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Isolation and screening of groundnut endophytic bacteria for multiple plant growth promoting characteristics

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Abstract

Groundnut is an important oilseed crop. Endophytic bacteria help plant growth and development through various mechanisms. The aim of the present study was to isolate and screen endophytic bacteria from groundnut for multiple plant growth promoting characteristics under in-vitro conditions. A total of 50 morphologically diverse endophytic bacteria were isolated from root and leaf samples of groundnut. These endophytic bacteria were screened for multiple plant growth promoting characteristics such as indole acetic acid (IAA) production, phosphorus, potassium and zinc solubilization, moisture stress tolerance and ACC deaminase synthesizing capabilities. The isolates GEB 32 (3.45µg/ml), GEB 58 (3.36µg/ml) and GEB 62 (3.31µg/ml) showed comparatively higher IAA synthesizing capabilities. Phosphorus, potassium and zinc solubilization was observed in 14, 12 and 9 isolates respectively. The isolates GEB 61, GEB 59 and GEB 48 have recorded maximum phosphorus, potassium and zinc solubilization indices as compared to other isolates. Relatively higher moisture stress tolerance in terms of growth in 30% PEG 6000 amended medium was observed in the isolate GEB 22. On the basis of in-vitro screening 07 best isolates were selected for further studies in pot experiments.

Keywords: Endophytic bacteria, groundnut, plant growth, rainfed, semi-arid

Introduction

Endophytic bacteria are microorganisms that colonize plant tissues without causing harm, benefiting plants through growth promotion, disease resistance, and stress tolerance (Santoyo *et al.* 2016, Omomowo and Babalola 2019) [37, 25]. They enhance plant growth by producing phytohormones like indole-3-acetic acid (IAA) and gibberellins (FAO, 2021). Certain species, such as *Rhizobium* and *Azospirillum* fix atmospheric nitrogen, making it available to plants (Bashan and de-Bashan 2010, Mus *et al.* 2016) [2, 20]. Additionally, endophytic *Bacillus* and *Pseudomonas* species act as biocontrol agents by producing antimicrobial compounds that protect against pathogens (USDA, 2020). These bacteria also help plants tolerate abiotic stresses like drought and salinity by regulating stress-responsive genes (Toomer, 2018) [42].

Groundnut, commonly known as peanut, is a globally significant legume valued for its nutritional and economic importance. Scientifically named as *Arachis hypogaea*, it belongs to the Fabaceae family and is widely cultivated in tropical and subtropical regions (FAO, 2021). Unlike most legumes, groundnut exhibits geocarpy, a unique process where pods develop underground after pollination (Nautiyal, 2022) [21]. It is an excellent source of nutrients, containing 25-30% protein, making it a crucial dietary component, especially in protein-deficient regions (Toomer, 2018) [42]. Additionally, it is rich in monounsaturated and polyunsaturated fats, which contribute to heart health (USDA, 2020). The crop is largely grown in rainfed conditions. In recent years long dry spells are common during the cropping season and are responsible for limiting the growth and yield of crop (Lobell *et al.*, 2011) [17]. Endophytic bacteria are known to impart moisture stress tolerance in several crops such as maize (Vurukonda *et al.*, 2016) [45], wheat (Naveed *et al.*, 2014) [22, 23], *Arabidopsis* sp. (Cohen *et al.*, 2008) [3], sugar beet, legumes (Paul and Lade 2014, Glick 2014, Vurukonda *et al.*, 2016) [30, 8, 45]. The aim of the present study was to isolate and characterize endophytic bacteria from groundnut plants.

Materials and Methods

Collection of plant samples

Groundnut plant samples were collected from Ananthapuramu district of Andhra Pradesh (India), wherein the crop is extensively grown under rainfed conditions. Plants were carefully uprooted, and loosely adhered soil was gently shaken off before placing in sterile polythene bags and transported to the laboratory in an ice box for further processing.

Isolation of endophytic bacteria

Surface sterilization and isolation of endophytic bacteria was done by following the methods described by Duhan *et al.* (2020)^[6] and Cao *et al.* (2003)^[3]. Briefly, the leaf and root samples were rinsed in running water, washed with Tween-20 and double-distilled water, and surface-sterilized in a laminar airflow chamber using 70% ethanol (5 min), 0.9% sodium hypochlorite (20 min), and sterile water (3 washes). To inhibit fungal growth, samples were soaked in 10% NaHCO₃ for 15 min. Sterilized samples were cut into 0.5-1.0 cm explants, plated on Nutrient Agar (NA) and LB Agar, and incubated at 29±1°C for 48 h. Morphologically distinct colonies were selected and purified by repeated streaking on respective media, and stored at 4°C for further use.

Morphological characterization

The morphological characteristics of the isolates were recorded as per the standard methods described in Bergey's manual of systematic bacteriology.

Screening of endophytic bacteria for plant growth promoting characteristics

In-vitro screening of the isolates was done for the following plant growth promoting characteristics;

Indole acetic acid (IAA) production

The procedure described by Gorden and Weber (1951)^[9] was followed to estimate IAA production capabilities of bacterial endophytes. Each isolate was raised in 5 ml nutrient broth incubated for 48 h in a shaker (110 rotations per minute) at 28±2°C. The broth culture was centrifuged at 10,000 rpm for 15 min of time and supernatant was collected. To 1 ml of supernatant, 2 ml of Salkowski reagent [1ml of FeCl₃ + 50 ml of perchloric acid (35%)] was added. Then 2 drops of Orthophosphoric acid was added and kept in the dark for about 30 min to develop colour. Development of pink colour by the samples was considered as positive for IAA production. The intensity of pink colour developed was measured by taking absorbance in a spectrophotometer at 530 nm. The quantitative estimation of IAA produced was calculated from standard curve.

Phosphate solubilization

Phosphate solubilization capabilities of the isolates was determined using Pikovskaya agar media (Pikovskaya, 1948)^[31]. The purified bacterial isolates were spot inoculated on Pikovskaya agar media containing tri-calcium phosphate and were incubated at 28°C for 3-5 days. A clear/halo zone around the colony was considered as positive for phosphate solubilization. The solubilization index was calculated by the following formula i.e.,

$$\text{Solubilization index} = \frac{\text{zone diameter (cm)} + \text{colony diameter (cm)}}{\text{colony diameter (cm)}}$$

(Pande *et al.*, 2017)^[28].

Potassium solubilization

To assess the potassium solubilization ability of the bacterial isolates Aleksandrov media was used (Hu *et al.*, 2006)^[12]. The Aleksandrov medium was prepared with the following constituents: glucose (0.5%), MgSO₄·7H₂O (0.05%), FeCl₃ (0.0005%), CaCO₃ (0.01%), calcium phosphate (0.2%) and potassium aluminium silicate (0.2%). The bacterial cultures were spot inoculated on the media and were incubated for 3-5 days at 28°C to observe the halo zones around the colonies. The solubilization index the colonies was calculated using the following formula i.e.,

$$\text{Solubilization index} = \frac{\text{zone diameter (cm)} + \text{colony diameter (cm)}}{\text{colony diameter (cm)}}$$

(Pande *et al.*, 2017)^[28].

Zinc solubilization

The tris minimal salt media amended with either zinc carbonate (ZnCO₃) or zinc oxide (ZnO) as a source of insoluble zinc compound was used to screen the isolates for zinc solubilization (Gandhi and Muralidharan 2016)^[7]. The bacterial isolates were streaked on agar plates and incubated at 28°C for 3-5 days to observe the formation of clear solubilization zones around the colonies. The solubilization index was calculated by using the formula:

$$\text{Solubilization index} = \frac{\text{zone diameter (cm)} + \text{colony diameter (cm)}}{\text{colony diameter (cm)}}$$

(Pande *et al.*, 2017)^[28].

1-aminocyclopropane-1-carboxylate (ACC) deaminase activity

To assess ACC deaminase activity of bacterial cultures, they were inoculated into a minimal salt medium supplemented with 5 mM of 1-aminocyclopropane-1-carboxylate (ACC) as the sole nitrogen source and incubated in a shaker at 28-30°C with for 48 h, the absorbance as a measure of growth of the cultures was measured spectrophotometrically at 600 nm. (Honma and Shimomura, 1978)^[11].

Moisture stress tolerance

Moisture stress tolerance of the endophytic bacterial isolates was measured by inoculating in a nutrient broth amended with 20% and 30% PEG (poly ethylene glycol) for 3 days. The growth of the isolates was recorded in terms of absorbance recorded spectrophotometrically at 600 nm (Sandhya *et al.*, 2009)^[36].

Statistical analyses

The data was statistically analysed. One way analysis of variance (ANOVA) was carried out using INDOSTAT software (version: 9.3 Year: 2017).

Results and Discussion

Isolation of endophytic bacteria

Bacterial endophytes were isolated from roots and leaves of groundnut crop. The pure cultures were labelled as GEB 1, GEB 4, GEB 7, GEB 8, GEB 9, GEB 10, GEB 18, GEB 19, GEB 20, GEB 22, GEB 25, GEB 27, GEB 32, GEB 35, GEB 37, GEB 38, GEB 39, GEB 40, GEB 41, GEB 42, GEB 43, GEB 44, GEB 45, GEB 46, GEB 47, GEB 48, GEB 49, GEB 50, GEB 51, GEB 52, GEB 53, GEB 54, GEB 55, GEB 56, GEB 57, GEB 58, GEB 59, GEB 60, GEB 61, GEB 62, GEB 63, GEB 64, GEB 65, GEB 66,

GEB 67, GEB 68, GEB 69, GEB 70, GEB 71 and GEB 72. Endophytic bacteria were isolated from groundnut plants (Li *et al.* 2019; Wang *et al.* 2013) ^[16, 46].

Morphological characteristics of the isolates

All the isolates viz., GEB 1, GEB 4, GEB 7, GEB 8, GEB 9, GEB 10, GEB 18, GEB 19, GEB 20, GEB 22, GEB 25, GEB 27, GEB 32, GEB 35, GEB 37, GEB 38, GEB 39, GEB 40, GEB 41, GEB 42, GEB 43, GEB 44, GEB 45, GEB 46, GEB 47, GEB 48, GEB 49, GEB 50, GEB 51, GEB 52, GEB 53, GEB 54, GEB 55, GEB 56, GEB 57, GEB 58, GEB 59, GEB 60, GEB 61, GEB 62, GEB 63, GEB 64, GEB 65, GEB 66, GEB 67, GEB 68, GEB 69, GEB 70, GEB 71, GEB 72 were streaked on Nutrient agar media to study morphological characteristics. The isolates showed the morphological, round with scalloped margin, raised, convex or wavy colonies

Screening of the endophytic bacterial isolates for plant growth promoting characteristics

Indole acetic acid (IAA) production

The isolates GEB 32 (3.45 µg/ml), GEB 58 (3.36 µg/ml) and GEB 62 (3.31 µg/ml) showed comparatively higher IAA synthesizing capabilities. The isolate GEB 1 (2.19 µg/ml) recorded lowest IAA as compared to other isolates (Table 2). Indole-3-acetic acid is a phytohormone that regulates cell division, elongation, and differentiation of plants. Endophytic bacteria like *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Burkholderia* are known to synthesize IAA and improve root development (Preyanga *et al.*, 2021; Rana *et al.*, 2023) ^[33, 35], nutrient uptake, and stress tolerance (Oviya *et al.*, 2023) ^[26]. Pal *et al.* (2021) ^[27] reported that, out of 56 endophytic isolates, 33 produced IAA. The production of IAA ranged from 1.65 (µg/mL) to 6.74 (µg/mL) with high production capacity observed in *Acinetobacter junii* J20N (6.74 µg/mL), *Pseudomonas pseudoalcaligenes* SEN29N (6.49 µg/mL), and *Bacillus firmus* J22N (5.82 µg/mL). Preyanga *et al.* (2021) ^[33] isolated 72 nodulating rhizobia and passenger endophytes from groundnut nodules in Tamil Nadu, India. Of these, 72% produced IAA (7.4-78.8 µg/ml), with *Rhizobium* and *Bacillus* species showing high IAA production. These isolates enhanced shoot and root growth, nodulation, and nutrient uptake in groundnut under field conditions (Prasanna *et al.* 2022) ^[32].

Phosphate solubilization

Of the 50 isolates screened, 14 isolates showed positive results for P solubilization (Table 3). The isolates GEB 61 (4.08) followed by GEB 70 (3.63), GEB 55 (3.33) and GEB 68 (3.22) have shown relatively higher phosphorous solubilization index compared to other isolates. Phosphorus is the 2nd most important nutrient required by the plants. Since significant amount of applied chemical fertilizers rapidly become unavailable to plants. Use of P solubilizing bacteria help in making available the unavailable/fixed phosphorus to plants. Occurrence of phosphate solubilizing microorganisms have been reported from different environmental niches (Chen *et al.*, 2006). Endophytic bacteria produce both organic acids like gluconic acid and acid phosphatases to solubilize both inorganic and organic phosphates (Hnini and Aurag, 2024) ^[10]. Inoculation of phosphate solubilising microorganisms under greenhouse as well as field conditions improved the plant growth of several crops. Use of PSB such as *Bacillus* and *Rhizobium* strains improved groundnut yield up to 20% through enhanced phosphorus uptake and soil enzyme activity (Vaishnav *et al.* 2023) ^[43]. Phosphorus solubilizing endophytic *Pseudomonas fluorescens* improved plant biomass of pea.

Potassium solubilization

A total of 12 isolates showed positive results for K solubilization. Among them, the isolates GEB 59 (3.40), GEB 49 (2.50) and GEB 69 (2.58) showed higher K solubilization index (Fig 2a and b). The bacteria belonging to different phyla such as Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, α -Proteobacteria, β -Proteobacteria, γ -Proteobacteria, and fungal phyla like Ascomycota and Glomeromycota were reported to solubilize potassium (Verma *et al.*, 2017). Potassium-solubilizing endophytic bacterial (KSB) strains improved germination and seedling vigour under salt stress (up to 120 mM NaCl), enhancing K⁺ assimilation and cytosolic K⁺/Na⁺ ratios, leading to increased plant growth and yield (Nawas *et al.*, 2023) ^[24].

Zinc solubilization

Nine isolates of total 50 showed positive results for Zn solubilization. Isolates GEB 48 (4.40), GEB 55, GEB 45 and GEB 68 (4.20) showed higher solubilization index in Tris minimal media with ZnO as prime source. Likewise, in ZnCO₃, isolates viz., GEB 71 (5.00) and GEB 68 (4.40) have showed higher SI (Table 6). Zinc is a crucial micronutrient for plant growth, development, disease suppression, and stress tolerance (Victoria *et al.*, 2025) ^[44]. Zinc-solubilizing bacteria provide an effective alternative to improve zinc availability in the soil (Upadhyay *et al.*, 2022; Saravanan, Kumar & Sa, 2011). Various zinc-solubilizing bacteria, such as *Pseudomonas protegens* RY2 (Yasmin *et al.*, 2021), *Bacillus megaterium* (Bhatt and Maheshwari, 2020) ^[18], and *Bacillus altitudinis* (Kushwaha *et al.*, 2021) ^[15], are recognized as plant growth-promoting bacteria. Inoculation of groundnut with zinc solubilizing endophytic *Pseudomonas aeruginosa* ABG5 significantly improved Zn content in groundnut shoots (Dhole *et al.*, 2016) ^[5].

Moisture stress tolerance

The isolate GEB 47 (1.60) has shown highest moisture stress tolerance in terms of growth in 20% PEG (6000) followed by the isolates GEB 39 (1.33), GEB 53 (1.30) and GEB 69 (1.30). Whereas isolate GEB 22 (1.01) has shown highest stress tolerance in 30% PEG (6000). The isolate GEB 44 (0.60), GEB 37 (0.55) has recorded least growth in 20% PEG and 30% PEG respectively indicating weak moisture stress tolerance capability as compared to other isolates (Table 7). Endophytic bacteria are more protected from environmental stresses than rhizosphere bacteria, because they live inside the plant tissues (Lata *et al.*, 2018; Rosenblueth and Martínez-Romero 2006). Plant-endophytic bacterial interactions enhance tolerance against drought stress (Paul and Lade 2014) ^[30]. Endophytic bacteria enhance drought tolerance by increasing compatible solutes like sugars, proline and RWC (Ngumbi and Kloepper, 2016). The isolates MKA2, MKA3, and MKA4 demonstrated drought tolerance by maintaining an O.D. value above 0.5 for 48 hours at 30% PEG-6000 (Meenakshi *et al.* 2019) ^[19]. Inoculation of *Trifolium arvense* with *Pseudomonas azotoformans* strain enhanced leaf RWC under both normal and drought conditions, demonstrating the role of endophytic bacteria in improving drought tolerance (Ma *et al.*, 2017). Application of endophytic *Bacillus subtilis* B26 capable of growing in a medium amended with 20% PEG enhanced the shoot biomass (30%), root length (25%), and relative water content (15%) of timothy seedlings.

ACC deaminase activity

The isolates GEB 58 (1.00), GEB 62 (1.00), GEB 72 (0.99) and GEB 60 (0.90) have shown highest growth measured in terms of

optical density at 600 nm on Dworkin and foster media amended with ACC compared to control with nitrogen source. The isolate GEB 67 (0.31) recorded lowest growth (Fig. 5). Ali *et al.* (2014) screened *Pseudomonas* isolates for ACC deaminase activity on DF minimal medium amended with 3 mM ACC as the sole nitrogen source and measured the growth by taking absorbance at 600 nm after incubation for 72 h at 28°C. ACC deaminase activity has been found in a wide range of gram positive and gram-negative bacteria (Nascimento *et al.*, 2014). These include *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, and *Rhizobium* (Kour *et al.*, 2024) ^[14]. It has been reported that ACC deaminase-producing bacteria promote plant growth under abiotic stress conditions (Rodríguez and Fraga., 1999; Fuentes-Ramirez *et al.*, 1993; Srinivasan *et al.*, 1996).

Biochemical characterization

Biochemical characterization of the selected isolates viz., GEB 22, GEB 45, GEB 50, GEB 55, GEB 65, GEB 68 and GEB 72 was done (Table 8). Raja *et al.* (2019) ^[34] characterized 20 bacterial isolates from groundnut nodules based on morphology and biochemical tests (Indole production, Methyl Red, Voges-Proskauer, Citrate utilization, Catalase oxidation, Starch hydrolysis, Urease, and Gelatin hydrolysis). Archana *et al.* (2020) ^[1] carried out biochemical tests like KOH, citrate utilization, indole, catalase, methyl red, Voges-Proskauer, urease, and gelatin liquefaction assays and identified seed endophytes based on colony traits, Gram staining, and biochemical tests conducted. Rana *et al.* (2023) ^[35] characterized nine endophytic bacteria using biochemical tests including indole production, catalase, urease, citrate utilization, and methyl red.

Table 1: Morphological characteristics of the isolated endophytic bacteria from groundnut

Isolate name	Configuration	Elevation	Margins
GEB 1	Round	Flat	Smooth (Entire)
GEB 4	Round with Scalloped Margin	Raised	Wooly
GEB 7	Round with Scalloped Margin	Convex	Smooth (Entire)
GEB 8	Round	Convex	Smooth (Entire)
GEB 9	Round with Scalloped Margin	Flat	Ciliate
GEB 10	Round with Scalloped Margin	Raised	Wooly
GEB 18	Round	Convex	Smooth (Entire)
GEB 19	Round with Scalloped Margin	Raised	Wavy (Undulate)
GEB 20	Round	Flat	Wavy (Undulate)
GEB 22	Round	Flat	Smooth (Entire)
GEB 25	Round	Raised	Wavy (Undulate)
GEB 27	Round	Convex	Smooth (Entire)
GEB 32	Round with Scalloped Margin	Convex	Wavy (Undulate)
GEB 35	Round	Convex	Branching
GEB 37	Round	Raised	Branching
GEB 38	Irregular and spreading	Flat	Irregular (Erose)
GEB 39	Round with Scalloped Margin	Raised	Wavy (Undulate)
GEB 40	Round	Flat	Smooth (Entire)
GEB 41	Round	Flat	Irregular (Erose)
GEB 42	Round with Scalloped Margin	Flat	Wavy (Undulate)
GEB 43	Round	Raised	Smooth (Entire)
GEB 44	Round with Scalloped Margin	Crateriform	Wavy (Undulate)
GEB 45	Round	Convex	Wavy (Undulate)
GEB 46	Round with Raised Margin	Drop-Like	Smooth (Entire)
GEB 47	Round with Raised Margin	Convex	Smooth (Entire)
GEB 48	Round with Scalloped Margin	Convex	Wavy (Undulate)
GEB 49	Round	Convex	Wavy (Undulate)
GEB 50	Round	Convex	Wavy (Undulate)
GEB 51	Round	Raised	Smooth (Entire)
GEB 52	Round	Flat	Smooth (Entire)
GEB 53	Round	Flat	Smooth (Entire)
GEB 54	Round	Raised	Smooth (Entire)
GEB 55	Round	Convex	Wavy (Undulate)
GEB 56	Round	Convex	Smooth (Entire)
GEB 57	Round	Convex	Smooth (Entire)
GEB 58	Round	Flat	Smooth (Entire)
GEB 59	Round	Raised	Smooth (Entire)
GEB 60	Round	Raised	Smooth (Entire)
GEB 61	Round	Convex	Smooth (Entire)
GEB 62	Irregular and spreading	Hilly	Wavy (Undulate)
GEB 63	Round	Convex	Smooth (Entire)
GEB 64	Round	Raised	Smooth (Entire)
GEB 65	Round	Raised	Smooth (Entire)
GEB 66	Round	Raised	Smooth (Entire)
GEB 67	Round with Scalloped Margin	Flat	Wavy (Undulate)
GEB 68	Round	Raised	Smooth (Entire)
GEB 69	Round	Convex	Smooth (Entire)
GEB 70	Round	Convex	Wavy (Undulate)
GEB 71	Round	Flat	Smooth (Entire)
GEB 72	Round	Convex	Wavy (Undulate)

Table 2: Screening of endophytic bacteria for indole acetic acid (IAA) production

Isolate name	IAA (µg/ml)	Isolate name	IAA (µg/ml)
GEB 1	2.16	GEB 48	2.56
GEB 4	2.87	GEB 49	2.68
GEB 7	2.72	GEB 50	2.92
GEB 8	2.95	GEB 51	2.72
GEB 9	2.59	GEB 52	2.87
GEB 10	2.71	GEB 53	2.39
GEB 18	2.41	GEB 54	2.66
GEB 19	2.52	GEB 55	2.69
GEB 20	2.53	GEB 56	3.08
GEB 22	3.09	GEB 57	2.71
GEB 25	2.79	GEB 58	3.36
GEB 27	2.35	GEB 59	3.17
GEB 32	3.45	GEB 60	2.57
GEB 35	2.29	GEB 61	3.00
GEB 37	2.64	GEB 62	3.31
GEB 38	3.18	GEB 63	2.53
GEB 39	2.49	GEB 64	2.18
GEB 40	2.48	GEB 65	2.72
GEB 41	2.70	GEB 66	2.76
GEB 42	2.49	GEB 67	2.37
GEB 43	2.56	GEB 68	2.32
GEB 44	2.51	GEB 69	2.83
GEB 45	2.70	GEB 70	2.29
GEB 46	2.50	GEB 71	2.95
GEB 47	3.01	GEB 72	2.49
GEB 48	2.56	S.Em ±	0.01
		C.D (p=0.05)	0.28

Table 3: Screening of endophytic bacteria for phosphorous solubilization

Isolate name	P solubilization	Isolate name	P solubilization
GEB 1	-ve	GEB 48	+ve
GEB 4	-ve	GEB 49	-ve
GEB 7	-ve	GEB 50	+ve
GEB 8	-ve	GEB 51	-ve
GEB 9	-ve	GEB 52	+ve
GEB 10	-ve	GEB 53	-ve
GEB 18	-ve	GEB 54	-ve
GEB 19	-ve	GEB 55	+ve
GEB 20	-ve	GEB 56	-ve
GEB 22	-ve	GEB 57	-ve
GEB 25	-ve	GEB 58	-ve
GEB 27	-ve	GEB 59	+ve
GEB 32	-ve	GEB 60	-ve
GEB 35	-ve	GEB 61	+ve
GEB 37	-ve	GEB 62	-ve
GEB 38	-ve	GEB 63	-ve
GEB 39	-ve	GEB 64	+ve
GEB 40	-ve	GEB 65	+ve
GEB 41	-ve	GEB 66	-ve
GEB 42	-ve	GEB 67	+ve
GEB 43	-ve	GEB 68	+ve
GEB 44	-ve	GEB 69	-ve
GEB 45	+ve	GEB 70	+ve
GEB 46	-ve	GEB 71	+ve
GEB 47	-ve	GEB 72	+ve

Table 4: Screening of endophytic bacteria for potassium solubilization

Isolate name	potassium solubilization	Isolate name	potassium solubilization
GEB 1	-ve	GEB 48	-ve
GEB 4	-ve	GEB 49	+ve
GEB 7	-ve	GEB 50	-ve
GEB 8	-ve	GEB 51	-ve
GEB 9	-ve	GEB 52	-ve
GEB 10	-ve	GEB 53	-ve
GEB 18	-ve	GEB 54	-ve
GEB 19	-ve	GEB 55	-ve
GEB 20	-ve	GEB 56	-ve
GEB 22	-ve	GEB 57	+ve
GEB 25	-ve	GEB 58	-ve
GEB 27	-ve	GEB 59	+ve
GEB 32	-ve	GEB 60	-ve
GEB 35	-ve	GEB 61	+ve
GEB 37	-ve	GEB 62	-ve
GEB 38	-ve	GEB 63	-ve
GEB 39	-ve	GEB 64	-ve
GEB 40	-ve	GEB 65	+ve
GEB 41	-ve	GEB 66	-ve
GEB 42	-ve	GEB 67	+ve
GEB 43	-ve	GEB 68	+ve
GEB 44	-ve	GEB 69	+ve
GEB 45	-ve	GEB 70	+ve
GEB 46	+ve	GEB 71	+ve
GEB 47	-ve	GEB 72	+ve

Table 5: Screening of endophytic bacteria for zinc (ZnO) solubilization

Isolate name	Zinc solubilization	Isolate name	Zinc solubilization
GEB 1	-ve	GEB 48	+ve
GEB 4	-ve	GEB 49	-ve
GEB 7	-ve	GEB 50	-ve
GEB 8	-ve	GEB 51	-ve
GEB 9	-ve	GEB 52	-ve
GEB 10	-ve	GEB 53	+ve
GEB 18	-ve	GEB 54	-ve
GEB 19	-ve	GEB 55	+ve
GEB 20	-ve	GEB 56	-ve
GEB 22	-ve	GEB 57	-ve
GEB 25	-ve	GEB 58	-ve
GEB 27	-ve	GEB 59	+ve
GEB 32	-ve	GEB 60	-ve
GEB 35	-ve	GEB 61	-ve
GEB 37	-ve	GEB 62	-ve
GEB 38	-ve	GEB 63	-ve
GEB 39	-ve	GEB 64	-ve
GEB 40	-ve	GEB 65	+ve
GEB 41	-ve	GEB 66	+ve
GEB 42	-ve	GEB 67	-ve
GEB 43	-ve	GEB 68	+ve
GEB 44	-ve	GEB 69	+ve
GEB 45	+ve	GEB 70	-ve
GEB 46	-ve	GEB 71	-ve
GEB 47	-ve	GEB 72	-ve

Table 6: Screening of endophytic bacteria for zinc solubilization (ZnCO₃)

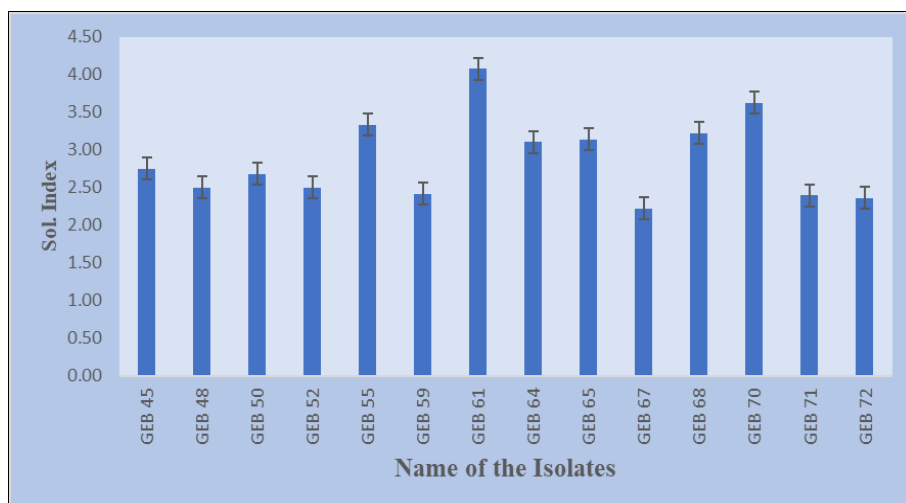
Isolate name	Zinc solubilization	Isolate name	Zinc solubilization
GEB 1	-ve	GEB 48	-ve
GEB 4	-ve	GEB 49	-ve
GEB 7	-ve	GEB 50	-ve
GEB 8	-ve	GEB 51	-ve
GEB 9	-ve	GEB 52	-ve
GEB 10	-ve	GEB 53	+ve
GEB 18	-ve	GEB 54	-ve
GEB 19	-ve	GEB 55	-ve
GEB 20	-ve	GEB 56	+ve
GEB 22	-ve	GEB 57	-ve
GEB 25	-ve	GEB 58	-ve
GEB 27	-ve	GEB 59	-ve
GEB 32	-ve	GEB 60	+ve
GEB 35	-ve	GEB 61	+ve
GEB 37	-ve	GEB 62	-ve
GEB 38	-ve	GEB 63	-ve
GEB 39	-ve	GEB 64	-ve
GEB 40	-ve	GEB 65	-ve
GEB 41	-ve	GEB 66	+ve
GEB 42	-ve	GEB 67	-ve
GEB 43	-ve	GEB 68	+ve
GEB 44	-ve	GEB 69	+ve
GEB 45	-ve	GEB 70	-ve
GEB 46	-ve	GEB 71	+ve
GEB 47	-ve	GEB 72	+ve

Table 7: Screening of endophytic bacteria for moisture stress tolerance

Isolate name	OD values at 600 nm (20% PEG)	OD values at 600 nm (30% PEG)	Isolate name	OD values at 600 nm (20% PEG)	Isolate name at 600 nm (30% PEG)
GEB 1	1.02	0.56	GEB 48	1.09	0.98
GEB 4	1.22	0.59	GEB 49	0.96	0.78
GEB 7	0.84	0.65	GEB 50	1.04	0.98
GEB 8	0.71	0.66	GEB 51	1.17	0.87
GEB 9	0.90	0.74	GEB 52	0.87	0.69
GEB 10	0.94	0.65	GEB 53	1.30	0.83
GEB 18	0.95	0.88	GEB 54	0.96	0.85
GEB 19	0.99	0.90	GEB 55	0.96	0.80
GEB 20	0.95	0.80	GEB 56	1.14	0.57
GEB 22	1.02	1.01	GEB 57	1.23	0.91
GEB 25	0.75	0.75	GEB 58	0.76	0.63
GEB 27	1.12	0.82	GEB 59	1.27	0.90
GEB 32	0.91	0.86	GEB 60	1.22	0.65
GEB 35	1.03	0.70	GEB 61	0.92	0.83
GEB 37	1.10	0.55	GEB 62	1.28	0.72
GEB 38	1.22	0.74	GEB 63	1.21	0.72
GEB 39	1.33	0.95	GEB 64	1.15	0.77
GEB 40	0.97	0.78	GEB 65	1.08	0.87
GEB 41	0.88	0.69	GEB 66	1.18	0.72
GEB 42	0.78	0.65	GEB 67	1.21	0.77
GEB 43	0.85	0.70	GEB 68	1.25	0.87
GEB 44	0.60	0.60	GEB 69	1.30	0.81
GEB 45	0.98	0.74	GEB 70	1.28	0.78
GEB 46	0.88	0.78	GEB 71	0.94	0.82
GEB 47	1.60	0.75	GEB 72	1.12	0.79

Table 8: Biochemical characterization of the selected endophytic bacterial isolates

Isolate name	Citrate utilization	Lysine utilization	Ornithine utilization	Urease detection	Phenyl alanine deamination	Nitrate reduction	H ₂ S production	Glucose	Aldonitol	Lactose	Arabinose	Sorbitol
GEB 22	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Positive
GEB 45	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive
GEB 50	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Negative	Negative	Negative	Positive	Positive
GEB 55	Positive	Positive	Positive	Negative	Negative	Negative	Positive	Negative	Positive	Positive	Positive	Positive
GEB 65	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive
GEB 68	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Positive
GEB 71	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Positive	Positive



*P solubilization index $a=C+Z/C$

Fig 1a: Phosphorous solubilization index of selected endophytic bacteria

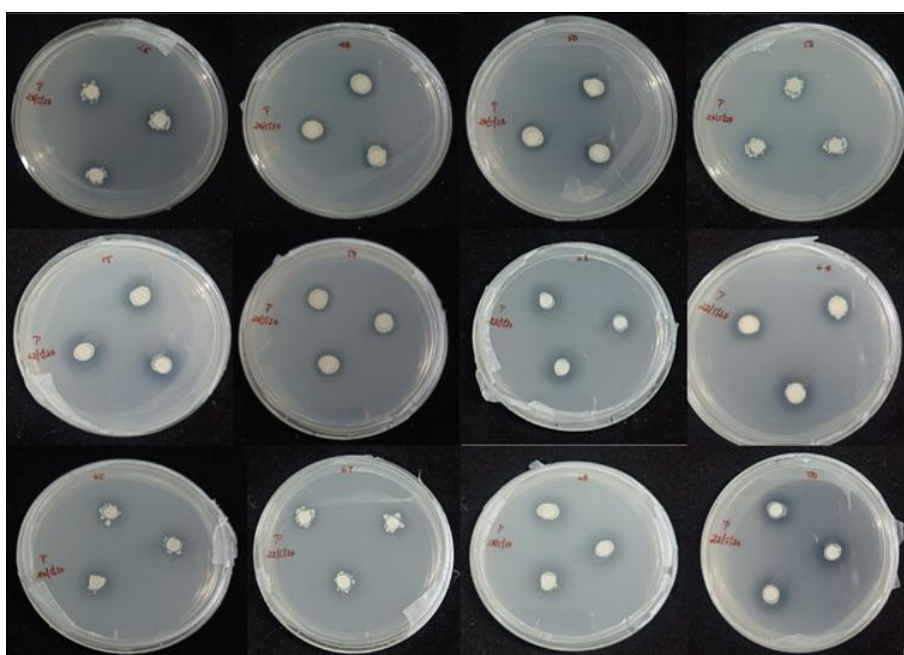
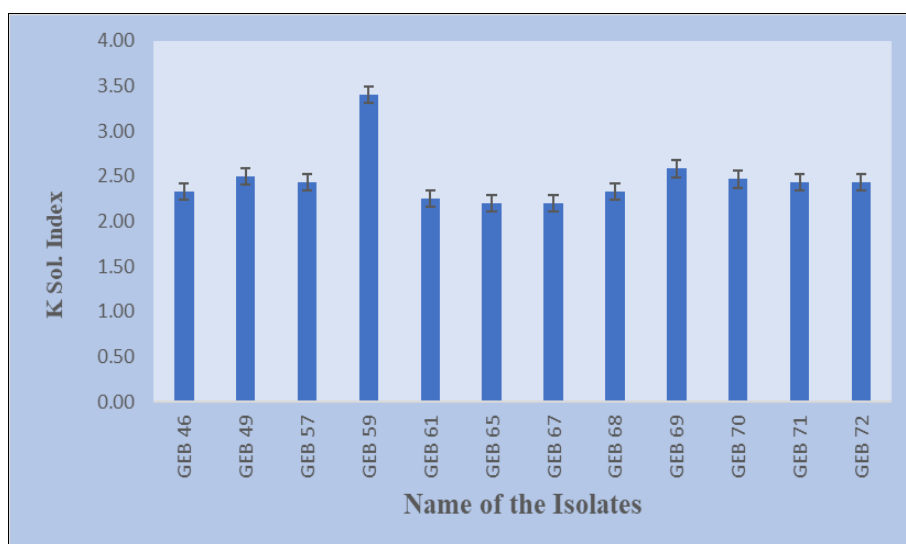


Fig 1b: Screening of bacterial endophytes for phosphorous solubilization



*K solubilization index $=C+Z/$

Fig 2a: Potassium solubilization index of selected endophytic bacteria

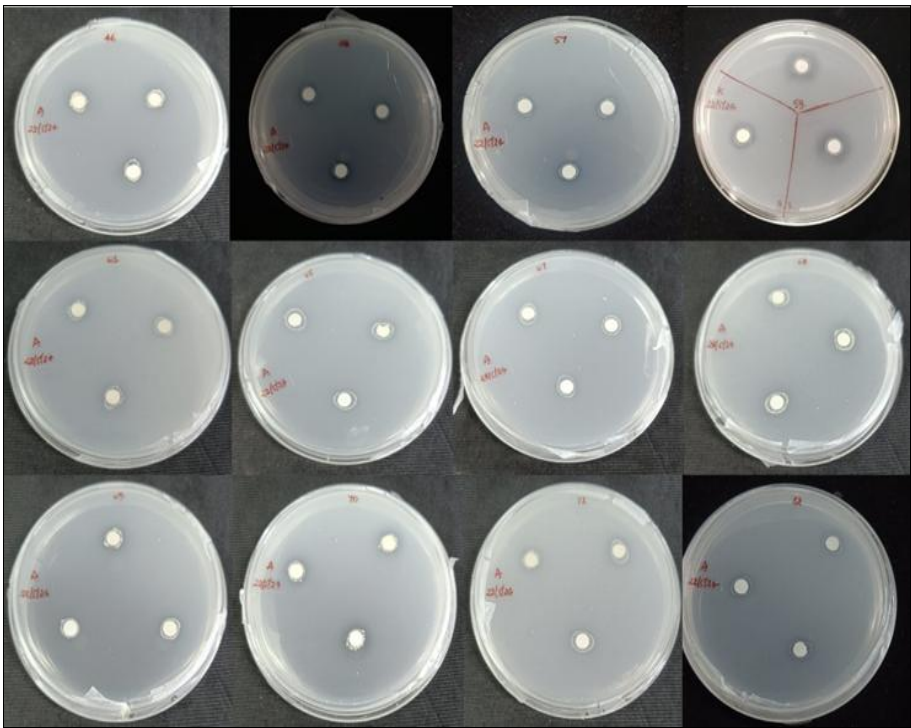
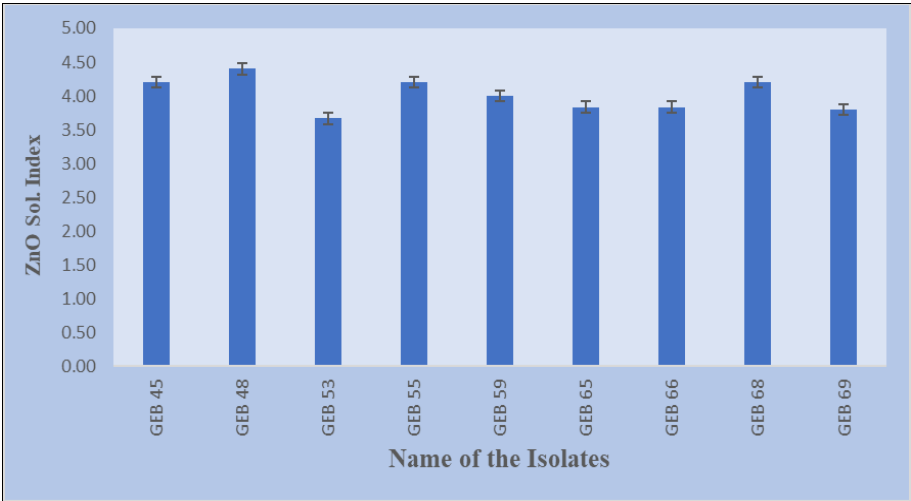


Fig 2b: Screening of bacterial endophytes for potassium solubilization



*Zinc solubilization index =C+Z/C

Fig. 3a: Zinc solubilization (ZnO) index of selected endophytic bacteria

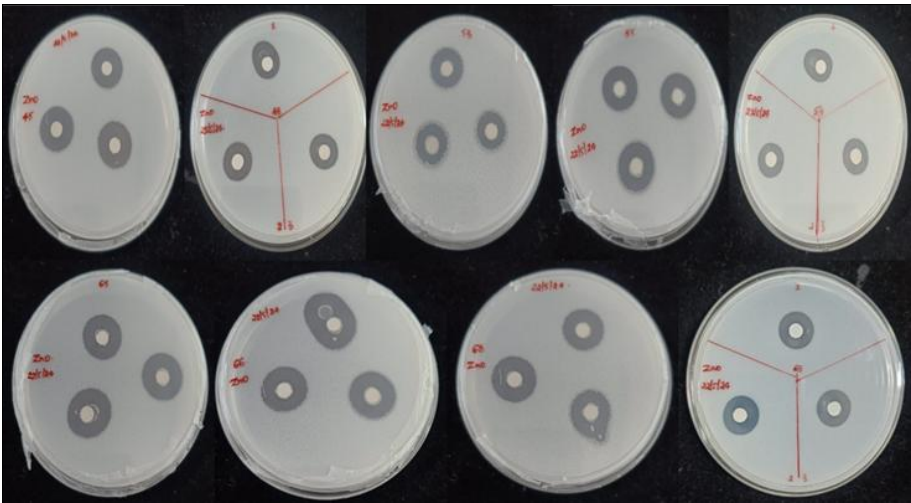
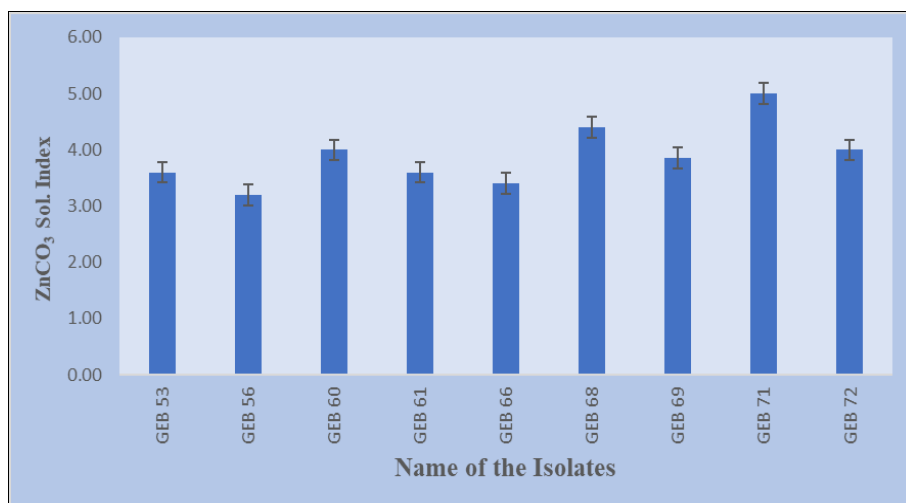


Fig 3b: Screening of bacterial endophytes for zinc (ZnO) solubilization



*ZnCO₃ solubilization index $a=C+Z/C$

Fig 4a: Zinc solubilization (ZnCO₃) index of endophytic bacteria

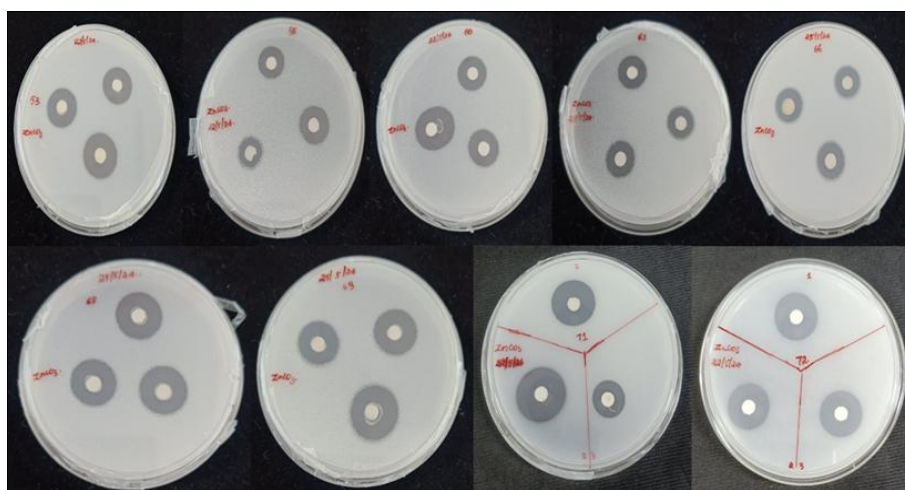


Fig 4b: Screening of bacterial endophytes for zinc (ZnCO₃) solubilization

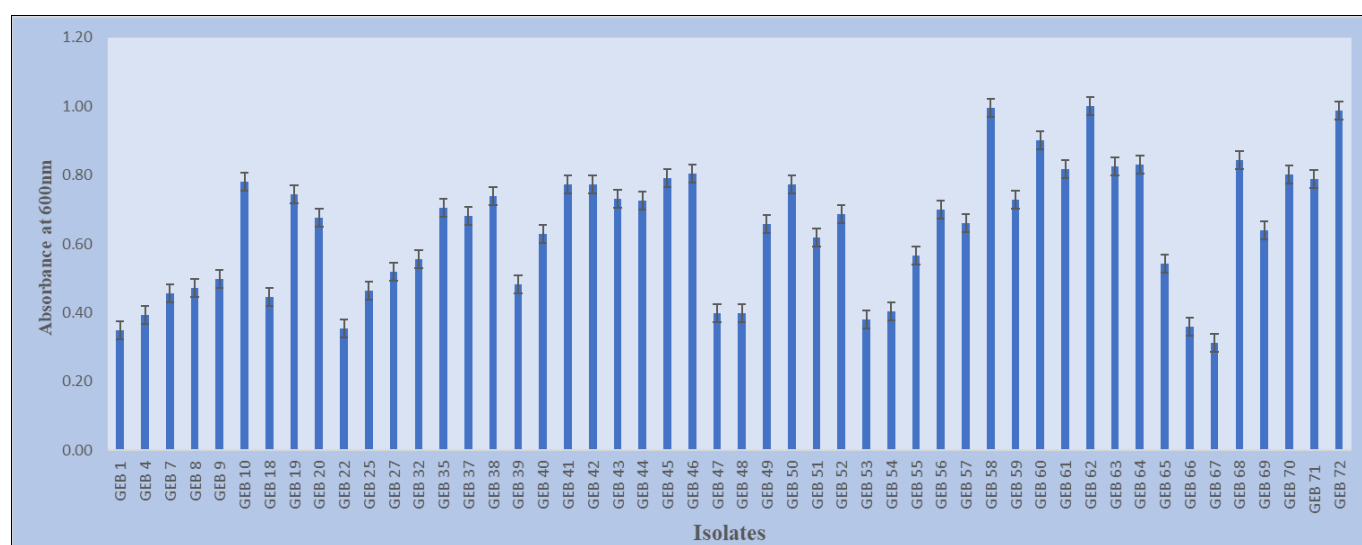


Fig 5: Screening of bacterial endophytes for 1- amino cyclopropane -1-carboxylate (ACC) deaminase activity

Conclusions

In the present study, 50 morphologically diverse endophytic bacteria were isolated from root and leaf samples of groundnut. The isolated endophytic bacteria were screened for various plant growth promoting features like multiple plant growth promoting characteristics such as phosphorus, potassium and zinc

solubilization, moisture stress tolerance, IAA production and ACC deaminase synthesizing capabilities. On the basis of screening under in-vitro conditions 07 isolates which were showing various plant growth promoting characteristics were selected for further evaluation under pot experiments. Further, best performing isolates will be identified by following

molecular approaches like 16S rRNA sequencing.

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