TRENDS IN SCIENCES 2026; 23(3): 11347
https://doi.org/10.48048/tis.2026.11347
RESEARCH ARTICLE

Four Newly Identified Endophytic *Bacillus megaterium* Strains with Biofertilizer Potential for Enhancing Maize Growth and Productivity

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Received: 21 July 2025, Revised: 25 July 2025, Accepted: 5 August 2025, Published: 1 December 2025

Abstract

Intensive maize farming in tropical regions has led to increasing reliance on chemical fertilizers, which negatively impact soil fertility and sustainability. To develop more eco-friendly alternatives, this study aimed to isolate and evaluate indigenous *Bacillus megaterium* strains with plant growth-promoting potential from maize roots grown in high-input fields of An Giang Province, Vietnam. Ten endophytic bacterial isolates were initially screened based on morphological and biochemical traits. Four promising strains of *Bacillus megaterium* KL-197, KSW-114, ROA 024, and KSW-113 were identified via 16S rRNA sequencing and phylogenetic analysis. These strains were further tested for nitrogenase activity using the acetylene reduction assay and for their ability to tolerate varying temperatures and salt concentrations. High nitrogen-fixing potential and ammonia production were observed, particularly in strains ROA 024 and KSW-113. Field experiments were conducted using a randomized block design with 5 treatments (4 bacterial strains and a control) to assess effects on maize growth, yield, and seed quality. Inoculation with all 4 strains significantly improved plant height, chlorophyll content, and biomass. Strains ROA 024 and KSW-113 outperformed others, resulting in increased ear length, seed number, 1000-grain weight, and fresh ear yield achieving up to a 12% yield increase over the control. Protein content in maize grains also improved with inoculation, while lipid content remained unaffected. These findings demonstrate that the 4 newly identified native *Bacillus megaterium* strains, especially ROA 024 and KSW-113, possess strong nitrogen-fixing and growth-promoting capabilities under field conditions. The study highlights the potential application of these strains in biofertilizer development, offering a sustainable solution to enhance maize productivity while reducing dependence on synthetic nitrogen inputs.

Keywords: Biofertilizer, Macronutrient, Maize productivity, Natural nitrogen-fixing, Soil nutrient

Introduction

Nitrogen is the 2nd most important macronutrient required by plants, accounting for approximately 1% - 5% of total plant dry matter. It plays a vital role in the formation of nucleic acids, such as DNA and RNA, and is essential for the synthesis of proteins and enzymes involved in plant growth and metabolism [18]. Despite its critical role, the current agricultural model heavily relies on synthetic nitrogen fertilizers to meet crop nutrient demands. While these fertilizers can enhance yields, their extensive use has resulted in escalating production costs, declining soil health, and negative environmental impacts, such as water contamination and greenhouse gas emissions [1,2]. Although inorganic fertilizers contain high nutrient concentrations and provide immediate nutrient availability, their long-term use has contributed to the deterioration of soil structure, reduced microbial diversity, and the disruption of natural biogeochemical cycles [3]. Furthermore, the efficiency of nitrogen use in current systems remains low, with a significant proportion lost through leaching, volatilization, or denitrification [4]. To address these challenges, there is a growing interest in sustainable nutrient management strategies that integrate organic amendments and biofertilizers, aiming to improve soil fertility, reduce chemical inputs, and maintain crop productivity [5].

Endophytic nitrogen-fixing bacteria (ENFB) have emerged as a promising tool in sustainable agriculture. These bacteria inhabit the internal tissues of plants without causing harm and can fix atmospheric nitrogen into forms usable by plants, such as ammonium (NH_4^+) and nitrate (NO_3^-). By encoding nitrogenase enzymes, ENFB convert atmospheric nitrogen (N_2) into biologically available nitrogen, contributing significantly to the nitrogen needs of crops [6,7]. In maize (*Zea mays L.*), ENFB have been reported to supply up to 30% of the plant's nitrogen requirement under field conditions [8,9]. The symbiotic relationship between ENFB and maize not only supports nitrogen uptake but also enhances plant growth through various mechanisms, including phytohormone production, phosphate and potassium solubilization, siderophore secretion, and pathogen suppression [10,11]. Several genera of ENFB, such as *Bacillus*, *Enterobacter*, and *Azospirillum*, have been successfully isolated from maize roots and rhizosphere environments [12,13]. Among them, *Bacillus megaterium* is particularly notable due to its large cell size, diverse metabolic capabilities, and ability to adapt to various environmental conditions [14].

Bacillus megaterium has been extensively studied for its role as a biofertilizer. It is capable of fixing nitrogen, solubilizing phosphorus and potassium, and producing plant growth-promoting substances such as indole acetic acid (IAA) and siderophores [15,16]. These traits enable it to enhance root growth, improve nutrient uptake, and increase crop resilience to abiotic stresses. Recent studies have demonstrated that *Bacillus megaterium* can enhance yields of crops like cucumber and corn while simultaneously improving the bioavailability of phosphorus and potassium in the soil [17,3]. A field trial combining varying doses of P and K fertilizers with *Bacillus megaterium* inoculation reported an 11.8% to 15.2% increase in cucumber yield and a 27.5% to 46.1% rise in nutrient accumulation in plant tissues. Furthermore, *Bacillus megaterium* inoculation has been shown to increase microbial diversity in the rhizosphere, including beneficial bacterial and fungal taxa, which play critical roles in nutrient cycling and plant health [18,19]. These findings underscore the potential of *Bacillus megaterium* not only as a nitrogen fixer but also as a comprehensive plant growth-promoting rhizobacterium (PGPR) capable of improving soil health and crop productivity.

Despite these promising results, there remains limited research focusing on the dual role of *Bacillus megaterium* as both an ENFB and a broader PGPR species in maize cultivation. Most existing studies treat nitrogen fixation and other growth-promoting mechanisms separately. This compartmental approach overlooks the potential synergistic effects that multifunctional bacteria like *Bacillus megaterium* can offer. Therefore, investigating the integrated nitrogen-fixing ability, phosphate and potassium solubilization, and plant growth-promoting traits of *Bacillus megaterium* is crucial to fully exploit its agricultural benefits [21].

Maize, one of the world's most important cereal crops, plays a central role in global food security and agricultural economies, especially in developing countries. Its high nutrient demand, particularly for nitrogen, phosphorus, and potassium, makes it an ideal model for evaluating the effectiveness of biofertilizers like *Bacillus megaterium* [2,22]. Enhancing nutrient use efficiency in maize through microbial inoculants can reduce the dependency on synthetic fertilizers and contribute to more resilient agroecosystems. Considering the above considerations, this study was conducted to isolate and identify 4 strains of *Bacillus megaterium* from maize roots and to evaluate their nitrogen-fixing capacity alongside other beneficial traits such as phosphate and potassium solubilization and plant growth-promoting potential. The goal is to determine the potential application of these strains as biofertilizers to support sustainable maize production, reduce chemical fertilizer inputs, and promote ecological balance in cropping systems.

Materials and methods

Collection and treatment of corn roots

The corn root samples were obtained from corn fields in the Cho Moi district, An Giang, Vietnam. The roots were carefully uprooted, placed in sterile plastic bags, and transported to the laboratory. Before isolation, maize roots were sterilized by surface of 1st thoroughly washing them with tap water to remove soil and debris. The roots were then immersed in 70% ethanol for 3 min for partial sterilization, followed by treatment with 2.5% sodium hypochlorite for 5 min to eliminate most surface bacteria. Finally, the roots were rinsed again with 70% ethanol and several times with sterile distilled water to remove any remaining sterilizing agents. To verify the effectiveness of surface sterilization, sterilized root segments were placed on agar plates. The absence of bacterial growth after incubation indicated successful sterilization [23,24].

Isolation and morphological observation of 4 *Bacillus megaterium* strains

The maize root samples were finely ground and diluted to 10^{-8}. Then, 0.1 mL from per dilution was spread on YMA plates to isolate ENFB, and the petri dishes were incubated at 32 °C for 60 h. Among the pure colonies that were Gram-stained and observed at 100× magnification, 10 colonies showing several traits like *Bacillus megaterium* were selected for subsequent molecular identification; these pure colonies exhibited sizes of 3 - 4 mm with regular margins, and all 10 selected colonies were large, rod-shaped, endospore-forming, and Gram-positive [25].

Identification of 10 selected colonies

The 10 selected colonies were identified with their 16S rRNA genes by sequencing. Gene amplification was carried out using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [26,27]. The obtained sequences were compared to the GenBank database using BLAST, and multiple sequence alignment was performed using alignment software (https://www.ncbi.nlm.nih.gov/). The evolutionary relationships were determined based on the method outlined by Saitou and Nei [28], and Tamura *et al*. [29], with a bootstrap tree generated from 1000 replicates (Felsenstein, 1985). All ambiguous sites were excluded using the pairwise deletion option, phylogenetic and molecular evolutionary analyses were carried out with MEGA 11 [30]. The sequence data for the 4 strains consisted of *Bacillus megaterium* KL-197, *Bacillus megaterium* KSW-114, *Bacillus megaterium* ROA024 and *Bacillus megaterium* KSW-113, which were thoroughly compared with reference sequences in GenBank via BLAST, and given that the identification thresholds were 100%, these sequences were subsequently submitted to the NCBI GenBank database (**Figure 1**).

Assessing thermal adaptation

The heat adaptation of the 4 *Bacillus megaterium* strains was assessed in 2 stages. First, 4 test tubes containing YMA nutrient medium were prepared for each strain. Second, the strains were streaked onto YMA agar medium in test tubes and incubated at temperatures of 30, 35, 40, and 45 °C. Each species was tested with 4 replicates, and colony growth was monitored for 1 week [31].

Assessing salt adaptation

The salt adaptation of the 4 selected trains was evaluated through the following procedure: Initially, test tubes containing YMA agar medium were prepared for each train. Next, NaCl solution was added to reach final concentrations of 1.0%, 2%, 3%, 4%, and 5%. Lastly, selected colonies were separately inoculated for each selected strain and incubated at 28 °C with 4 replicates. The results were recorded after 1 week [31,32].

Ammonia production

The ammonia production capability of the identified strains was assessed qualitatively. Each colony was inoculated into peptone water and incubated on YMA medium at 30 °C for 60 to 80 h. The ammonia production was indicated by a color change from brown to yellow after adding Nessler's reagent [33].

Nitrogenase activity

The nitrogenase activity was assessed using the acetylene reduction assay. Four selected and identified strains were grown in YMA liquid medium and incubated for 24 h. The resulting suspension was then used to inoculate a nitrogen-free liquid medium. A control sample was prepared using the N-free liquid medium without the addition of 4 selected *Bacillus megaterium* strains. The cell density of the selected *Bacillus megaterium* suspension, determined using spectrophotometry at a wavelength of 600 nm, reached 0.8 under incubation conditions of 30 °C and 160 rpm [34].

Nitrogen concentration determination

The selected strains were cultured in an N-free medium with 0.05% malate as the primary carbon source and incubated at 30 °C. After centrifugation at 3000 rpm for 1 min, the supernatant was collected for nitrogen determination following the method of Li *et al.* [35].

Density augmentation of selected strains

The selected strains were inoculated into sterile YMA medium and incubated at 32 °C for 60 h. Following incubation, the cultures were centrifuged at 6,000 rpm for 5 min, and the supernatant was discarded. The cells were then washed with sterile saline to achieve a concentration of 10^8 CFU mL^{-1}. The resulting suspensions of the 5 selected strains were prepared as a 1.0% (v/v) inoculum to assess factors affecting plant growth. In the field experiment, 100 mL of this cell suspension was applied to peanut seeds prior to planting [36]. Given their strong nitrogen-fixing capacity and consistent enhancement of maize growth and yield under field conditions, the 4 *Bacillus megaterium* strains (KL-197, KSW-144, ROA024, and KSW-113) have been preserved under cold storage for future formulation and application as biofertilizers. Their potential use as sustainable alternatives to chemical nitrogen inputs highlights their value in promoting eco-friendly maize production and long-term soil fertility.

Study area

The field experiment was conducted in Cho Moi Commune, An Giang Province, Vietnam. This area is situated in the Mekong Delta region, characterized by high agricultural intensity and tropical monsoon climate conditions. The estimated geographical coordinates of the study site are approximately 10.50° N latitude and 105.40° E longitude. The region is representative of intensive maize cultivation systems, making it a suitable location for evaluating the agronomic potential of plant growth-promoting bacterial strains.

Experimental design

An arranged treatments of field experiment was in a completely randomized design with 5 treatments and 4 replications. The 5 treatments included C0, C1, C2, C3, and C4, with the arrangements shown in **Table 1**. Inorganic fertilizers used were single fertilizers such as urea, superphosphate, and potassium chloride. All fertilizer quantities were converted to N, P, and K weight per hectare, applied at a rate of 150 kg Urea $[CO(NH_2)_2]$ ha^{-1} + 80 kg P_2O_5 ha^{-1} + 80 kg KCl ha^{-1} for all experimental treatments (**Table 1**). The area of arranged treatments was 400 m² (2.0 m×10 m×5 treatments×4 replications) and the distance between 2 plots was 0.5 m. The hybrid corn variety F1 LION 10 from Hai Mui Ten Do Company, Vietnam were used during the experiment, which were planted in single rows with a spacing of 30 cm (2 seeds per hole). The corn seeds (F1 LION 10) were manually sown from November 2024 to February 2025. The corn was harvested 90 days after sowing (DAS). One healthy plant was kept in each hole to monitor throughout the experiment.

Table 1 The layout of the experimental treatments under field conditions.

Treatments	ENFB (10^8 CFU mL^{-1})	Chemical fertilizers (kg ha^{-1})
C0 (control)	Without ENFB	150Urea + 80 P_2O_5 + 80KCl
C1	*Bacillus megaterium* KL-197	
C2	*Bacillus megaterium* KSW-114	
C3	*Bacillus megaterium* ROA024	
C4	*Bacillus megaterium* KSW-133	

Data analysis

Data were processed using Statgraphite XVI software to determine the mean and perform variance analysis. Statistical analysis was carried out, and comparisons were made using Duncan's test, considering a significant level of $p \leq 0.05$.

Results and discussion

Morphological and biochemical testing of 4 selected colonies

The 4 colonies were selected from the initial 10 isolates based on molecular identification results. All 4 strains were 4 *Bacillus megaterium* strains, they included *Bacillus megaterium* KL-197, *Bacillus megaterium* KSW-114, *Bacillus megaterium* ROA 024, and *Bacillus megaterium* KSW-113, which were confirmed to exhibit genetic similarity (**Table 2**). In the next step, their morphology was examined to assess their nitrogen-fixing potential before conducting field experiments. Four strains, including *Bacillus megaterium* KL-197, *Bacillus megaterium* KSW-144, *Bacillus megaterium* ROA 024, and *Bacillus megaterium* KSW-113, were noted by BM1, BM2, and BM4, respectively.

Table 2 Similar percentage of 4 strains and code number.

Note	Genera	Species	Strains	Similar (%)	Code number
BM1	*Bacillus*	*megaterium*	KL-197	100	AY030338.1
BM2	*Bacillus*	*megaterium*	KSW-114		OR 514194.1
BM3	*Bacillus*	*megaterium*	ROA 024		MT 510154.1
BM4	*Bacillus*	*megaterium*	KSW-113		OR514193.1

Table 3 The physical and chemical characterization of 4 *Bacillus megaterium* strains was determined using the VITEK 2 analyzer.

Biochemical test	BM1	BM2	BM3	BM4	Biochemical test	BM1	BM2	BM3	BM4
Beta–Xyloidine	+	+	+	+	NaCl (1 - 5%)	++	++	++	++
D–Mannose	–	–	–	–	Temperature (20 - 50 °C)	+	+	+	+
D–Glucose	–	–	–	–	pH (4.0 - 8.0)	++	++	++	++
D–Galactose	–	–	–	–	Citrate use	+	+	+	+
D–Ribose	–	–	–	–	β–Glucosidase	+	+	+	+
Catalase	++	++	++	++	Mannitol	+	+	+	+
Oxidase	–	–	–	–	Raffinose	+	+	+	+
Starch hydrolysis	+	+	+	+	Glucose oxidation	+	+	+	+

Notes: (–): Negative reaction; (+) Weak reaction; (++) Strong reaction.

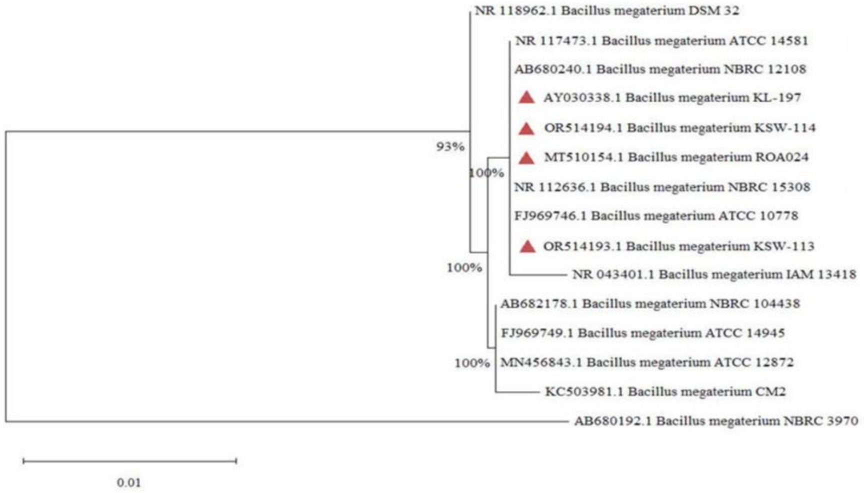

Figure 1 The phylogenetic tree for the 16S rRNA sequence of 4 *Bacillus megaterium* strains was constructed using the neighbor-joining method.

The VITEK 2 analysis system (BioMérieux, France) that provided a 92% probability and were confirmed using Illumina gene analysis, was utilized to identify the morphological and biochemical characteristics of 4 selected strains. Morphologically, before identification was sent out the identification, the 4 colonies of *Bacillus megaterium* appeared cream-yellow, 3 mm in diameter, non-pigmented, and ranged from round to irregular with smooth to wavy edges on YMA medium. All 4 *Bacillus megaterium* strains were Gram-positive, motile rod-shaped bacteria, catalase-positive, and grew aerobically at temperatures of 20 - 40 °C, with optimal growth at pH 4 - 7, a temperature range of 20 - 50 °C, and tolerance to salt concentrations up to 5%. Four *Bacillus megaterium* strains were mainly identified through the API 50 BCL system, and some of the results from the VITEK 2 analyzer are presented in **Table 3**. Furthermore, the species phylogenetic analysis based on the 16S rRNA sequence with a total branch length of 0.01 confirmed the isolated strains as *Bacillus megaterium* KL-197, *Bacillus megaterium* KSW-144, *Bacillus megaterium* ROA 024, and *Bacillus megaterium* KSW-113, with the respective accession numbers AY030338.1, OR 514194.1, MT510154.1 and OR514193.1, all showing 100% similarity with the NCBI reference sequence as shown in **Figure 1**.

Determination of ammonia activity and concentration

The reduction process of C_2H_2 to C_2H_4 using the C_2H_2 gas injection system initially showed a delay after introducing C_2H_2 into the system and then became stable over a period of 0 to 72 h. The rate of C_2H_2 to C_2H_4 reduction in the experimental setups, conducted with 4 replications in the laboratory, was measured by tracking the increase in C_2H_4 concentration during the stable period, which lasted around 72 h.

Results of **Figure 2** show that 4 *Bacillus megaterium* strains were monitored for acetylene reduction tests to evaluate the nitrogenase activity. **Figure 2** shows that *Bacillus megaterium* ROA024 (BM3) obtained the highest value of ethylene (330 nmol C_2H_4/h/mL), followed by *Bacillus megaterium* KSW-113 (BM4: 270 nmol C_2H_4/h/mL) and *Bacillus megaterium* KSW-114 (BM2: 213 nmol C_2H_4/h/mL), while *Bacillus megaterium* KL-197 (BM1:198 nm/h/mL) had the lowest value. The Kjeldahl method is commonly used to estimate nitrogen concentration. The research results from **Figure 2** indicate that *Bacillus megaterium* strains actively participate in the nitrogen fixation [13]. The amount of nitrogen was produced by the 4 strains ranging from 164 to 312 mg N/100 mL. *Bacillus megaterium* ROA 024 produced the highest amount of nitrogen (312 mg N/100 mL), followed by *Bacillus megaterium* KL-197 (187 mg N/100 mL), while *Bacillus megaterium* KSW-113 and *Bacillus megaterium* KSW-114 had the lowest result (164 and 165 mg N/100 mL), with a statistically significant difference at the 1% level.

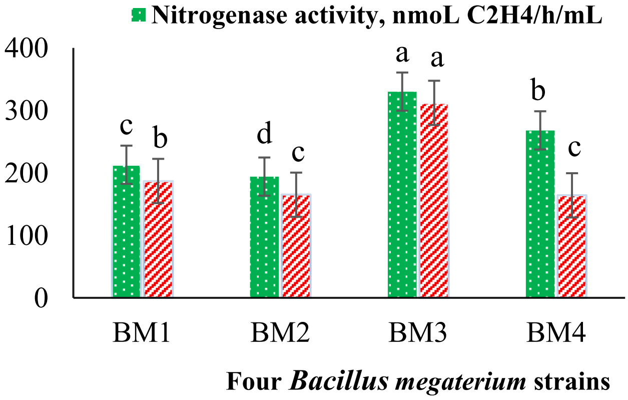

Figure 2 Nitrogenase activity of 4 *Bacillus megaterium* strains measured by the acetylene reduction assay, along with corresponding nitrogen concentrations. Data are presented as mean ± standard deviation. Different lowercase letters (a, b, c, and d) indicate statistically significant differences among treatments, as determined by one-way ANOVA followed by Duncan's multiple range test at $p < 0.01$.

However, the results from **Figure 2** show that all 4 strains can produce high nitrogen concentrations after just 72 h of experimentation. In this research, 4 novel *Bacillus megaterium* strains were isolated from maize roots using YMA nutrient medium. Phylogenetic analysis of their 16S rRNA gene sequences confirmed that they belong to the *Bacillus* genus and show close genetic relationships to known *megaterium* species and strains, which consisted of KL-197, KSW-144, ROA024 and KWS-113 (**Table 3**). Through biochemical tests, it showed that all 4 strains could produce ammonia, synthesize the nitrogenase enzyme, and possess nitrogen-related genes involved in nitrogen fixation. Their growth capability using ammonia gas as the sole nitrogen source and acetylene-reducing activity were successfully demonstrated for these 4 newly isolated strains [2,37].

The aim of this study is not only to isolate and identify new *Bacillus megaterium* strains from maize roots, but more importantly, to select strains with strong plant growth-promoting potential. These selected endophytic strains were further evaluated for their effectiveness as biofertilizers through field experiments on maize. Nitrogen fixation of ENFB is an ecologically important role, which contributed to a natural N source as an input of nitrogen into the environment and represents the nitrogen-fixing capability of the *Bacillus megaterium* strains [38,39]. All 4 strains exhibited high nitrogen-fixing mechanisms, making them suitable for use as biofertilizers (**Table 3** and **Figure 2**). The production of nitrogenase enzymes plays a crucial role in nitrogen fixation, raising the natural N content in the soil, reducing chemical nitrogen usage, increasing available nutrients, and promoting plant growth, especially in nitrogen-deficient soils [40].

Effect of 4 *Bacillus megaterium* strains on corn growth traits

All 4 *Bacillus megaterium* strains promoted corn agronomy traits compared to those of the control (**Figure 3**). Analyzing ANOVA to compare each strain to the uninoculated treatment, 4 strains significantly increased 3 agronomy parameters: Leaf number, chlorophyll content, and plant height. *Bacillus megaterium* ROA024 (C3) exhibited the highest stimulation of these 3 parameters, followed by *Bacillus megaterium* KSW-113 (C4), *Bacillus megaterium* KSW-144 (C2), and *Bacillus megaterium* KL-197 (C1). Meanwhile, no strain had the lowest values for all 3 parameters, and all differences were highly significant ($p < 0.01$). Bacterial treatments had significant influences on leaf number, chlorophyll content, and plant height. *Bacillus megaterium* strains observed during the field experiment demonstrated the potential for plant growth promotion in corn experiments. *Bacillus megaterium* has been shown to have broad-spectrum activity, as it has been effective in promoting the growth of soybean and wheat. The plant growth stimulation by *Bacillus megaterium* species observed in this research aligns with prior studies indicating that *Bacillus megaterium* strains effectively enhanced growth across various crop species [41]. According to Akinrinlola *et al.* [42], corn exhibits a stronger response to *Bacillus megaterium* strains compared to soybean or wheat. Similarly, Lee *et al.* [43], assessed the plant growth-promoting abilities of thirty bacterial strains on wheat seedlings and found that only 4 *Bacillus megaterium* strains were effective. Furthermore, *Bacillus megaterium* strains demonstrated the highest growth-promoting effects on corn compared to other strains [44].

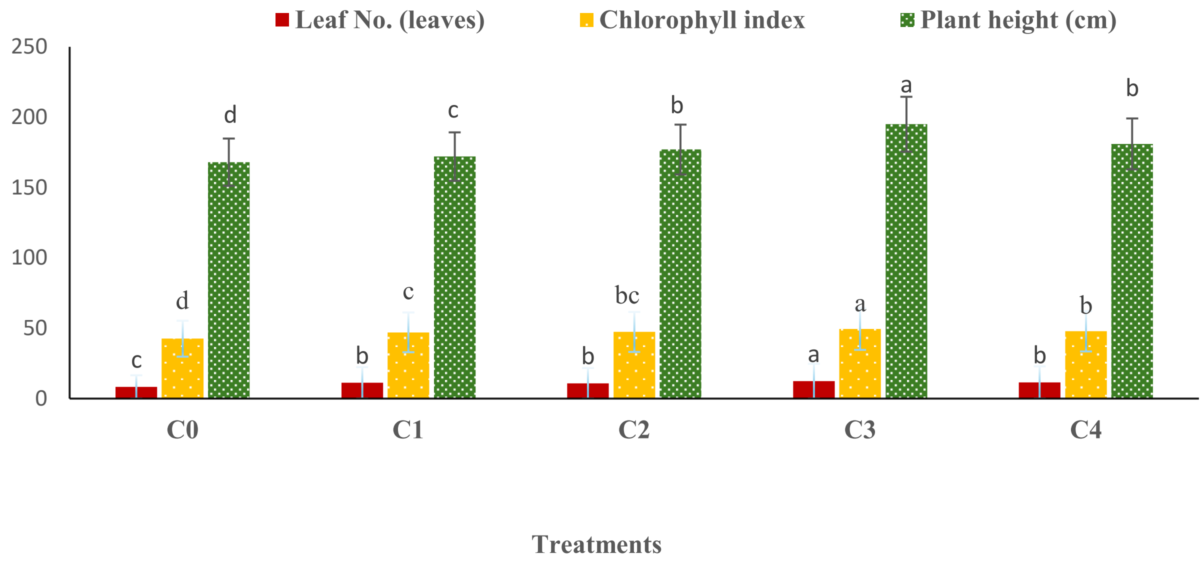

Figure 3 Effects of 4 *Bacillus megaterium* strains on maize agronomic traits. Values are expressed as mean ± standard deviation. Different lowercase letters (a, b, c and d) denote significant differences among treatments based on 1-way ANOVA with Duncan's multiple range test at a significance level of $p < 0.01$.

Effects of 4 *Bacillus megaterium* strains on yield component, productivity and nutrient compositions

The addition of the 4 *Bacillus megaterium* strains resulted in higher values for all yield parameters, including fruit length and diameter, the number of rows per fruit, the number of seeds per row, and plant biomass, compared to the control without strains. Among them, treatment C3 (*Bacillus megaterium* ROA024) and C4 (*Bacillus megaterium* KSW-113) showed the highest improvement in yield components, followed by *Bacillus megaterium* KSW-144 (C2), and *Bacillus megaterium* KL-197 (C1), while the control (C0) had the lowest values among the 5 treatments (**Table 4**).

The ENFB application as biofertilizers to regulate rhizosphere microbial ecosystems and boost nutrient absorption is a potential strategy for enhancing crop development in nutrient-deficient soils [45,46]. In a recent study, A successfully isolated a novel *Bacillus megaterium* strain with plant growth-promoting capabilities. The findings revealed that *Bacillus megaterium* OQ560352 influenced the fungal and bacterial communities in the maize rhizosphere and was positively associated with phosphorus solubilization activity in alkaline soil. The combination of NPK fertilizer with *Bacillus megaterium* resulted in a 4% increase in shoot biomass, a 6% rise in shoot phosphorus concentration, a 10% boost in soil phosphorus concentration, and a 13% enhancement in phosphorus solubilization activity. In conclusion, this study emphasizes the potential of the newly isolated *Bacillus S megaterium* strain as an effective plant growth-promoting rhizobacterium capable of enhancing maize growth and yield through modulation of the microbial community [47,48]. Similarly, Li *et al.* [49], recently identified 3 endophytic bacterial strains, consisting of *Bacillus megaterium*, *Bacillus flexus*, and *Bacillus subtilis*, which were isolated from maize roots and applied as bio-stimulants to assess their impact on maize development.

Table 4 The effect of 4 *Bacillus megaterium* strains on corn yield traits.

Treatment	**Ear length (cm)**	**Ear diameter (cm)**	**No. of rows per ear (rows)**	**No. of seed per ear (seed)**	**Plant biomass (t ha^{-1})**
C0	13.9^{c} ± 0.35	3.60^{c} ± 0.04	11.9 ± 0.37	30.0^{b} ± 1.33	24.8^{c} ± 1.11
C1	17.0^{b} ± 0.32	4.08^{b} ± 0.12	12.1 ± 0.26	29.6^{b} ± 0.60	32.1^{b} ± 0.96
C2	16.9^{b} ± 0.51	4.09^{b} ± 0.15	11.8 ± 0.55	34.1^{a} ± 0.32	32.0^{b} ± 1.33

Treatment	Ear length (cm)	Ear diameter (cm)	No. of rows per ear (rows)	No. of seed per ear (seed)	Plant biomass (t ha^{-1})
C3	17.9^{a} ± 0.50	4.82^{a} ± 0.26	12.1 ± 0.39	34.2^{a} ± 0.61	35.8^{a} ± 0.68
C4	18.3^{a} ± 0.42	4.80^{a} ± 0.06	12.3 ± 0.42	35.1^{a} ± 0.63	35.3^{a} ± 0.65
F	**	**	ns	**	**
CV (%)	9.82	11.7	3.36	7.59	13.0

Notes: ±: The standard error of the mean is based on 4 replicates per treatment. Different superscript letters (a, b, and c) in the same column denote significant differences among treatments according to Duncan's multiple range test at $p < 0.01$ (**), and ns indicates no significant difference.

Table 5 The effect of 4 *Bacillus megaterium* strains on yield traits, yield and seed-nutrient composition.

Treatment	Weight of 1,000 seeds (g)	Fresh ear yield (t ha^{-1})	Lipid (%)	Protein (%)
C0	369^{b} ± 2.97	12.5^{b} ± 0.32	2.78 ± 0.02	4.03^{c} ± 0.16
C1	371^{b} ± 3.58	12.9^{b} ± 0.29	2.79 ± 0.02	4.55^{b} ± 0.12
C2	385^{a} ± 3.07	13.8^{a} ± 0.22	2.78 ± 0.03	4.75^{b} ± 0.15
C3	387^{a} ± 3.50	13.8^{a} ± 0.21	2.80 ± 0.03	5.12^{a} ± 0.16
C4	389^{a} ± 4.11	14.2^{a} ± 0.25	2.81± 0.02	5.11^{a} ± 0.19
F	**	**	ns	**
CV (%)	2.44	5.12	0.88	9.32

Notes: ±: The standard error of the mean is based on 4 replicates per treatment. Different superscript letters (a, b, and c) in the same column denote significant differences among treatments according to Duncan's multiple range test at $p < 0.01$ (**), and ns indicates no significant difference.

Four *Bacillus megaterium* strains demonstrated favorable outcomes across multiple parameters during the experiment. During the harvest season, corn ears and seeds were harvested to assess the weight of 1,000 seeds, ear yield, and the percentage of lipid and protein content in the seeds. As shown in **Table 5**, the weight of 1,000-seeds and fresh ear yield. The treatment C2, C3, and C4 were higher than those in C0 and C1. While lipid concentration showed no significant difference, protein content was highest in C3 and C4, followed by C2 and C1, with the lowest in C0. Compared to the control (C0), the average yield increase in treatments of *Bacillus megaterium* addition reached up to 12%, with a statistically significant difference at the 1% level (**Table 5**).

In recent years, there has been growing interest in examining the influence of various *Bacillus megaterium* strains on different crop species. According to a recent study of Oliveira-Paiva *et al.* [50] used 40 ENFB species to assess their impact on maize and sorghum growth. Among these species, *Bacillus megaterium* exhibited a greater increase in plant height, shoot dry weight, and chlorophyll content compared to the control group without *Bacillus megaterium*. This aligns with our findings, as the 4 *Bacillus megaterium* strains also demonstrated significant improvements in multiple parameters: 1) Agronomic traits (**Figure 3**), including plant height, leaf number, chlorophyll concentration, and plant biomass; 2) Yield components such as 1000-seeds weight and fresh ear yield; 3) Grain nutritional composition including percentage of lipid and protein content percentages (**Table 5**). These values were significantly higher in maize plants inoculated with *Bacillus megaterium* compared to the non-inoculated control. This consistency in results further confirms that *Bacillus megaterium* strains exhibit high performance and have a positive impact on maize growth [50].

Conclusions

This study successfully isolated and identified 4 indigenous *Bacillus megaterium* strains (KL-197, KSW-144, ROA024, and KSW-113) from maize root tissues cultivated in intensive farming areas of Cho Moi commune, An Giang, Vietnam. These strains demonstrated strong nitrogen-fixing capabilities under laboratory conditions, with notable ammonia production and nitrogen-fixing activity. Field trials further validated their agronomic potential, where all 4 strains significantly enhanced maize growth, yield, and seed protein content compared to the uninoculated control. Among them, strains ROA024 and KSW-113 consistently outperformed the others, contributing to a yield increase of up to 12%. The isolated strains also improved key yield components, including ear length, seed number, biomass, and 1000-seed weight, while maintaining stable lipid content. The findings highlight the dual function of these native strains as both endophytic nitrogen-fixers and plant growth-promoting rhizobacteria. This research introduces novel strains adapted to local agro-ecological conditions, offering an eco-friendly and sustainable alternative to chemical nitrogen fertilizers. The promising performance of these isolates supports their application in biofertilizer development, particularly for improving maize productivity in tropical agroecosystems.

Acknowledgements

The authors would like to thank An Giang University, Vietnam, for providing laboratory equipment and research facilities that enabled us to complete this study.

Declaration of Generative AI in Scientific Writing

The authors declare that generative AI tools were used solely to enhance the readability and language clarity of the manuscript. These tools were applied under full human oversight and control. The authors take complete responsibility for all scientific content, analyses, and conclusions presented in the article. No AI tool was credited as an author or co-author in this work.

CRediT author statement

Nguyen Van Thuan, Tran Le Kim Tri, Tran Thanh Liem, Trinh Van Tuan Em, and Nguyen Ngoc Phuong Trang contributed to the study design, conducted *in vivo* and *in vitro* experiments, and were involved in data collection, data analysis, result interpretation, and preparation of figures and tables. Nguyen Van Chuong provided critical revisions to the manuscript, contributed to the interpretation of results within the physiological context, supervised the overall research process, coordinated team activities, and finalized the manuscript.

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