetric Ninhydrin Method:

A colorimetric assay using ninhydrin was performed to quantify the degradation of ACC by the bacterial isolates. A standard solution of α-ketobutyrate (1 mg/mL) was prepared. The assay involved adding 1 mL of ACC working solution and 2 mL of freshly prepared ninhydrin reagent into glass tubes. The tubes were tightly capped, shaken, and immersed in a boiling water bath for 15 minutes. After heating, tubes were cooled at 37 °C in a water bath for 2 minutes, then shaken for 30 seconds. The samples were allowed to stand at room temperature for 10 minutes. Absorbance was then measured at 570 nm using a UV-visible spectrophotometer at 15-minute intervals. DF medium served as the blank control. One unit of ACC deaminase activity was defined as the amount of enzyme that produces 1 mg of α-ketobutyrate per 15 minutes. All experiments were conducted in triplicate to ensure reproducibility (Li et al., 2011).

Morphological and Biochemical Characterization of selected isolates:

The selected bacterial isolates were subjected to morphological examination and a series of biochemical tests to support their preliminary identification. Gram staining was performed to determine cell wall characteristics. Biochemical assays included the Methyl Red (MR) and Voges-Proskauer (VP) tests, citrate utilization, urease activity, triple sugar iron (TSI) test, catalase activity, hydrogen sulfide (H_2S) production, nitrate reduction, and ammonia production. These tests were carried out following standard microbiological protocols described by Elbeltagy et al., 2001.

Statistical Analysis:

The effects of potential endophytes used as bioinoculants were statistically analyzed using one-way Analysis of Variance (ANOVA) was conducted, followed by Tukey HSD with a significance level of $p < 0.05$. The growth parameters of Vigna unguiculata treated with bioinoculants were statistically compared to the control to evaluate their effectiveness.

RESULTS:

Collection of Sample Plants:

A total of eleven endophytic bacterial isolates were obtained from the root tissues of two weed species—Eichhornia crassipes and Wedelia urticifolia collected from ecologically diverse regions. These eleven isolates were then evaluated for their potential as bio-inoculants by applying them to Vigna unguiculata plants under controlled conditions. Among all tested isolates, three strains exhibited significantly enhanced plant growth when compared to the untreated control. These promising strains were subsequently selected for further analysis of plant growth promoting (PGP) traits. The selected isolates included two from E. crassipes (designated as ECR3 and ECR4), one from W. urticifolia (WUR5). These selected isolates were later subjected to molecular identification. These three strains, showing distinct colony morphologies, were purified and maintained on nutrient agar slants for downstream characterization. Although the remaining eight isolates were also studied for their plant growth promoting traits. The three highlighted strains demonstrated superior capabilities in promoting seed germination and early seedling development, suggesting their potential application as bio inoculants in sustainable agriculture.

Bioinoculant Preparation:

Bioinoculants were prepared for all eleven selected endophytic bacterial strains and evaluated for their plant growth-promoting potential through a controlled pot experiment. Plant growth parameters from inoculated treatments were compared against a non-inoculated control. Among the tested strains, ECR3, ECR4, and WUR5 consistently outperformed others in promoting plant growth, as reflected in enhanced shoot and root development. Based on their superior performance in the pot assay, these three strains were selected for further molecular identification and detailed in vitro characterization of plant growth-promoting (PGP) traits.

Bioassay-Based Plant Growth Promotion Ability in Cowpea (Vigna unguiculata):

A total of 15 treatments (T1–T14) were evaluated to assess the plant growth-promoting potential of bacterial isolates on cowpea (Vigna unguiculata PGCP-14) under glasshouse conditions. Treatments included non-inoculated control (T1), chemical fertilizer (T2), farmyard manure (T3), and bioinoculant-treated seeds (T4–T14). Among the inoculated treatments, T6 (ECR3), T7 (ECR4), and T13 (WUR5) significantly enhanced plant growth parameters including seedling height, root length as well as SVI I and SVI II compared to other treatments (Figure 1). Based on their superior performance, these three strains were shortlisted for further molecular identification and in vitro characterization of plant growth-promoting traits.

A

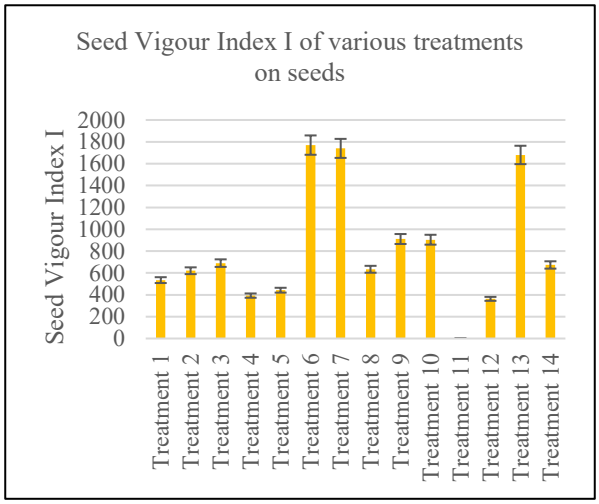

Figure 1A: Seedling Vigour Index I

B

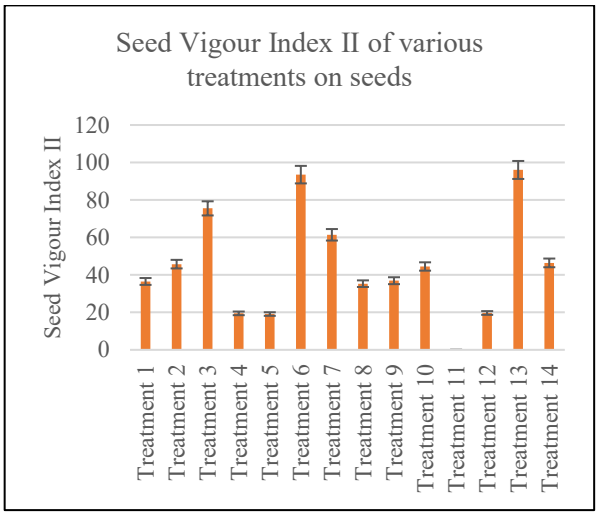

Figure 1B: Seedling Vigour Index II

Figure 1: Comparison of SVI I and SVI II with applied treatments

Molecular characterization of selected isolate:

The three most promising endophytic bacterial isolates—ECR3, ECR4, and WUR5—were subjected to 16S rRNA gene sequencing for molecular identification. Sequence analysis revealed that ECR3, isolated from Eichhornia crassipes, showed 99.72% similarity with Paracoccus aestuarii (GenBank Accession No. QR554009) (Figure 2). Isolate ECR4, also from E. crassipes, was identified as Stenotrophomonas maltophilia with 99.92% similarity (Accession No. QR554015) (Figure 3). The third isolate, WUR5, obtained from Wedelia urticifolia, was closely related to Bacillus paramycoides, showing 99.87% similarity (Accession No. QR554128) (Figure 4). Table 2 summarize these identifications confirmed the taxonomic diversity of the selected endophytes.

Table 2: Identity of endophytic isolates based on 16S rRNA gene sequencing

Isolate label	Plant name	Source	Strain	Similarity %	Accession number
ECR3	Eichhornia crassipes	Endophyte	Paracoccus aestuarii	99.72	QR 554009
ECR4	Eichhornia crassipes	Endophyte	Stenotrophomonas maltophilia	99.92	QR 554015
WUR5	Wedelia urticifolia	Endophyte	Bacillus paramycoides	99.87	QR 554128

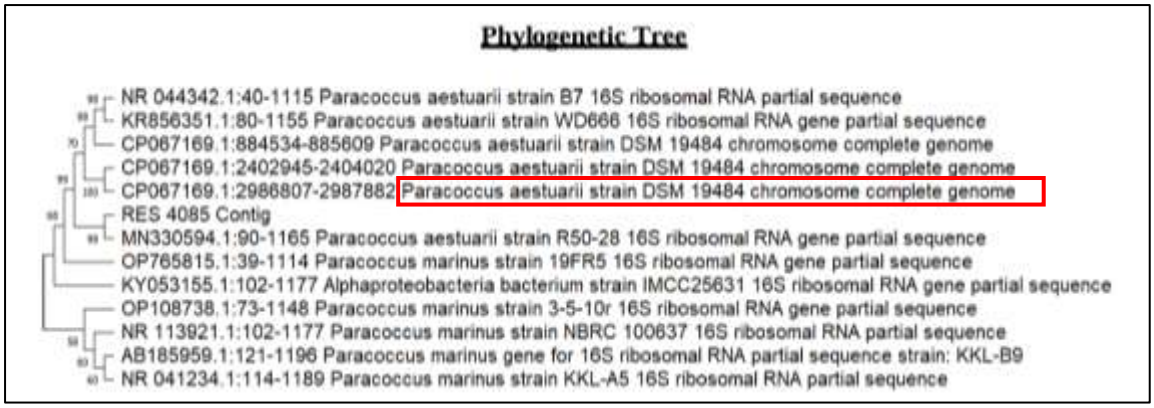

Figure 2: Phylogenetic tree of Paracoccus aestuarii ECR3 isolates based on a partial 16S rRNA nucleotide sequences constructed with neighbour joining method

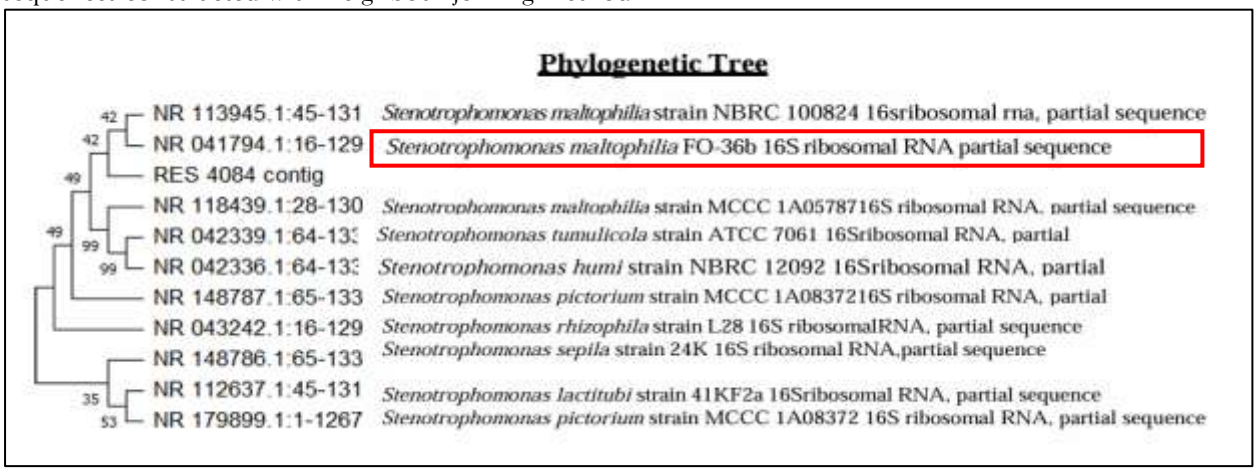

Figure 3: Phylogenetic tree of Stenotrophomonas maltophilia ECR4 isolates based on a partial 16S rRNA nucleotide sequences constructed with neighbour joining method

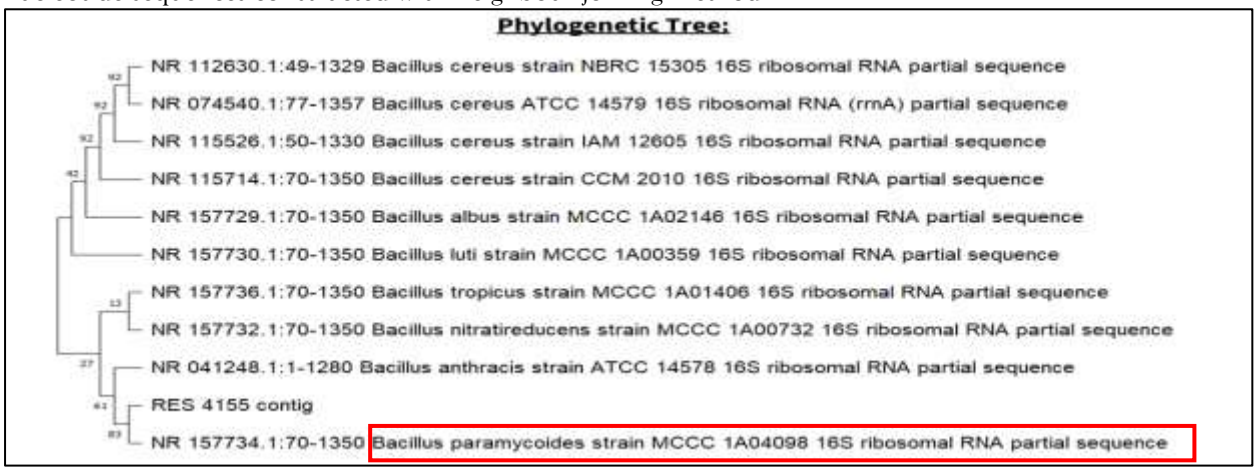

Figure 4: Phylogenetic tree of Bacillus paramycoides WUR5 isolates based on a partial 16S rRNA nucleotide sequences constructed with neighbour joining method

Screening for Plant Growth-Promoting Traits via Qualitative Assays and Quantitative Assays:

The selected endophytic strains—ECR3, ECR4, and WUR5—were characterized for their plant growth-promoting (PGP) traits, including nutrient solubilization and siderophore production (Table 3). All three strains exhibited growth on nitrogen-free media, indicating their potential for nitrogen fixation. They also showed positive activity for zinc and calcium solubilization.

Phosphate solubilization zone diameters ranged from 4.26 cm (ECR3) to 5.25 cm (WUR5). Among the strains, WUR5 recorded the highest phosphate concentration in the culture supernatant (0.783 ± 0.043 mg/mL), followed by ECR3 (0.643 ± 0.023 mg/mL) and ECR4 (0.382 ± 0.034 mg/mL). In terms of potassium solubilization, all isolates except WUR5 were positive. ECR3 exhibited the highest potassium

solubilization (0.713 ± 0.014 mg/mL), while ECR4 showed lower solubilization (0.334 ± 0.023 mg/mL). Siderophore production was observed in all strains, with solubilization efficiency ranging from 34.88% (ECR3) to 54.65% (ECR4). The type of siderophore produced varied, with ECR3 and WUR5 producing hydroxamate-type siderophores, while ECR4 produced catecholate-type siderophores (Table 3).

Table 3: Characterization of selected bacterial strains for qualitative and quantitative plant growth promoting traits

Endophyte strain	**Growth on nitrogen free media**	**Zinc**	**Calcium**	**Phosphate**		**Potassium**		**Siderophore**	
				Zone (cm)	**Conc (mg/ml)**	**Zone (cm)**	**Conc (mg/ml)**	**% SU**	**Type**
ECR3	+	+	+	4.26	0.643 ±0.023	0.35	0.713 ± 0.014	34.88	Hydroxamate
ECR4	+	+	+	4.57	0.382 ± 0.034	0.4	0.334 ±0.023	54.65	Catecholate
WUR5	+	+	-	5.25	0.783 ± 0.043	0.35	0.346 ±0.032	50.00	Hydroxamate

(Values represent mean ± standard error (n = 3). Data are based on three independent replicates. Key: "+" indicates positive activity; "−" indicates no activity.)

The selected bacterial endophytes were evaluated for their ability to produce plant growth hormones—indole-3-acetic acid (IAA) and gibberellic acid (GA)—under in vitro conditions. All three strains exhibited hormone production to varying extents. Among them, strain WUR5 recorded the highest levels of both IAA (0.217 ± 0.029 mg/mL) and GA (0.415 ± 0.041 mg/mL). Strain ECR3 produced moderate amounts of IAA (0.089 ± 0.021 mg/mL) and GA (0.035 ± 0.024 mg/mL), while ECR4 showed relatively lower levels of IAA (0.038 ± 0.025 mg/mL) but slightly higher GA production (0.049 ± 0.031 mg/mL) compared to ECR3. These results indicate that WUR5 is a potent phytohormone-producing strain with promising plant growth-promoting potential (Table 4).

Table 4: Characterization of selected bacterial strains for qualitative and quantitative plant growth hormone production

Endophyte strain	**IAA (mg/ml)**	**GA (mg/ml)**
ECR3	0.089±0.021	0.035±0.024
ECR4	0.038±0.025	0.049±0.031
WUR5	0.217±0.029	0.415±0.041

(Values represent mean ± standard error (n = 3). Data are based on three independent replicates.)

The selected bacterial strains ECR3, ECR4, and WUR5 were evaluated for biocontrol traits, including hydrogen cyanide (HCN) production and antifungal activity against Fusarium oxysporum (Table). All three isolates tested positive for HCN production. In antifungal assays, ECR3 exhibited the highest inhibition zone (20.13 ± 0.12 mm), followed by WUR5 (17.24 ± 0.17 mm) and ECR4 (15.09 ± 0.31 mm), indicating their potential role in suppressing phytopathogens (Table 5).

Table 5: Characterization of selected bacterial strains for biocontrol activity

Endophyte strain	**HCN**	**Antifungal activity with inhibition zones in mm (Fusarium oxysporum)**
ECR3	+	20.13±0.12
ECR4	+	15.09±0.31
WUR5	+	17.24±0.17

The ACC deaminase activity of selected bacterial strains was evaluated based on their growth on Dworkin and Foster (DF) minimal media supplemented with 1-aminocyclopropane-1-carboxylate (ACC) as the sole nitrogen source, and quantified by α-ketobutyrate (α-KB) production (Table 7). Among the three strains, only WUR5 exhibited growth on DF + ACC medium and showed measurable ACC deaminase activity (0.513 nmol α-KB/15 min/mL). Strains ECR3 and ECR4 did not grow on ACC-supplemented media and showed no detectable enzyme activity, although all isolates were able to grow on DF + $(NH_4)_2SO_4$, confirming their viability (Table 6).

Table 6: Characterization of selected bacterial strains for qualitative and quantitative for ACC deaminase activity

Endophyte strain	Growth on DF media	Growth on DF + $(NH_4)_2SO_4$ media	Growth on DF + ACC media	ACC Deaminase (nmol α-KB/15 min/ml)
ECR3	-	+	-	-
ECR4	-	+	-	-
WUR5	+	+	+	0.513

Biochemical profiling of the three selected endophytic bacterial strains revealed diverse physiological traits (Table X). ECR3 and ECR4 were Gram-negative, whereas WUR5 was Gram-positive. All three strains tested positive for citrate utilization, urea hydrolysis, catalase activity, nitrate reduction, and ammonia production, and exhibited typical positive reactions in TSI (Triple Sugar Iron) tests. ECR3 and WUR5 were methyl red (MR) positive and Voges–Proskauer (VP) negative, while ECR4 showed the opposite pattern, being MR-negative and VP-positive. Hydrogen sulfide (H_2S) production was observed only in ECR4. These results further supported the physiological diversity among the selected endophytes (Table 7).

Table 7: Biochemical characterization of selected bacterial strains

Endophyte Strain	Gram staining	MR	VP	Citrate utilization	Urea hydrolysis	TSI	Catalase	Nitrate	H_2S	Ammonia production
ECR3	-ve	+	-	+	+	+	+	+	-	+
ECR4	-ve	-	+	+	+	+	+	+	+	+
WUR5	+ve	+	-	+	+	+	+	+	-	+

To evaluate the effects of different endophytic bioinoculants on seedling vigor, a one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test was performed. The treatments included ECR3, ECR4, and WUR5, compared with the untreated control for Seedling Vigor Index I (SVI-I) and Seedling Vigor Index II (SVI-II).The results indicated that all three selected bioinoculants (ECR3, ECR4, and WUR5) significantly enhanced both SVI-I and SVI-II compared to the control ($p < 0.05$). Among these, ECR3 treatment exhibited the highest mean SVI-I (1770) and SVI-II (93.5), followed closely by ECR4 and WUR5. Tukey HSD post hoc analysis confirmed significant differences between the control and all three treatments, indicating a strong potential of these endophytic strains to improve seedling vigor in cowpea under glasshouse conditions.

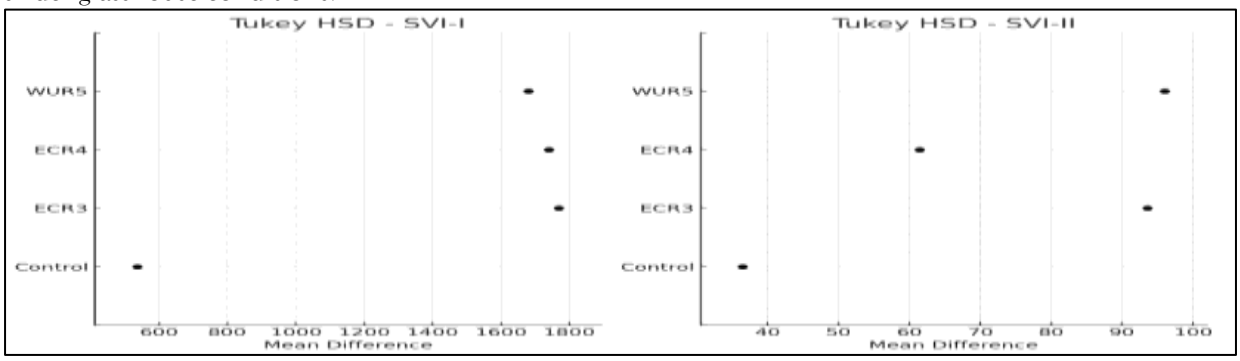

Figure 5: Tukey HSD analysis for Seedling Vigor Indices (SVI) in cowpea seedlings treated with selected endophytic bacterial strains. [Left: Comparison of SVI-I mean differences among treatments; Right: Comparison of SVI-II mean differences. Treatments include Control, ECR3, ECR4, and WUR5. The

analysis shows significant improvement in seedling vigor indices in treated groups compared to control ($p < 0.05$), confirming the effectiveness of endophytic bioinoculants in enhancing early seedling growth.]

DISCUSSION:

In the context of sustainable agriculture, the use of microbial bioinoculants has gained significant attention as an eco-friendly alternative to chemical fertilizers and pesticides. Endophytic bacteria, which reside within plant tissues without causing harm, offer several benefits including nutrient solubilization, hormone production, and disease suppression. Their ability to establish close associations with host plants makes them ideal candidates for enhancing crop productivity in an environmentally sustainable manner. In this study, selected endophytic strains demonstrated promising plant growth-promoting (PGP) traits such as nitrogen fixation, solubilization of phosphorus, potassium, and zinc, production of phytohormones like IAA and GA, antifungal activity, and ACC deaminase production. These traits collectively support plant development and stress resilience, underscoring their potential as bioinoculants for improved crop management.Weed plants were intentionally chosen as the source for endophyte isolation in this study due to their hardy nature and ability to thrive in diverse, often nutrient-poor environments. Unlike cultivated crops, weeds are typically exposed to minimal human intervention, making them a reservoir of robust microbial communities with potential stress-tolerant traits. Despite their ecological significance, there is a noticeable gap in research focusing on endophyte isolation from weed species such as Eichhornia crassipes, and Wedelia urticifolia. This study contributes novel insights by isolating and characterizing endophytic strains from these underexplored hosts. The effective performance of the resulting bioinoculants in promoting cowpea growth highlights the potential of weed-associated endophytes in sustainable agriculture, especially as low-cost, eco-compatible inputs for smallholder and organic farming systems.The formulation of bioinoculants using selected bacterial isolates derived from weed-associated endophytes represents a novel and sustainable approach to enhancing plant growth. In this study, bacterial strains isolated from Eichhornia crassipes, Wedelia urticifolia, and Lantana camara were cultured and standardized using McFarland turbidity standards to prepare bioinoculant suspensions. These were subsequently applied to cowpea seeds to assess their plant growth-promoting potential. The use of such endophytic bacteria, especially those adapted to marginal or stress-prone habitats like weeds, provides a unique microbial reservoir that may possess traits beneficial for crop development and resilience under challenging conditions. The bioassay conducted on Vigna unguiculata (cowpea) under controlled glasshouse conditions clearly demonstrated the efficacy of the formulated bioinoculants. Compared with untreated control, chemical fertilizer (CF), and farmyard manure (FYM), the bioinoculants significantly improved seedling performance, as evident from the Seedling Vigor Index I and II (SVI-I and SVI-II) values. Among the eleven initial strains tested, three strains—ECR3, ECR4, and WUR5—showed markedly higher seedling vigor compared to control and conventional treatments. These strains were thus shortlisted for further molecular characterization and PGP trait profiling.Similar plant growth promotion using endophytic bacteria has been documented in previous studies. For instance, Kumar et al. (2020) reported enhanced growth and stress tolerance in chickpea inoculated with endophytes from Withania somnifera. Likewise, Ramesh et al. (2014) observed improved nutrient uptake and biomass accumulation in rice treated with native endophytes. However, unlike these earlier works that primarily focused on medicinal or cultivated plants, our study uniquely targets weed-associated endophytes—an underexplored reservoir with potential agricultural applications. To our knowledge, there is limited prior work on isolating and characterizing endophytic bioinoculants from weed species, highlighting the novelty and significance of this approach. The use of such bioinoculants may offer an effective and sustainable alternative to synthetic inputs in crop production systems. The molecular identification of the three most promising endophytic isolates—ECR3, ECR4, and WUR5—through 16S rRNA gene sequencing provided insight into their taxonomic affiliations and potential functional roles. ECR3, isolated from Eichhornia crassipes, was identified as Paracoccus aestuarii with a 99.72% similarity. Members of the genus Paracoccus are known for their versatile metabolic capabilities, including nitrogen cycling and tolerance to environmental stressors (Zhang et al., 2019), which may contribute to the observed plant growth-promoting effects. ECR4, also from E. crassipes, showed close similarity to Stenotrophomonas maltophilia (99.92%). S. maltophilia is a well-documented endophyte and rhizobacterium that can promote plant growth through the production of phytohormones, phosphate solubilization, and biocontrol activity (Ryan et al., 2009). The

third isolate, WUR5, derived from Wedelia urticifolia, was affiliated with Bacillus paramycoides (99.87% similarity), a species within the Bacillus cereus group known for plant growth promotion and biocontrol activity (Gupta et al., 2020). These identifications not only validate the physiological and biochemical results observed during PGP trait screening but also reinforce the potential of endophytes from weed plants as effective bioinoculants for sustainable agriculture. The selected endophytic bacterial strains ECR3, ECR4, and WUR5 demonstrated a broad spectrum of plant growth-promoting traits, particularly in nutrient solubilization and siderophore production, highlighting their potential as efficient bioinoculants. All three isolates exhibited growth on nitrogen-free media, suggesting their possible nitrogen-fixation ability, a key attribute in enhancing nitrogen availability to plants (Bhattacharyya & Jha, 2012). Their capacity to solubilize essential micronutrients such as zinc and calcium further indicates their role in improving nutrient uptake in crops. Phosphate solubilization is a critical trait for sustainable agriculture, given the limited bioavailability of phosphorus in soils. WUR5 showed the highest solubilization potential, both in zone diameter (5.25 cm) and soluble phosphate concentration (0.783 ± 0.043 mg/mL), underscoring its strong capability to mobilize phosphorus, followed by ECR3 and ECR4. Potassium solubilization was notable in ECR3, which recorded the highest concentration among the strains, aligning with the findings of Meena et al. (2016), who reported enhanced growth in legumes due to potassium-solubilizing rhizobacteria. Siderophore production by all three strains indicates their ability to chelate iron and compete with plant pathogens in the rhizosphere. ECR4 produced catecholate-type siderophores with the highest solubilization efficiency (54.65%), whereas ECR3 and WUR5 produced hydroxamate-type siderophores, which are known for their high iron affinity and biocontrol efficacy (Saha et al., 2016). These PGP traits collectively suggest that these endophytic bacteria contribute to improved plant nutrition and growth, both directly by supplying nutrients and indirectly by suppressing pathogens through iron competition. Such multifunctional endophytes can serve as eco-friendly alternatives to synthetic agrochemicals, supporting the broader goals of sustainable agriculture. The selected endophytic strains ECR3, ECR4, and WUR5 exhibited multiple plant growth-promoting (PGP) and biocontrol traits, reinforcing their potential as multifunctional bioinoculants. All three strains produced phytohormones such as indole-3-acetic acid (IAA) and gibberellic acid (GA), which are critical regulators of plant development. WUR5, in particular, stood out with the highest levels of both IAA and GA, suggesting a stronger influence on root elongation, seed germination, and overall plant vigor (Patten & Glick, 2002; Spaepen et al., 2007). The ability to produce these hormones is especially important in stressful environments, where microbial IAA can compensate for plant hormone deficits, and GA can promote cell elongation and division. In terms of biocontrol attributes, the strains showed promising antagonistic activity against Fusarium oxysporum, a widespread soilborne pathogen. ECR3 exhibited the strongest antifungal activity, with a significant inhibition zone (20.13 mm), likely due to the synergistic effect of hydrogen cyanide (HCN) production and siderophore-mediated iron competition. These mechanisms are well-documented in microbial biocontrol (Kloepper et al., 2004; Raaijmakers et al., 2009). Additionally, ACC deaminase activity, an important trait for mitigating plant stress through ethylene regulation, was exclusively observed in WUR5. This enzyme enables microbes to lower ethylene levels in plants, thereby enhancing growth under abiotic stress (Glick, 2014). The absence of ACC deaminase in ECR3 and ECR4 suggests that WUR5 could be particularly valuable in stress-prone agroecosystems. Biochemical profiling revealed notable physiological diversity among the isolates. While ECR3 and ECR4 were Gram-negative, WUR5 was Gram-positive, and all three were catalase positive, indicating tolerance to oxidative stress. Their ability to utilize citrate, hydrolyze urea, reduce nitrate, and produce ammonia further supports their metabolic versatility and survival in varied soil environments (Kavamura et al., 2013). Such multifaceted traits are advantageous for rhizosphere competence and effective colonization, which are essential for the success of microbial inoculants in the field. Biochemical profiling of microbial isolates provides essential insights into their taxonomy, metabolic versatility, and potential applications as bioinoculants. In this study, the selected endophytic bacterial strains—ECR3, ECR4, and WUR5—demonstrated diverse biochemical traits, indicating their functional variability and ecological adaptability. Gram staining revealed that ECR3 and ECR4 were Gram-negative, whereas WUR5 was Gram-positive, reflecting phylogenetic diversity that aligns with their molecular identification as Paracoccus aestuarii, Stenotrophomonas maltophilia, and Bacillus paramycoides, respectively. These genera are commonly reported in association with plant tissues and are known for their plant growth-promoting and biocontrol capabilities (Khan et al., 2016; Glick, 2014).

All three isolates tested positive for catalase activity, urea hydrolysis, citrate utilization, nitrate reduction, and ammonia production. These traits are indicative of active nitrogen cycling and adaptability to rhizospheric and endophytic environments. For example, catalase activity is linked to oxidative stress tolerance and survival within plant tissues (Kumar et al., 2017), while ammonia production contributes to nitrogen enrichment in the rhizosphere, promoting plant growth. Positive results for Triple Sugar Iron (TSI) reactions further suggest the metabolic flexibility of these strains to utilize multiple sugars, an advantage for colonization and persistence in plant systems. Variability was observed in methyl red (MR) and Voges–Proskauer (VP) tests. ECR3 and WUR5 were MR-positive and VP-negative, indicating a mixed acid fermentation pathway, while ECR4 displayed the opposite pattern, favoring butanediol fermentation. Additionally, H_2S production was recorded only in ECR4, which may reflect its broader enzymatic repertoire for sulfur metabolism. These differences highlight the metabolic plasticity of the strains, an important consideration for their formulation as multifunctional bioinoculants under diverse agroecological conditions (Bhattacharyya & Jha, 2012). The statistical evaluation of seedling vigor indices (SVI-I and SVI-II) demonstrated that treatments with selected endophytic bacterial strains—ECR3, ECR4, and WUR5—significantly enhanced seedling growth parameters in cowpea compared to the untreated control. Among the treatments, ECR3 exhibited the most pronounced improvement, followed by ECR4 and WUR5. The significant differences confirmed by Tukey's HSD test ($p < 0.05$) highlight the consistency and reliability of the bioinoculant effects. These findings underscore the potential of endophytic bacteria to act as effective growth enhancers during early seedling establishment, a critical phase for overall crop performance.

CONCLUSION:

This study highlights the potential of endophytic bacteria isolated from weed plants as sustainable bioinoculants to promote plant growth in an environmentally friendly manner. Among the tested strains, Paracoccus aestuarii ECR3 demonstrated the most promising traits, including superior seedling vigor, phosphate and potassium solubilization, antifungal activity, and overall biochemical performance, indicating its strong suitability for agricultural applications. The use of such beneficial endophytes not only enhances crop productivity but also reduces dependence on chemical fertilizers, aligning with the goals of sustainable agriculture. Future research should focus on field-scale validation and formulation development of these bioinoculants. Additionally, advanced molecular approaches such as Whole Genome Sequencing (WGS) and Next-Generation Sequencing (NGS) can be employed to further explore the genomic basis of plant growth promotion and stress resilience in these strains, paving the way for targeted strain improvement and application in diverse cropping systems.

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Authors' contributions

SZ collected samples, conducted experiments, and compiled the data. SDG supervised the research, drafted the manuscript, and performed the review.

Compliance with ethical standards

Conflict of interest:

Authors do not have any conflict of interests to declare.

Ethical issues: None

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