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POTENTIAL ENDOPHYTIC BACTERIA FROM *E. elatior*'s ROOTS FOR PLANT GROWTH-PROMOTING ACTIVITIES

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Abstract: Plant Growth-promoting Endophytic (PGPE) bacteria contribute to various developmental processes such as nitrogen fixation, plant disease resistance, nutrient cycling improvement, and the production of plant hormones and enzymes. *Etlingera elatior* is a plant from the Zingiberaceae family that has been used for medicinal, bioactivities, ornamental, culinary, and floral arrangement purposes. Diverse microorganisms with unknown potential reside in the roots of *E. elatior*. However, research on the presence of PGPE and its plant growth-promoting activities is still green. Therefore, this study aims to isolate and characterise the beneficial endophytic bacteria with plant growth-promoting activities. Results showed that six bacteria were successfully isolated from the roots of the plant. These bacteria exhibit a positive activity for phosphate solubilisation, indole-3-acetic acid production, nitrogen, fixation, and plant growth-promoting properties. Three of the six endophytes with the best results were molecularly identified. The results of 16S ribosomal ribonucleic acid sequence analysis identified endophyte *A3* as *Enterobacter cloacae*, endophyte *A4* as *Bacillus velezensis*, and endophyte *B2* as *Bacillus siamensis*. Endophyte *A3* exhibited the highest phosphate solubilising activity, with an index value of 3.13±0.03. The isolated PGPE from this study demonstrated significant potential as plant growth-promoting amendments.

Keywords: Sustainable agriculture, beneficial microbes, phosphate solubilisation, indole-3-acetic acid, nitrogen fixation.

Introduction

Plant growth-promoting endophytic (PGPE) bacteria are organisms that reside within plant tissues without causing harm to their hosts. The interaction between endophytic bacteria and plants is mutually beneficial and characterised as a symbiotic relationship (Mohd Rosli *et al*., 2024). The plants provide protection niches for the microorganisms, which, in return, enhance nutrient absorption, stimulate plant development, and induce resistance to pathogens. This interaction is influenced by various factors, leading to an intimate relationship through changes at cellular and molecular levels (Santos *et al*., 2018).

PGPE bacteria are widespread and can be found in all plant species, contributing to plant growth both directly and indirectly (Biswas *et al*., 2023). Typically, these bacteria directly enhance plant development through phytohormone production, fixing nitrogen, and improving the absorption of minerals such as phosphate, zinc, and potassium (Adeniji *et al*., 2018; Omotayo & Babalola, 2021). They produce indole-3-acetic acid (IAA), an important hormone which influences plant development by stimulating cell elongation, differentiation, lateral root formation, and chlorophyll production (Santoyo *et al*., 2016; Eid *et al*., 2021). PGPE bacteria also boost the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which helps plants cope with stress (Biswas *et al*., 2023).

A study by Anisha *et al*. (2013) revealed that the *Klebsiella* sp. bacteria present in the rhizome of turmeric can increase host plant growth by converting insoluble phosphate into a soluble form. This suggests that these bacteria

could be beneficial for improving phosphate utilisation in various plant species. Recent research found that phosphate-solubilising endophytes in peanut plants, *Enterobacter* sp. J49 and *Serratia* sp. S119, significantly enhanced the soybean and maize growth in microcosm experiments (Lucero *et al*., 2021). Endophytic nitrogen-fixing bacteria are found in many plants and are utilised as biofertilisers to lessen the demand for inorganic fertilisers. They improve nutrient use efficiency, increase crop output, and aid in reducing environmental pollution (Qin *et al*., 2022). According to Du *et al*. (2022), nitrogen-fixing bacteria have been found to not only stimulate nitrogen uptake in crops, but also improve photorespiration and carbohydrate metabolism.

Etlingera elatior is a clumping herbaceous flora belonging to the Zingiberaceae family, known for its fragrance and showy inflorescence (Yunus *et al*., 2021). This ginger species is commonly grown in Southeast Asia, with the centre of its concentration in Malaysia, Indonesia, and Thailand (Krajarng *et al*., 2017). *E. elatior* is a promising horticultural plant with multiple uses such as culinary, medicinal, bioactivities, ornamental, and floral arrangement purposes. It is commonly used traditionally due to the presence of biochemical compounds, including phenols, flavonoids, glycosides, saponins, tannins, steroids, and terpenoids (Yunus *et al*., 2022).

Plant organs host unique endophytic bacterial communities with varying diversity and composition. The root endosphere exhibits significantly lower microbial diversity compared with the rhizosphere (Liu *et al*., 2017). Bulgarelli *et al*. (2012) suggested that roots act as habitat filters, selecting specific bacterial lineages as conditions shift from soil to roots. Researchers have explored the potential of various root-associated microorganisms for their potential as plant growth promoters. However, there are no reports on the presence of the PGPE bacteria isolated from any part of *E. elatior.* This study provides the first documentation of *E. cloacae*, *B. velezensis*, and *B. siamensis* residing within the roots of *E. elatior*. The findings confirm that *E. elatior* roots host a variety of PGPE bacteria species. Moreover, these three isolated endophytic bacteria exhibit promising plant growth-promoting characteristics and could potentially be developed as environmentally sustainable biofertilisers for *E. elatior* and other significant plant species in the future.

Materials and Methods

Collection of Plant Root Samples

The plant samples were obtained from the Glasshouse and Nursery Complex, Kulliyyah of Science (KOS), International Islamic University Malaysia (IIUM), Kuantan. Healthy *E. elatior* plants without disease symptoms were selected for the isolation of endophytic bacteria [Figure 1 (A)]. The entire plant was excavated, and the shoots and roots were separated. The root samples [Figure 1 (B)] were transported to the Microbiology Laboratory, KOS, IIUM Kuantan.

Isolation of Endophytic Bacteria from *E. elatior* Roots

Endophytic bacteria were isolated under sterile conditions from the secondary roots of *E. elatior*. The roots were cut into 2 cm segments using a sterile blade and washed with tap water to remove dirt. They were then immersed in 20 mL of sterile distilled water for one minute. Surface sterilisation was performed by immersing the samples in 70% (v/v) ethanol for 10 seconds, followed by immediate rinsing with sterile distilled water. This process was repeated two to three times to ensure effective sterilisation. The root samples were then aseptically dissected into thin pieces to expose the inner part. The thin-cut roots were then placed on nutrient agar (NA) and incubated at 37°C. After three days of incubation, distinct endophytic bacterial colonies were recorded and isolated. The isolated bacteria underwent routine Gram staining for further morphological analysis. All the isolates were preserved at -40°C in equal volumes of nutrient broth (NB) and 50% (v/v) glycerol for further analysis.

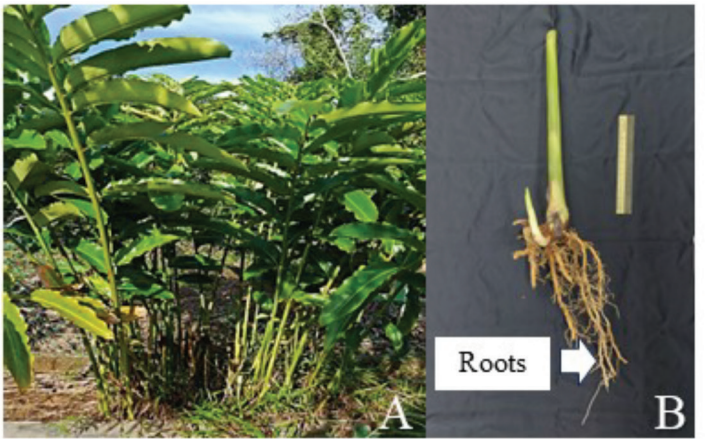


Figure 1: *E. elatior* from the GNC, IIUM. (A) Health plants reaching a maximum height of 4 metres. (B) Healthy root samples used for bacterial isolation

Evaluation of the Efficacy of the Surface Sterilisation Method

To determine the effectiveness of the surface sterilisation method, 100 µL of the final rinse water was streaked onto NA plate. The method was considered successful if no bacterial growth was observed on the media after incubation at 37°C for three days.

Phosphate Solubilising Activity Screening

The phosphate-solubilising ability of endophytic bacteria isolates was assayed using Pikovskaya's agar media, consisting of 5 g of tricalcium phosphate (TCP) as a phosphorus source. A loopful of every isolate was inoculated at the centre of the agar plates and incubated at 30±0.1°C for five days (Prasad *et al*., 2014). For the analysis, three technical replicates were examined. The halo zone and colony diameter of isolates were observed and recorded. Kalayu (2019) stated that the Phosphate Solubilising Index (PSI) was determined using the formula PSI = (Colony diameter + Halozone diameter)/ Colony diameter.

***In Vitro* Indole Acetic Acid (IAA) Analysis**

Initially, 50 mL of NB media consisting of 0.1% DL-tryptophan were augmented with 500 µL of 24-hour-old bacterial cultures. The mixtures were then placed in a shaker set at 30±0.1°C and 180 rpm for 48 hours. Following incubation, the bacterial cultures underwent centrifugation at 10,000 rpm (BIO-RAD) for 10 minutes at 4°C. Then, 1 mL of supernatant was added to 4 mL of Salkowski reagent and incubated for 30 minutes under dark conditions. The pink colour solution due to the IAA production was measured for its absorbance at 535 nm using a UV/Visible Spectrophotometer. The concentration of IAA was determined using the regression equation derived from a standard curve (Sundaram & Murali, 2018). The trials were designed using a completely randomised design. All experiments had three replicates. The differences between tryptophan and no tryptophan treatment were calculated and compared using Independent Samples T-Test at $p \leq 0.05$.

***In Vitro* Screening of Isolates for Nitrogen Fixation**

Endophytic bacteria were examined for nitrogen-fixing activity using the method developed by Ahmad *et al*. (2008). In this study, endophytic bacteria were investigated for nitrogen fixation by inoculation on Jensen's nitrogen-free medium supplemented with bromothymol blue dye (0.01%) as a colour indicator. Jensen's medium is specifically formulated for the identification and cultivation of nitrogen-fixing bacteria.

Molecular Identification of Endophytic Bacteria

For the identification of the isolates, the PGPE cultures were sent to Apical Scientific Sdn. Bhd. (Seri Kembangan, Selangor, Malaysia). The partial sequences of 16S rRNA obtained from Apical Scientific were aligned with the Basic Local Alignment Search Tool (BLAST) program in the National Centre for Biotechnology Information (NCBI) GenBank Database. Next, the sequences were aligned using the "Muscle" function in MEGA 11 software. A phylogenetic tree was constructed to determine the evolutionary relationships among closely related microorganisms. The tree was generated using Kimura 2-parameter model with 1,000 bootstrap replicates and the Maximum Likelihood (ML) method.

Results and Discussion

Morphological Analysis and Microscopic Characterisation

The emergence of bacteria from the sterilised root explants can be observed on the NA medium after 24 hours of incubation at 37°C (Figure 2). Six endophytic bacteria, designated as A3, A4, B1, B2, C3, and C4 were successfully isolated from *E. elatior* roots based on the observation of various distinct morphological characteristics (Table 1). Gram staining was performed to classify the endophytes into Gram-positive or Gram-negative bacteria. Staining and microscopic observation revealed that three endophytes, specifically A4, B2, and C4 exhibited traits characteristic of Gram-positive bacteria (Figure 3). The rest, A3, B1, and C3 were identified as Gram-negative bacteria (Figure 3). Notably, all six isolates were rod-shaped.

Analysis of Phosphate-solubilisation Activity

The results in Figure 4 show that the *A3*, *B2*, and *A4* isolated endophytes exhibit good phosphate-solubilising agents. The *A3* endophyte recorded the highest PSI value of 3.13±0.03, followed by *B2* and *A4*, with PSI values of 2.97±0.17 and 2.56±0.05, respectively. Phosphorus is

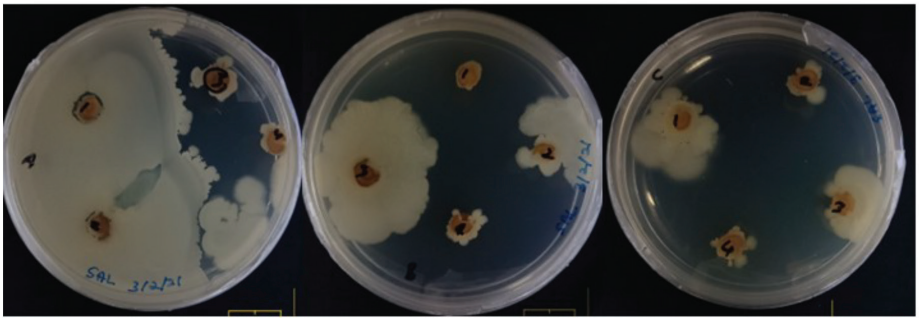
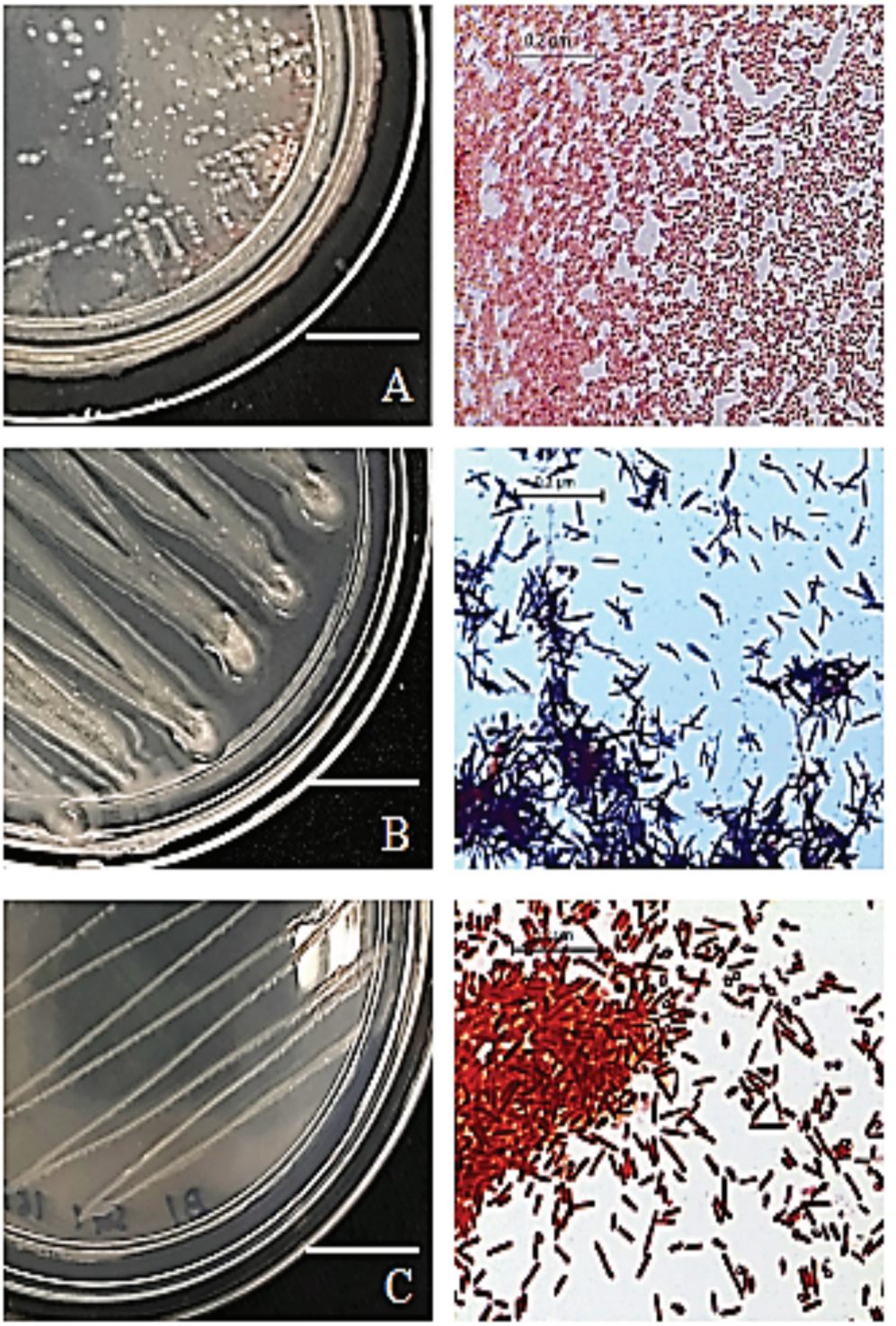


Figure 2: The emergence of bacteria from sterilised root explants on three NA medium plates after 72 hours of incubation at 37°C

Table 1: Colony morphological and microscopic characteristics of the six endophytic bacterial isolates

Endophytes	Colour	Elevation	Form	Texture	Cell Shape	Opacity	Gram
A3	White	Raised	Irregular	Creamy	Short rods	Translucent	-
A4	White	Flat	Irregular	Slimy	Rods	Translucent	+
B1	White	Flat	Entire	Creamy	Rods	Opaque	-
B2	White	Flat	Irregular	Creamy	Short rods	Translucent	+
C3	White	Raised	Rhizoid	Creamy	Short rods	Translucent	-
C4	White	Flat	Irregular	Creamy	Short rods	Opaque	+


A
B
C

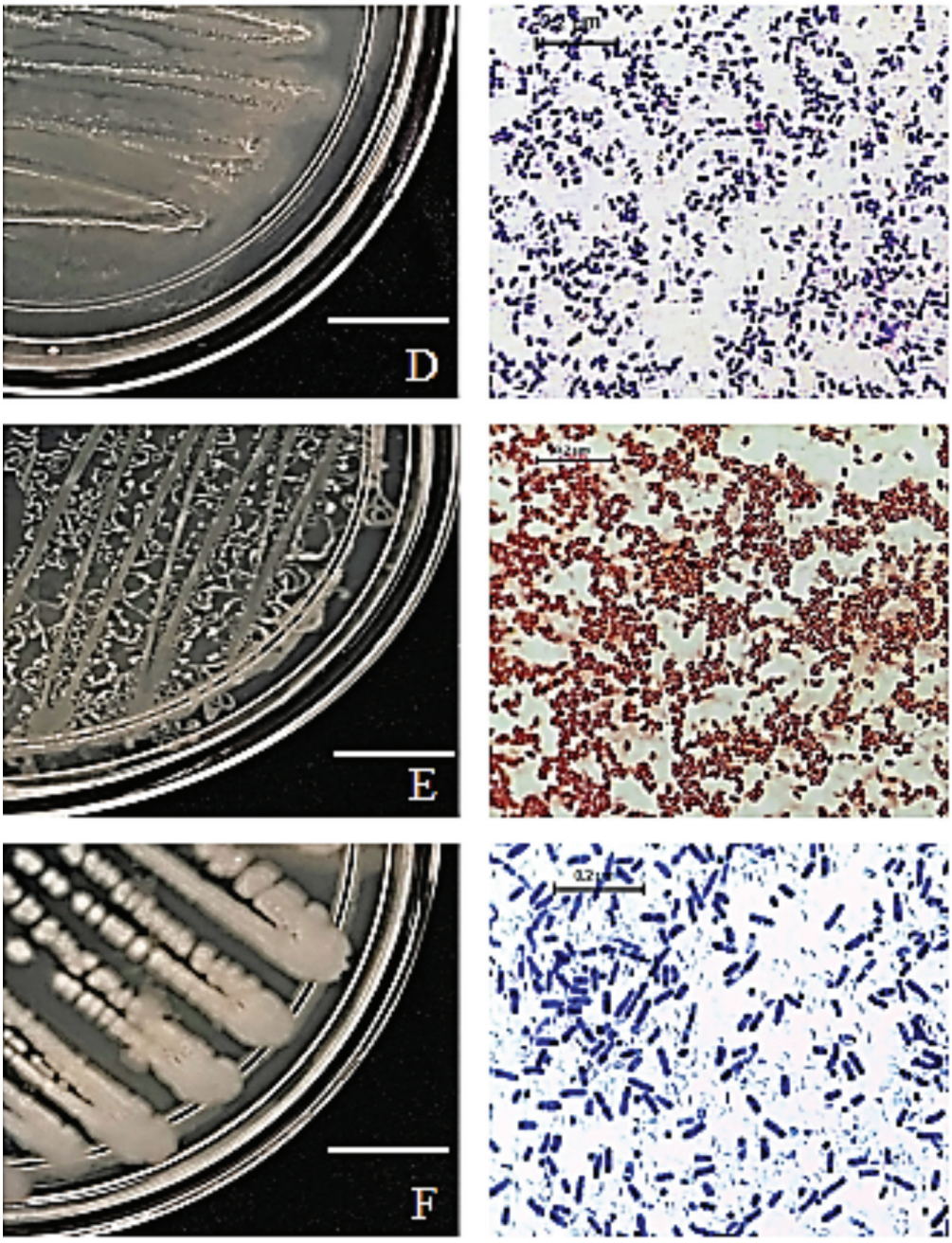


Figure 3: Morphological characteristics of the six endophytic bacterial isolates. (A) Endophyte A3, (B) Endophyte A4, (C) Endophyte B1, (D) Endophyte B2, (E) Endophyte C3, and (F) Endophyte C4. The left side shows the morphology of the colonies of the endophytes grown on nutrient agar while the right side shows the Gram-staining colour of the respective endophytes. White scale bar: 1 cm

crucial for plants, but is often inaccessible due to its tendency to form metal complexes in soil, binding to minerals or organic matter. PGPE microbes play a vital role in solubilising inorganic phosphate for plant development. Through mineral solubilisation, endophytic microorganisms can aid non-nodulating plants thrive in low-nutrient conditions by increasing the availability of essential minerals, particularly phosphate (Varga *et al*., 2020).

Solubilisation of the inorganic phosphate by the PGPE bacteria was evaluated through Pikovskaya's medium, which contains insoluble calcium phosphate $(Ca_3PO_4)_2$. Following a five-day incubation period, the presence of a halo zone surrounding all endophytic bacterial colonies was observed, indicating the successful solubilisation of calcium phosphate by these bacteria (Figure 5). Mardad *et al*. (2013) stated that the emergence of these clear halo zones surrounding the bacterial colonies results from the production of organic acids.

According to Silva and Vidor (2000), PSI values under 1.0 indicate very low solubilisation, 2.0 to 3.0 indicate medium solubilisation, and values above 3.0 indicate high solubilisation. Based on this criteria, *A3* is a high-solubiliser endophytic bacteria while isolates *B2* and *A4* are considered medium solubilisers. These findings align with previous studies on phosphate solubilisation by *Bacillus cereus ECL1*, *Bacillus* sp. ECL3, *Bacillus pumilis ECL4*, and *Pseudomonas putida* ECL5 isolated from the rhizomes of *C. longa* (Kumar *et al*., 2016). Saryanah *et al*. (2021) reported that isolate *TAT2*, an endophytic bacterium isolated from *Curcuma xanthorrhiza* shows the highest PSI index of 5.60. Among 57 bacterial isolates tested, 29 demonstrated the ability to solubilise phosphate with a PSI index ranging from 1.06 to 5.60. Another study found that out of 15 endophytic bacteria isolated from *Z. officinale*, four distinct strains, namely *GS2*, *GS5*, *GS8*, and *GS10*, demonstrated phosphate-solubilising capabilities (Jabborova *et al*., 2020).

The synthesis of organic acids from phosphate-solubilising microorganisms leads to an acidification of its surroundings, causing the discharge of phosphate ions. This discharge occurs as hydrogen ions (H^+) will substitute with phosphate-bound H^+ cations. As a result, phosphate ions become more accessible for plant uptake (Mardad *et al*., 2013). Therefore, this study successfully demonstrated the ability of *A3*, *A4*, and *B2* endophytes to solubilise phosphorus, making it more accessible for plant absorption.

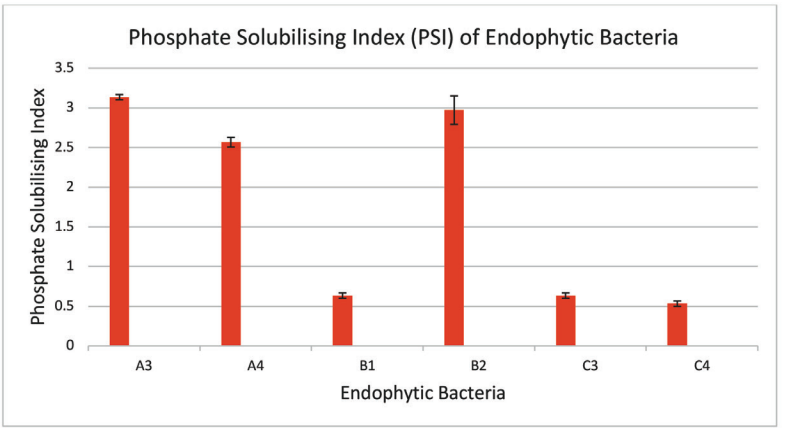


Figure 4: The phosphate solubilisation index of the six different endophytic bacteria isolates. Values represent the mean of three replicates

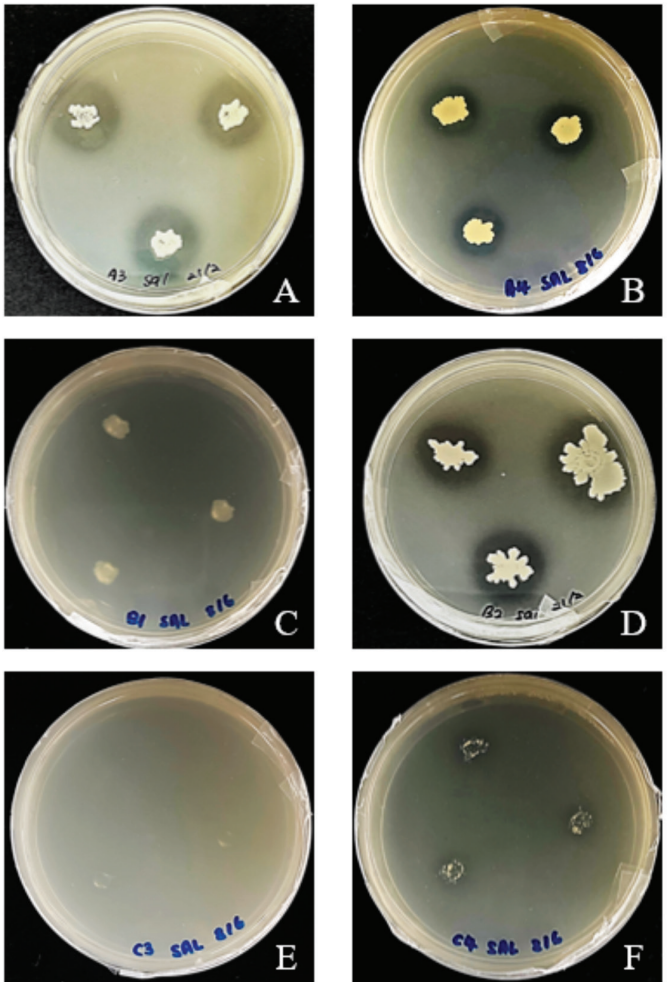


Figure 5: The phosphate solubilisation activity of the six different endophytic bacteria on Pikovskaya's media. (A) Endophyte *A3*, (B) Endophyte *A4*, (C) Endophyte *B1*, (D) Endophyte *B2*, (E) Endophyte *C3*, and (F) Endophyte *C4*. Endophytes *A3*, *A4*, and *B2* show large halo zone formation on Pikovskaya's media

Analysis of Indole Acetic Acid (IAA) Production

PGPE bacteria were cultured with and without the presence of tryptophan to assess their dependency on tryptophan availability for IAA biosynthesis. It was found that all endophytes produced IAA with varying concentrations, ranging from 9.72 µg/ml to 21.32 µg/ml. Endophyte *B2* was found to synthesise a high concentration of IAA, both in the presence or absence of tryptophan. Notably, all isolates were able to synthesise IAA, even in the absence of the tryptophan, indicating their ability to produce the phytohormone through both tryptophan-dependent and independent pathways (Figure 6). Isolate *B2* produces the highest IAA concentration, 19.79 µg/ml, in the presence of 0.1% tryptophan, and 21.32 µg/ml without the supply of tryptophan. In brief, based on the statistical analysis of IAA production, there was no significant difference between tryptophan-supplement and non-supplemented treatments across all six isolates.

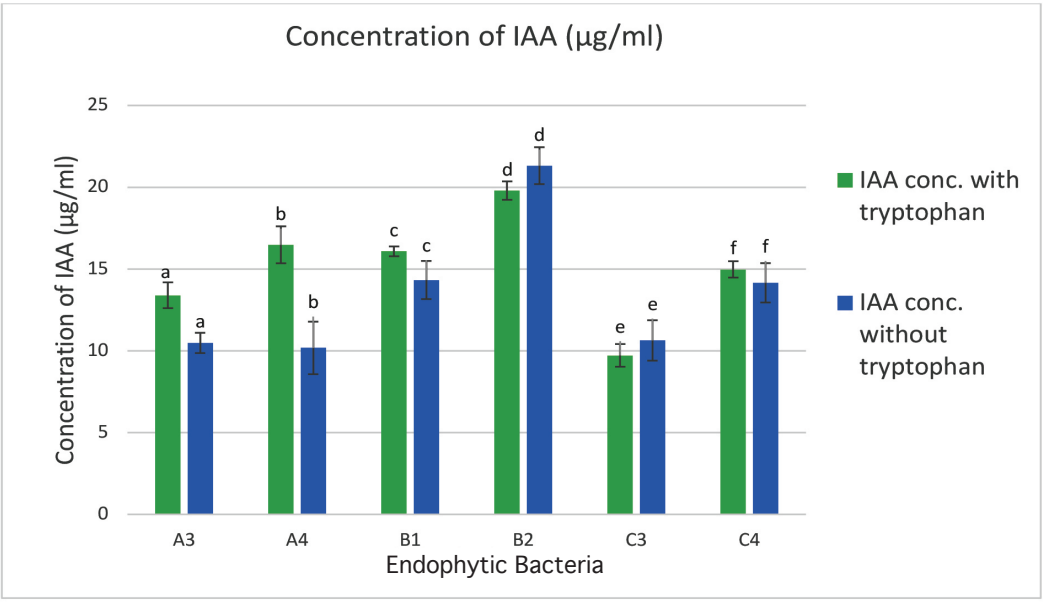


Figure 6: Production of IAA by endophytic bacteria with and without tryptophan. Values represent the mean of three replicates. Means with different letters indicate significant differences ($p \leq 0.05$)

IAA, a key phytohormone, regulates several physiological processes, including cell division, differentiation, and protein synthesis (Lestari *et al*., 2015). IAA can be synthesised by both plants and diverse microorganisms. This phytohormone not only promotes plant growth, but also facilitates communication and mutual symbiosis between plants and microorganisms (Lin *et al*., 2012). Tryptophan serves as a precursor for auxin production in both plants and microorganisms, providing bioactive compounds that promote the growth of rhizosphere microbes and endophytes. In soil, the microbial synthesis of IAA can be influenced by the presence of tryptophan, which may originate from root exudates or damaged cells (Waqas *et al*., 2012).

Wang *et al.* (2015) reported that IAA can be synthesised through both tryptophan-dependent and tryptophan-independent pathways. However, tryptophan may not always be available or be in a sufficient quantity for endophytic bacteria to synthesise IAA. While most microorganisms require tryptophan as a precursor for the synthesis of IAA, the isolates from *E. elatior* in this study demonstrated the ability to synthesise IAA without the need for supplemental tryptophan, suggesting that all isolates produce IAA through a tryptophan-independent pathway.

This finding aligns with Oliveira *et al*. (2020), who reported that all 12 diazotrophic bacteria isolated from the soil samples and roots of *E. elatior* could produce IAA, both in the presence and absence of tryptophan. Interestingly, five isolates (*UNIFENAS 100-340*, *UNIFENAS 100-342*, *UNIFENAS 100-343*, *UNIFENAS 100-346*, and *UNIFENAS 100-347*) produced more IAA in the absence of tryptophan. Furthermore, isolate *UNIFENAS 100-346* produced 82.17 µg/L of IAA with tryptophan and 40.03 µg/L without it (Oliveira *et al*., 2020). Conversely, Haque *et al*. (2023) found that *E. cloacae*, *E. ludwigii*, and *Bacillus* sp. isolated from rice produced approximately 7.5 µg/L of IAA. Additionally, Azri *et al*. (2024) reported that inoculation with IAA-synthesising bacteria significantly increased root growth, thereby enhancing fertiliser absorption.

Analysis of Nitrogen Fixation

Observations on Jensen's medium revealed visible colony growth for isolates *A3*, *A4*, *B2*, *C3*, and *C4*, indicating a positive nitrogen-fixation activity (Figure 7). The growth of bacteria on the culture medium indicates its ability to fix atmospheric nitrogen (Ogale *et al*., 2018). In general, colour changes from blue to yellow on the media added with bromothymol blue suggested that organic acids have been produced by the growing bacteria (Sulistiyani & Meliah, 2017; Aryantha & Hidiyah, 2018). Microorganisms from the Enterobacteriaceae and Bacillaceae families are known for their nitrogen-fixing activities and nitrogen assimilation processes (Li *et al*., 2022; Haque *et al*., 2023; Thakur & Gauba, 2024). The secretion of nitrate reductase, a key enzume, catalyses the nitrate-nitrite reduction process, where the nitrite, an organic acid, contributes to the observed colour change.

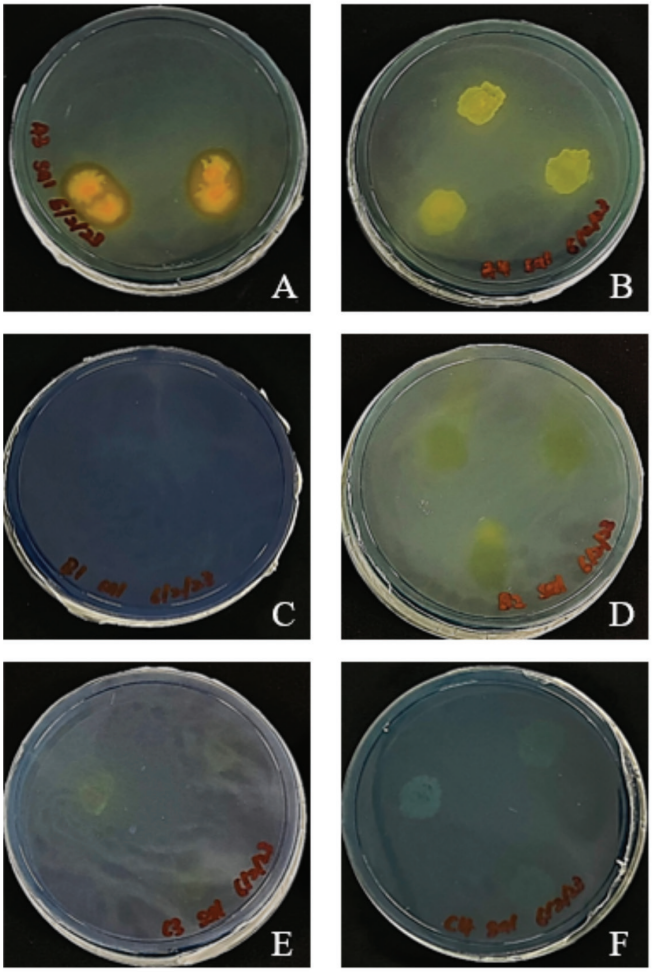


Figure 7: Six different endophytic bacteria were cultured on a Jensen's medium agar plate supplemented with bromothymol blue. Bromothymol blue was used as an indicator to detect the ability of nitrogen-fixing bacteria to survive in nitrogen-free media. Endophytes *A3*, *A4*, *B2*, *C3*, and *C4* showed the potential as nitrogen fixers. (A) Endophyte *A3*, (B) Endophyte *A4*, (C) Endophyte *B1*, (D) Endophyte *B2*, (E) Endophyte *C3*, and (F) Endophyte *C4*

Molecular Identification and Phylogenetic Tree Analysis

A detailed analysis revealed that endophytes *A3*, *A4*, and *B2* displayed the most pronounced potential in enhancing plant growth. Based on this observation, a comprehensive genotypic characterisation analysis was conducted for these isolates to determine their phylogenetic relationships.

The partial 16S rRNA sequences obtained were aligned with the Basic Local Alignment Search Tool (BLAST) programme from the National Centre for Biotechnology Information (NCBI) GenBank database. The BLAST analysis identified endophyte *A3* as closely related to *E. cloacae* subsp. *dissolvens* strain (accession number: NR_118011.1) with 99.51% similarity (Table 2). To gain a deeper understanding, phylogenetic trees were constructed for both endophytes to determine the relationship between microbial species. The 16S rRNA sequence-based phylogenetic tree analysis showed that *A3* was affiliated with the family Enterobacteriaceae and clustered with the *E. cloacae* clade (Figure 8). From the results, endophyte *A3* was renamed *E. cloacae* strain UIA3.

Table 2: Top 10 homology search results for endophytic bacteria *A3* using BLASTN

Accession Number	Description	Identity (%)	Total Score	E Value
NR_118011.1	*Enterobacter cloacae* subsp. dissolvens strain ATCC 23373 16S ribosomal RNA, partial sequence	99.51	2580	0.0
NR_044978.1	*Enterobacter cloacae* subsp. dissolvens strain LMG 2683 16S ribosomal RNA, partial sequence	99.38	2587	0.0
NR_028912.1	*Enterobacter cloacae* strain 279-56 16S ribosomal RNA, partial sequence	99.25	2591	0.0
NR_117405.1	*Leclercia adecarboxylata* ATCC 23216=NBRC 102595 strain LMG 2803 16S ribosomal RNA, complete sequence	99.25	2587	0.0
NR_102794.2	*Enterobacter cloacae* strain ATCC 13047 16S ribosomal RNA, complete sequence	99.00	2574	0.0
NR_024640.1	*Enterobacter asburiae* strain JCM6051 16S ribosomal RNA, partial sequence	98.62	2532	0.0
NR_044977.1	*Enterobacter cancerogenus* strain LMG 2693 16S ribosomal RNA, partial sequence	98.37	2558	0.0
NR_117547.1	*Enterobacter soli* ATCC BAA-2102 strain LF7 16S ribosomal RNA, partial sequence	98.25	2556	0.0
NR_146667.2	*Enterobacter mori* strain YIM Hb-3 16S ribosomal RNA, partial sequence	98.25	2569	0.0
NR_148649.1	*Enterobacter bugandensis* strain 247BMC 16S ribosomal RNA, partial sequence	98.85	2532	0.0

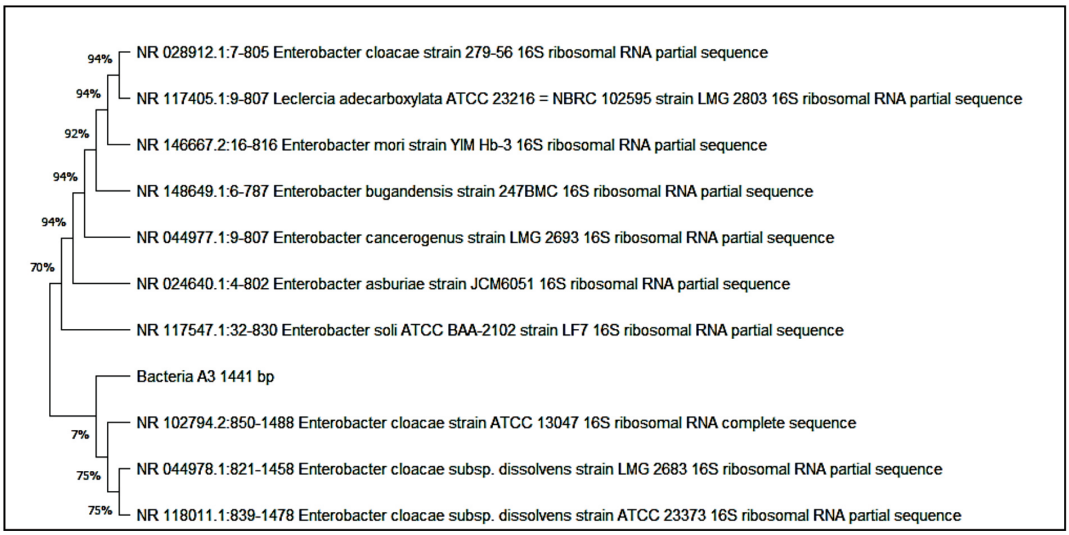


Figure 8: Phylogenetic tree analysis of the bacterial endophyte *A3* isolated from *E. elatior*. The 16S rRNA sequences were aligned using "Muscle" in MEGA 11 software. A phylogenetic tree was constructed using the maximum likelihood method. The numbers at the nodes indicate bootstrap values of 100% with 1,000 bootstrap replications

According to Macedo-Raygoza *et al*. (2019), *E. cloacae* strains obtained from both banana roots and leaves can inhibit the pathogen black Sigatoka fungus while also promoting the growth of plants in soil lacking organic matter. This finding aligns with Khalifa *et al*. (2016), who stated that *E. cloacae MSR1* extracted from non-nodulating Alfalfa roots demonstrated beneficial properties for plants. *MSR1* had several plant growth-promoting traits, including phosphate solubility, production of phytohormones like acetoin, and synthesis of bioactive compounds. Inoculating *Pisum sativum* with *MSR1* led to significant improvements in leaf length and dry weight compared with untreated plants (Khalifa *et al*., 2016). Additionally, *E. cloacae* has been identified as a PGPE bacteria in Kenyan basmati rice, exhibiting beneficial attributes such as the production of IAA and phosphate solubilisation. This finding highlights the significant impact bacterial endophytes like *E. cloacae* can have on plant growth, particularly through the production of IAA (Mbai *et al*., 2013). Further supporting this, Panigrahi *et al.* (2020) reported that the endophytic bacterium *E. cloacae* obtained from *Ocimum sanctum* produced IAA when cultivated in a medium supplemented with tryptophan.

Rijavec *et al*. (2007) identified *Enterobacter* sp. as an endophyte found in *Zea mays*, acknowledging its various plant growth-promoting effects (Deepa *et al*., 2010). Interestingly, *Enterobacter* sp. has been associated with its ability to produce siderophores (Giongo *et al*., 2010) and atmospheric nitrogen fixation (James, 2000). Additionaly, *E. cloacae* strain UIA3 exhibited similar plant growth-promoting potential to *Enterobacter* sp. (accession number: KY924600) isolated from rhizomes of *C. longa*. The latter demonstrated phosphate solubilisation, IAA synthesis, hydrogen cyanide production, siderophore production, and protease activity (Vinayarani & Prakash, 2018). Supporting this, Mowafy *et al*. (2021) reported that *Enterobacter* strain E1S2 isolated from *Phragmites australis* demonstrated the production of IAA, ammonia, and siderophores. Futhermore, *Enterobacter* strain E1S2 is also compatible with *Zea mays* and was successfully used as biostimulants in both lab and field experiments.

The sequencing of endophytes *A4* and *B2* was aligned with other bacterial sequences from BLAST in the NCBI GenBank database. Similar to endophyte *A3*, phylogenetic trees were developed to determine the relationship between microbial species. Interestingly, BLAST analysis revealed that *A4* had the closest relationship with *Bacillus velezensis* strain FZB42 (accession number: NR_075005.2), sharing 99.66% identity with the strain (Table 3). The phylogenetic tree analysis showed that endophyte *A4* was associated with the family Bacillaceae and clustered with the *B. velezensis* clade (Figure 9). Based on these results, endophyte *A4* was renamed *B. velezensis* strain UIA4.

In general, the generus *Bacillus* consists of Gram-positive, rod-shaped bacteria (Shahadat *et al*., 2023). *B. velezensis* is found in various ecological niches and the majority of the strains isolated from the rhizosphere could colonise plant roots and significantly contribute to the inhibition of pathogenic bacteria (Wu *et al*., 2015). According to Meng *et al*. (2016), *B. velezensis* generates compounds like IAA and ammonia, which stimulate the growth of various crops while also suppressing *Streptomyces scabies* pathogen. Multiple research works have demonstrated that *B. velezensis* can synthesise IAA and ammonia (Meng *et al*., 2016), siderophore (Kim *et al*., 2017), bacteriostatic substances (Fan *et al*., 2017), and lipopeptide antibiotics (Ait Kaki *et al*., 2013). These compounds contribute to the promotion of root architecture, optimising the absorption of nutrients, and stimulating plant development and survivability.

Rabbee *et al*. (2019) reported that *B. velezensis* has been utilised to enhance plant development and provide protection against a broad spectrum of phytopathogens. The result of the plant growth-promoting analysis revealed that endophyte *A4* exhibited similar capabilities to *B. velezensis* strain Lle-9 isolated from *Lilium leucanthum*. *B. velenzensis* strain Lle-9 exhibited beneficial properties like organic acid synthesis, ACC deaminase activity, siderophores production, IAA synthesis, nitrogen fixation, and phosphate solubilisation (Khan *et al*., 2020). Ji *et al*. (2021) reported that an endophytic *B. velezensis* JC-K3 strain originating from *Triticum aestivum* cultivated in soils with high

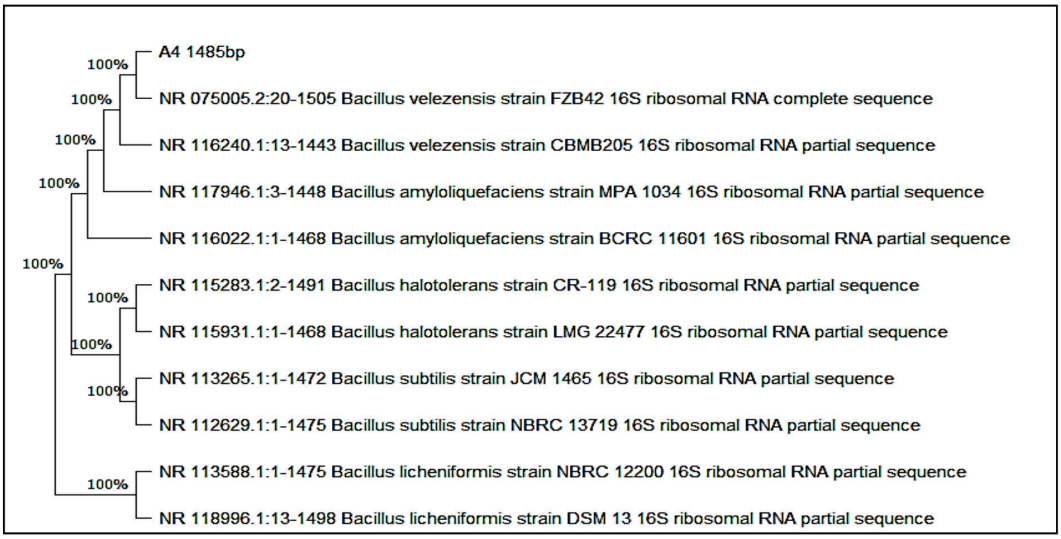


Figure 9: Phylogenetic tree analysis of the bacterial endophyte *A4* isolated from *E. elatior*. 16S rRNA sequences were aligned using "Muscle" in MEGA 11 software. A phylogenetic tree was constructed using the maximum likelihood method. The numbers at the nodes indicate bootstrap values of 100% with 1,000 bootstrap replications

Table 3: Top 10 BLASTN homology search results for endophytic bacteria *A4*

Accession Number	Description	Identity (%)	Total Score	E Value
NR_075005.2	*Bacillus velezensis* strain FZB42 16S ribosomal RNA, complete sequence	99.66	2715	0.0
NR_112629.1	*Bacillus subtilis* strain NBRC 13719 16S ribosomal RNA, partial sequence	99.39	2673	0.0
NR_113265.1	*Bacillus subtilis* strain JCM 1465 16S ribosomal RNA, partial sequence	99.39	2667	0.0
NR_116022.1	*Bacillus amyloliquefaciens* strain BCRC 11601 16S ribosomal RNA, partial sequence	99.46	2665	0.0
NR_115931.1	*Bacillus hatolerans* strain LMG 22477 16S ribosomal RNA, partial sequence	99.05	2632	0.0
NR_117946.1	*Bacillus amyloliquefaciens* strain MPA 1034 16S ribosomal RNA, partial sequence	99.52	2630	0.0
NR_116240.1	*Bacillus velezensis* strain CBMB205 16S ribosomal RNA, partial sequence	99.58	2608	0.0
NR_115283.1	*Bacillus hatolerans* strain CR-119 16S ribosomal RNA, partial sequence	98.05	2580	0.0
NR_118996.1	*Bacillus licheniformis* strain DSM 13 16S ribosomal RNA, partial sequence	97.71	2555	0.0
NR_113588.1	*Bacillus licheniformis* strain NBRC 12200 16S ribosomal RNA, partial sequence	97.70	2540	0.0

salinity, exhibited alkali tolerance, and produced high levels of IAA, siderophore, proline, soluble sugar, protease, cellulase, and glucanase.

Furthermore, BLAST analysis showed that endophyte *B2* had the closest relationship with three species, *Bacillus siamensis* strain IHB B 15617 (accession number: KU605232.1), *Bacillus siamensis* strain IHB B 15618 (accession number: KU605231.1), and *Bacillus siamensis* strain IHB B 14741 (accession number: KM817230.1), where *B2* shared 100% of its identity (Table 4). The 16S rRNA sequence-based phylogenetic tree analysis revealed that endophyte *B2* was affiliated with the family Bacillaceae and clustered with the *B. siamensis* clade (Figure 10). From the results, endophyte *B2* was renamed *B. siamensis* strain UIA5.

B. siamensis is recognised as a bacterium that promotes plant growth, fosters plant health, diminishes oxidative stress, and enhances antioxidant enzyme activity in *Triticum aestivum* (Awan *et al*., 2020), as well as enhancing nitrogen utilisation efficiency in *Capsicum annuum* (Pastor-Bueis *et al*., 2017). According to Feng *et al*. (2017), the rice endophyte *B. siamensis*

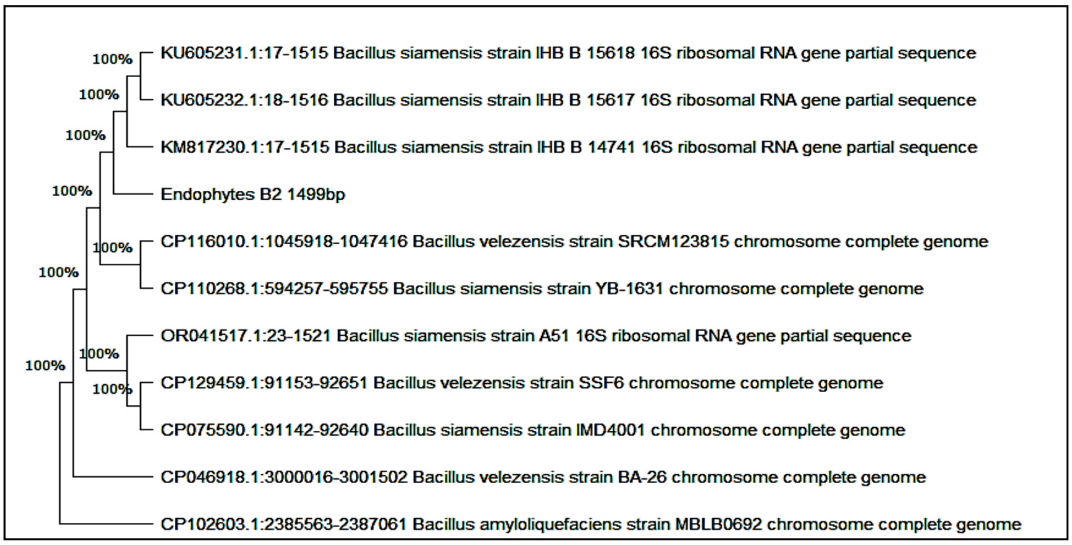


Figure 10: Phylogenetic tree analysis of the bacterial endophyte *B2* isolated from *E. elatior*. The 16S rRNA sequences were aligned using "Muscle" in MEGA 11 software. A phylogenetic tree was constructed using the maximum likelihood method. The numbers at the nodes indicate bootstrap values of 100% with 1,000 bootstrap replications

was found to enhance root growth through the production of volatile compounds, acting independently of hormonal pathways involving auxins, ethylene, or jasmonic acid. Gorai *et al*. (2021) stated that *B. siamensis* CNE6 isolated from *Cicer arietinum* exhibited many beneficial characteristics, including the production of IAA and siderophore, phosphate solubilisation, nitrogen fixation, and ACC deaminase activity.

Conclusions

The isolation, characterisation, and identification of endophytic bacteria from *E. elatior* hold significant promise for various agricultural and biotechnological applications. By exploring the diverse bacterial communities within this plant species, this study has identified strains with potential benefits for plant growth promotion, disease suppression, and environmental sustainability. Specifically, three PGPE bacteria, endophytes *A3*, *A4*, and *B2*, identified as *E. cloacae* strain UIA3, *B. velezensis* strain UIA4, and *B. siamensis* strain UIA5, exhibit several promising activities. Further studies are needed to fully explore the potential of these bacteria. Investigating their biochemical properties such as oxidase and catalase activities would provide valuable insights. Additionally, analysing the production of ACC deaminase is necessary to confirm their ability to manage ethylene stress. Examining their capacity to ferment carbon sources such as lactose and maltose would also be of interest. The study serves as a foundation for the formulation of growth media containing PGPE for better plant growth performance. Continued research in this field offers hope for harnessing the potential of endophytic bacteria from *E. elatior* to enhance crop productivity, improve agricultural practices, and contribute to the development of sustainable eco-friendly solutions for farming challenges.

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Table 4: Top 10 homology search results for endophytic bacteria *B2* using BLASTN

Accession Number	Description	Identity (%)	Total Score	E Value
KU605232.1	*Bacillus siamensis* strain IHB B 15617 16S ribosomal RNA gene, partial sequence	100	2769	0.0
KU605231.1	*Bacillus siamensis* strain IHB B 15618 16S ribosomal RNA gene, partial sequence	100	2769	0.0
KM817230.1	*Bacillus siamensis* strain IHB B 14741 16S ribosomal RNA gene, partial sequence	100	2769	0.0
CP046918.1	*Bacillus velezensis* strain BA-26 chromosome, complete genome	99.93	24570	0.0
CP129459.1	*Bacillus velezensis* strain SSF6 chromosome, complete genome	99.87	24790	0.0
OR041517.1	*Bacillus siamensis* strain A51 16S ribosomal RNA gene, partial sequence	99.87	2758	0.0
CP102603.1	*Bacillus amyloliquefaciens* strain MBLB0692 chromosome, complete genome	99.87	24734	0.0
CP116010.1	*Bacillus velezensis* strain SRCM123815 chromosome, complete genome	99.87	24733	0.0
CP110268.1	*Bacillus siamensis* strain YB-1631 chromosome, complete genome	99.87	24718	0.0
CP075590.1	*Bacillus siamensis* strain IMD4001 chromosome, complete genome	99.87	24733	0.0

Conflict of Interest Statement

The authors declare no conflict of interest.

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