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Enhanced soil fertility and baby maize yield through *Bacillus megaterium* CM2 under reduced nitrogen input

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Abstract

This present study evaluated the effectiveness of *Bacillus megaterium* CM2 (strain CM2) as a biofertilizer for the enhancement of soil fertility and baby maize (*Zea mays L.*) productivity under reduced nitrogen (N) input in the Mekong Delta, Vietnam. A field experiment was conducted using a randomized complete block design (RCBD) featuring five treatments combining strain CM2 inoculation and varying nitrogen fertilizer rates. Each treatment was replicated four times. The treatments included a non-inoculated control receiving the full recommended N rate (350 kg urea ha^{-1}) and four strain CM2-inoculated treatments supplied with 100%, 75%, 50%, and 25% of the recommended N dose (350, 262.5, 175, and 87.5 kg urea ha^{-1}, respectively). Laboratory characterization demonstrated that strain CM2 possessed high nitrogenase activity, strong thermotolerance, and broad adaptability, confirming its suitability for field application. In comparison with the non-inoculated control, strain CM2 inoculation under reduced N regimes significantly improved soil chemical properties, including soil pH, cation exchange capacity, soil organic matter, total nitrogen, available phosphorus, and exchangeable potassium. This present study revealed overall soil fertility indicators exhibited an increase by approximately 10–95%, with the lowest responses as observed in under treatments subjected to 25% and 57% reductions in nitrogen fertilizer application. Whilst the most pronounced enhancement were recorded at the 50% N reduction level, particularly for pH (6.99), CEC (14.8 $cmol^{+}$ kg^{-1}), and SOM (2.99%). Thirty days after sowing, CM2-inoculated plants exhibited substantial increases in leaf number (32%), chlorophyll content (17%), and plant height (19%) relative to uninoculated control. Furthermore, yield performance was also substantially enhanced, with total edible cob yield reaching 2.98 t ha^{-1} and the proportion of grade-1 cobs increasing to 65.8%, corresponding to yield gains of 12.9% and 27.6%, respectively. The enhancement observed were attributed to enhanced biological nitrogen fixation, phytohormone-mediated growth promotion, and improved nutrient acquisition efficiency. The integration of strain CM2 with 25–50% reduced N fertilization-maintained yield and improved soil fertility, highlighting its potential as an eco-friendly microbial inoculant for sustainable baby maize cultivation.

Keywords: Bacillus megaterium; baby corn; biofertilizer; nitrogen fixation; sustainable agriculture

1. Introduction

Baby maize (Zea mays L.), when harvested at the immature stage, has gained increasing prominence as a short-duration, high-value crop in Asia and globally. The harvesting period of this crop occurs within 65–75 days post-sowing provides tender ears that are commonly consumed as vegetables or salad components across diverse cuisines [1]. Baby maize is characterized by a relatively brief growth cycle and high market demand. These factors enable the cultivation of baby corn to be performed multiple times per year, either as a sole crop or as part of intercropping system. This approach has been shown to enhance land-use efficiency and increase profitability [2]. Nitrogen (N) is a pivotal determinant of maize growth, affecting chlorophyll synthesis, leaf expansion, ear development, and kernel filling [3,4]. The provision of adequate levels of nitrogen has been demonstrated to enhance kernel number and weight, delay senescence, and increase yield [5]. However, N demand varies with soil type, climate, and cropping system [6,2]. Synthetic fertilizers have been shown to ensure high yields; nevertheless, their intensive use has led to soil degradation, declining nutrient-use efficiency, and serious environmental issues such as greenhouse gas emissions and water eutrophication [7]. Therefore, reducing reliance on chemical fertilizers while maintaining yield has become a central priority for sustainable agriculture.

Endophytic bacteria (EB) are critical partners in nutrient

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cycling and crop health. By colonizing the rhizosphere, they metabolize root exudates and release compounds that enhance plant performance [8]. The functions of these bacteria include biological nitrogen fixation, phosphorus solubilization, phytohormone production, and pathogen suppression [9]. Numerous studies have demonstrated that ER improves nutrient uptake, yield components, and soil enzymatic activity, thus promoting agroecosystem sustainability [10]. For instance, vermicompost integrated with PGPR enhanced peanut yield and nodulation when compared to chemical fertilizers alone [11], underscoring the potential of microbial inoculants as alternatives to mineral inputs. Within EB, the Bacillus species have been identified as a particularly promising subset due to their spore-forming capacity, stress tolerance, and multiple growth-promotion mechanisms [12,13]. Several Bacillus strains have been shown to enhance the performance of maize crops by facilitating nutrients solubilization, producing indole-3-acetic acid (IAA), and optimizing nitrogen-use efficiency [14]. Recently, B. megaterium has emerged as a potential EB [15]. It has been demonstrated that B megaterium has the capacity to promote the growth of wheat and alleviate salt stress, indicating both nutrient cycling and stress mitigation potential. When isolated from baby corn roots, B. megaterium exhibits nitrogen fixation, phosphorus solubilization, and IAA production, which collectively improve crop performance [16,17]. However, the integration of this technology with reduced N fertilization in baby corn systems remains unexplored, particularly under the agroecological conditions of the Mekong Delta. In An Giang province, Vietnam, intensive cultivation practices characterized with excessive fertilizer inputs has caused soil acidification, nutrient imbalances, and declining crop responsiveness. The excessive use of nitrogen and phosphorus has reduced fertility level and increased the risks of nutrient leaching [18]. The utilization of microbial inoculants such as B. megaterium could provide an eco-friendly pathway to optimize fertilizer inputs, restore soil health, and maintain productivity in these systems. Furthermore, the integration of EB with reduced N inputs contributes to climate change mitigation. As demonstrated in field trials, the utilization of EB with reduced N fertilizer has been shown to be effective in maintaining maize yields while also reducing nitrous oxide emissions and enhancing nitrogen-use efficiency [19]. These practices further enhance soil biological activity, stabilize organic matter, and improve overall soil quality [20]. For short-duration crops such as baby corn, the implementation of such strategies reduce production costs, maintain export-quality yields, and align sustainable intensification goals [21].

Endophytic bacteria have emerged as critical biological resources in sustainable agriculture in view of their unique ability to colonize internal plant tissues and establish intimate associations with the host, thereby providing functions that extend well beyond those of free-living rhizobacteria [22]. Their capacity to fix atmospheric nitrogen directly within plant tissues not only reduces reliance on chemical fertilizers but also ensures a more efficient and stable nutrient supply in diverse environmental conditions [23]. In addition, endophytic strains can synthesize phytohormones, enhance plant vigor, and confer protection against a wide range of biotic stresses, thereby integrating growth promotion with resilience to pests and diseases (Pandey and Saharan, 2025) [24]. The multifunctionality of these organisms emphasizes their indispensable role as both nutrient providers and biological control agents. For instance, in baby corn systems, seed inoculation with endophytic nitrogen-fixing bacteria has been shown to decrease nitrogen fertilizer requirements by 50%, while simultaneously improving soil nitrogen pools, yield parameters, and cob quality [25]. These outcomes demonstrate the dual agronomic and ecological significance of endophytes, as they not only support productivity but also reduce input-related environmental burdens. Nevertheless, the sustainability of baby corn cultivation remains constrained by persistent biotic challenges, with more than 110 maize-associated diseases documented globally [26]. Against this backdrop, it is deemed pivotal to regard endophytic bacteria as central components of integrated crop management strategies. The combination of microbial inoculants and optimized nutrient regimes has been identified as a pivotal strategy to ensure the safeguarding of yields, the enhancement of soil fertility, and security of prolonged production sustainability [27].

This study investigated the integration of strain CM2 with reduced nitrogen (N) fertilization in baby corn production in Cho Moi commune, An Giang province, Vietnam. The objectives of this study include: (i) to assess the effects of strain CM2 inoculation combined with reduced nitrogen inputs on baby corn growth, yield attributes, and productivity; and (ii) to evaluate changes in soil physicochemical properties., this research provides empirical evidence to support safe, sustainable, and productive baby corn cultivation in the Mekong Delta by addressing the synergistic inoculated effects of strain CM2 and reduced fertilizer inputs.

2. Materials and Methods

2.1. Source and characterization of B. megaterium CM2

B. megaterium CM2 was isolated from the roots of baby corn 55 days after sowing on yeast mannitol agar (YMA) medium, following the procedure described by Chuong and Tri [28]. A bacterial suspension containing approximately 10^8 CFU mL^{-1} was sent to Phu Sa Company, Vietnam, for molecular identification and phylogenetic analysis. The genomic DNA was extracted from pure colonies using the Thermo Scientific™ GeneJET Genomic DNA Purification Kit, and the 16S rDNA region was amplified via PCR with primers 20F and 1500R [29,30]. The 16S rRNA gene sequences obtained were analyzed using MEGA X software (version 11.0.13) prior to be compared with closely related sequences deposited in the GenBank database using the BLAST algorithm. This analysis revealed a sequence similarity of ≥99.9%. In addition, biochemical characterization demonstrated that strain CM2 exhibited a strong nitrogen-fixing capacity when cultured in liquid yeast mannitol agar (YMA) medium.

2.2. Thermotolerance, salt, and pH response

Thermal tolerance was examined in YMA broth and agar slants incubated at 25, 37, and 45°C, with four replicates per temperature treatment. Colony growth was monitored over a period of seven days [28]. The evaluation of salt tolerance was conducted by supplementing liquid YMA with NaCl concentrations of 0.5%, 2%, 3%, 4%, and 5%, followed by incubation at 28°C. In this experiment, growth performance was recorded after one week. For pH tolerance, the YMA broth was adjusted to pH 4.5 (0.1 N HCl), 7.0, and 8.5 (0.1 N NaOH). Subsequently, the pH was verified using a calibrated pH meter. Cultures were incubated at 28°C, and the assessment of bacterial growth was conducted after a period of seven days [28].

2.3. Ammonia production, nitrogenase activity and nitrogen content determination

Ammonia production was qualitatively determined using peptone water cultures, which were incubated at the temperature of 30°C for 60–80 hours. The addition of Nessler's reagent to these cultures resulted in a color shift from brown to yellow, indicating positive ammonia production [32]. Meanwhile, nitrogenase activity was quantified via the acetylene reduction assay (ARA) as previously described by Soper et al. (2021) [33]. Briefly, strain Y74 was grown in YMA broth for 24 hours prior to be transferred to nitrogen-free medium (NFM). In this process, non-inoculated strain CM2 served as a control. The cell density was then adjusted to OD_{600} = 0.8 and incubated at a temperature of 30°C with shaking at 160 rpm [33,28]. For total nitrogen quantification, strain CM2 was cultured in NFM containing 0.05% malate as the carbon source at 30°C. After centrifugation at 3000 rpm for 1 minute, the supernatant was collected and analyzed in accordance with the procedure of Wang et al. [34].

2.4. Experimental design

A field experiment was conducted using a randomized complete block design (RCBD) comprising five treatments with the combination of different nitrogen (N) fertilizer rates and *Bacillus megaterium* CM2 inoculation. Each treatment was subjected to replication four times to ensure the reliability of the results. Baby corn was planted at a spacing of 25 cm × 25 cm (row × plant), corresponding to an area of 0.0625 m^2 per plant; thus, each 10 m^2 plot contained approximately 160 plants. The treatments were defined as follows: BM1, non-inoculated control receiving the full recommended N rate (350 kg urea ha^{-1}); BM2, CM2 inoculation + 350 kg urea ha^{-1}; BM3, CM2 inoculation + 262.5 kg urea ha^{-1}; BM4, CM2 inoculation + 175 kg urea ha^{-1}; and BM5, CM2 inoculation + 87.5 kg urea ha^{-1}.

The application of nitrogen, phosphorus, and potassium fertilizers were conducted separately in which the rate of the application of each element did not exceed the locally recommended rates (350 kg urea, 400 kg P_2O_5, and 80 kg KCl ha^{-1}). The total experimental area covered 200 m^2, consisting of individual plots measuring 1 × 10 m, separated by 2.5 m buffer zones. The seeds were sown at 20 cm intervals within planting holes, and one healthy seedling was retained per hill at the three-leaf stage. Prior to experiment initiation, soil analysis was carried out and indicated a pH of 5.97, cation exchange capacity of 12.2 $cmol^+ kg^{-1}$, soil organic matter content of 2.14%, total nitrogen of 0.141%, available phosphorus of 120 mg kg^{-1}, and exchangeable potassium of 135 mg kg^{-1}. The soil texture was classified as clay loam (60.0% silt, 9.7% sand, and 30.3% clay), which is considered suitable for the cultivation of baby corn.

2.5 Culture Preparation and Seed inoculation

The strain CM2 grew in YMA medium at 28 °C for 24 hours under agitation (100 rpm) to obtain active cell suspensions, in accordance with Gram-positive culture protocols [35]. Spores were collected by means of centrifugation at 10,000 × g for 5 min at 4 °C, washed three times with sterile water, and resuspended up to 10^8 CFU mL^{-1}. The seeds of baby corn were uniformly coated with the suspension and incubated in darkness for eight hours before sowing. This aimed to ensure that approximately 10^8 CFU were present per seed. Control seeds were then subjected to sterile distilled water.

2.6. Field management and supplementary inoculation

In this study, the Thai cultivar Pacific 421, renowned for its high yield and pest tolerance, was employed. The spacing of the plants within rows was 20 centimeters and the distance between replications was 250 centimeters. To maintain microbial density, supplementary inoculations of strain CM2 (10^8 CFU mL^{-1}) were applied at 20 and 40 days after sowing (DAS). The process of fertilizer application followed four stages: (i) basal: full P_2O_5, (ii) 10 DAS: ⅓ urea, (iii) 20 DAS: ⅓ urea + ½ KCl, (iv) 35 DAS: ⅓ urea + ½ KCl. Weeds were manually controlled, and pest management targeted major insect and fungal infestations. Irrigation was supplied twice in a day during dry periods and adjusted to maintain optimal level of soil moisture, in particular during flowering phase.

2.7. Growth and yield evaluation

Tassel removal was performed at 50 DAS (days after sowing) to enhance ear development and yield. Growth parameters, including plant height, leaf number, biomass, and reproductive traits (silk, husk, tassel, cob length, and diameter) were recorded at 45 DAS (days after sowing). At maturity, ten representative plants per treatment were sampled to determine the marketable ear number, ear yield, length, diameter, and weight, following Kumar et al. [36].

2.8. Soil physicochemical analysis

A series of composite soil samples were collected before and after the experiment. The pH of the soil (1:2.5 w/v) was measured using a digital pH meter. Mineral N was determined via the Kjeldahl method, and available P was extracted using Bray II (0.1 N HCl + 0.03 N NH_4F; 1:7 w/v) and quantified spectrophotometrically at 880 nm. The determination of soil organic matter was conducted through the utilization of dichromate oxidation and titration with $FeSO_4$ 0.5 N [37,38].

2.9. Statistical analysis

Prior to analysis, all data were tested for normality and homogeneity of variances. Subsequently, the data were analyzed using Statgraphics Centurion XVI. Treatment effects were evaluated by analysis of variance (ANOVA), and mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at p ≤ 0

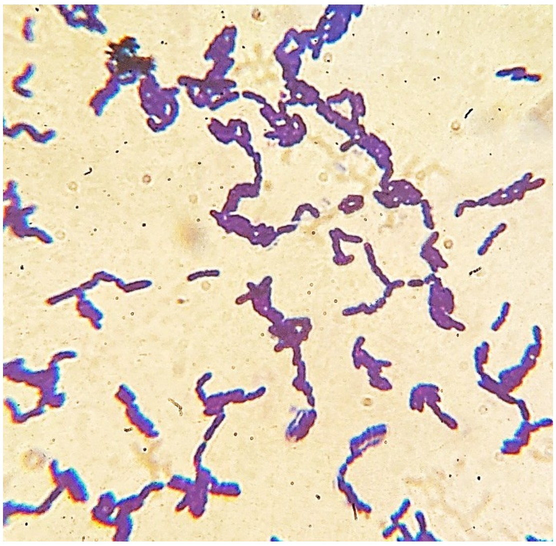
3. Results and Discussion

3.1 Morphological and biochemical characterization of B. megaterium CM2

As illustrated in Fig. 1, strain CM2 exhibited distinct morphological characteristics typical of the genus Bacillus. The colonies observed on nutrient agar (Fig. 1(a)) included opaque, creamy-white, and circular with slightly irregular margins, features consistent with Bacillus species reported by recent studies [39,40]. Microscopic examination (Fig. 1(b)) revealed Gram-positive, rod-shaped cells with centrally or subterminally located endospores, confirming its spore-forming capability, defining trait of Bacillus spp. [41,28].

These morphological traits not only support the classification of B. megaterium CM2 within the Bacillus genus but also suggest its potential resilience under adverse environmental conditions, as endospore formation enables long-term survival [42]. The colony morphology of the strain was creamy white and opaque, characteristics which aligns with reports describing *Bacillus megaterium* isolates from rhizosphere environments [43]. Collectively, these observations provide a preliminary identification consistent with Bacillus morphology, which was later confirmed through molecular characterization.

(a)



(b)

Fig. 1. Morphological characteristics of strain CM2:(a) Pure cultures grown on nutrient agar showing creamy-white, opaque, circular colonies with slightly irregular margins. (b) Gram-stained cells observed under oil immersion at 100× magnification, showing Gram-positive rod-shaped bacteria occurring singly or in short chains; oval endospores are clearly visible within the bacterial cells (indicated in the central or subterminal region of the rods)

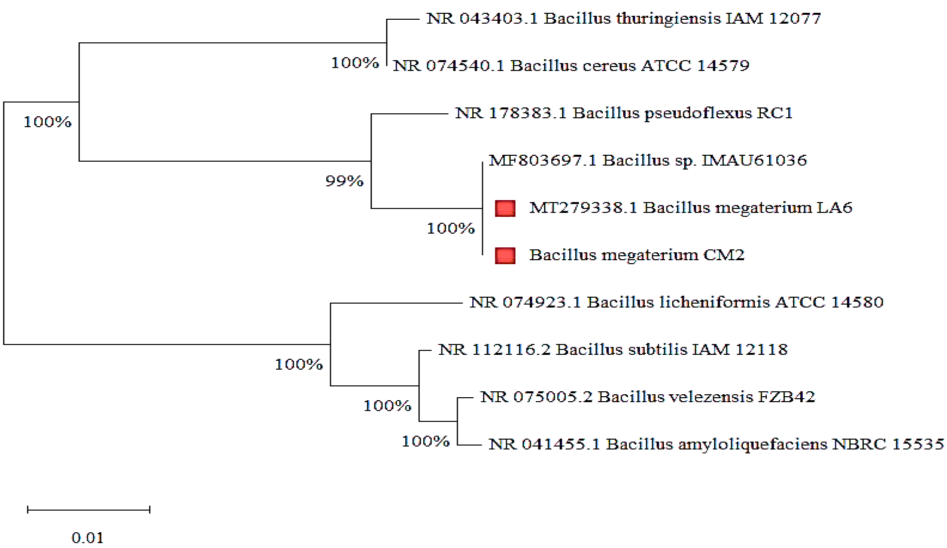


Fig. 2. Neighbor-joining phylogenetic tree constructed from B. megaterium CM2 16S ribosomal RNA gene, partial sequence (1445 bp), isolated from baby corn roots, in comparison with representative reference strains. Bootstrap support values are displayed at branch points, and the scale bar represents 0.01 nucleotide substitutions per site

B. megaterium CM2 demonstrated strong tolerance to salinity (NaCl 0.5–5%), broad pH (4.5–8.5), and temperature (25–45°C) ranges, in addition to diverse enzymatic and metabolic activities (see Table 1). These features are characteristic of plant growth-promoting rhizobacteria (PGPR) and endophytic bacteria that enhance plant performance under abiotic stress conditions. Recent studies have highlighted that bacterial strains with similar traits can mitigate salt stress through ion regulation and Osmo protectant synthesis [44]. The catalase and oxidase activities detected in strain CM2 indicate an antioxidant defense mechanism that reduces reactive oxygen species, a key factor in improving plant resilience to oxidative stress [45]. Furthermore, the presence of β-glucosidase and starch hydrolysis activities suggest the capacity to decompose complex carbohydrates, thereby facilitating nutrient mobilization in the rhizosphere and supporting plant metabolic balance [11]. Such enzymatic profiles have been identified as functional markers for beneficial soil microbes with potential use as bioinoculants in sustainable agriculture [46]. Overall, the biochemical profile of strain CM2 supports its potential role as a biofertilizer or bioprotective agent, warranting further in planta evaluation and genomic characterization to confirm its stress tolerance and plant growth-promoting genes [47].

The findings from B. megaterium CM2 demonstrated a steady increase in nitrogenase activity over a 72-hour period, accompanied by an increase in total nitrogen content, thus indicating efficient atmospheric nitrogen fixation. This pattern aligns with the characteristics of endophytic or rhizospheric diazotrophic bacteria, which have the capacity to enhance soil fertility and plant growth without forming typical root nodules, thus rendering them valuable candidates for biofertilizers [48]. Recent studies have reported that Bacillus spp. and other diazotrophs show significant nitrogenase activity, which correlates with improved growth and nitrogen content in cereals and legumes [49]. Additionally, the capacity of such strains to tolerate environmental stresses while maintaining nitrogen-fixing activity enhances their adaptability and practical applicability in diverse agricultural systems [50]. The observed results suggest that strain CM2 could serve as a sustainable microbial inoculant to partially replace chemical nitrogen fertilizers, promoting eco-friendly farming practices while maintaining or increasing crop productivity. These findings are consistent with current trends emphasizing the use of beneficial microbes to improve nutrient cycling and reduce environmental impact in modern agriculture.

Table 1. Biochemical Characteristics of B. megaterium strain CM2

Biochemical Test / Condition	Reaction of strain CM2	Biochemical Test / Condition	Reaction of strain CM2
NaCl (0.5, 2, 3, 4, 5) %	++	Voges–Proskauer test	+
Temperature (25, 37, 45) °C	++	Nitrate reduction	+
pH (4.5, 7.0, 8.5)	++	Ornithine decarboxylase	–
Citrate utilization	+	D–Mannose	++
β–Glucosidase activity	++	D–Glucose	++
Catalase	++	D–Galactose	–
Oxidase	–	Beta–Xyloidine	++
Glucose oxidation	++	Mannitol	+
Starch hydrolysis	++	Raffinose	+
Methyl Red test	–	Esculin hydrolysis	++

Note: The table presents the physicochemical tolerance and biochemical properties of B. megaterium CM2 under different culture conditions. Symbols indicate the reaction intensity observed in standard biochemical assays: (–) no reaction or negative result; (+) weak or moderate positive reaction; (++) strong positive reaction. Tests include tolerance to salinity (NaCl 0.5–5%), temperature (25–45 °C), and pH (4.5–8.5), as well as utilization of various carbon sources (e.g., D-mannose, D-glucose, raffinose, mannitol), and enzyme activities such as catalase, oxidase, nitrate reduction, β-glucosidase, and starch hydrolysis.

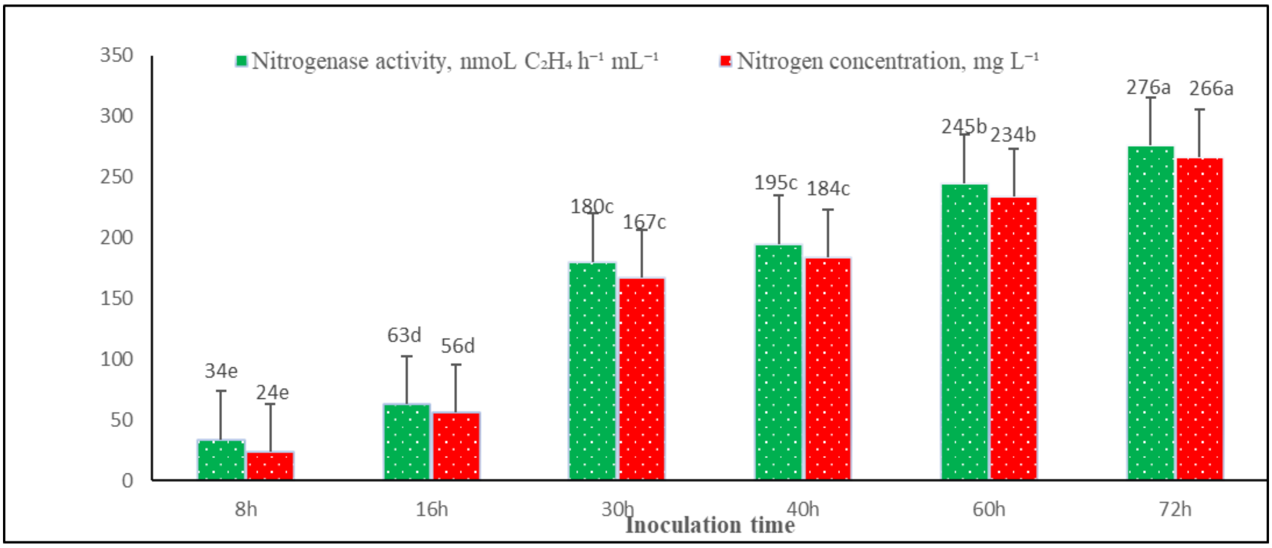


Fig. 3. Nitrogenase activity of B. megaterium CM2 determined by the acetylene reduction assay (ARA) and corresponding total nitrogen concentration measured at different incubation times (8, 16, 30, 40, 60, and 72 h). Nitrogenase activity is expressed as nmol C_2H_4 mg^{-1} protein h^{-1}, while total nitrogen concentration is expressed as mg L^{-1}. Bars represent mean values ± standard deviation (SD) (n = 4). Different lowercase letters indicate statistically significant differences among incubation times within the same parameter at $p \leq 0.01$. Treatments showing the same letter are not significantly different

3.2. Soil chemical characteristics at the time of harvest

The findings, as depicted in Table 2, demonstrated that the application of B. megaterium CM2 in combination with different nitrogen levels had a positive impact on key chemical soil indicators. The treatment BM4 consistently exhibited the highest value across the majority of measured parameters. The pH level substantially increased, reaching the highest value of 6.99 in treatment BM4. This finding suggests that the inclusion of B. megaterium CM2 contributes to the maintenance of our buffer soil pH. CEC values were observed to be significantly higher in the treated soils, with the maximum value of 14.8 cmol$^+$ kg^{-1} as observed in treatment BM4. The enhancement of CEC is indicative of an improved capacity for nutrient retention. The content of both SOM and TN were improved, reaching the peaks at 2.99% and 0.31%, respectively in treatment BM4. These increases are likely attributable to the microbial activity of the B. megaterium CM2, which contributes to organic matter turnover and nitrogen cycling. The availability of essential nutrients, AP and EK, also exhibited a significant increase. The highest AP (218.08 mg kg^{-1}) and EK (167.71 mg kg^{-1}) were both recorded in treatment BM4, suggesting that the strain promotes the solubilization of AP and enhances the availability of EK. Overall, the integration of the B. megaterium CM2 with N fertilizer application not only sustained but also enhanced several critical soil fertility indicators. The superior results seen observed in treatment BM4 emphasize the potential of utilizing bio-inoculants as a sustainable strategy to enhance soil quality and potentially optimize fertilizer use in agriculture.

These findings suggest that B. megaterium CM2 may contribute to buffering soil acidity via microbial metabolic processes, including ammonium assimilation and the breakdown of organic acids [51]. The observed increase in CEC is likely to reflect enhanced nutrient retention, driven by microbial enrichment of SOM and the production of metabolites that stabilize soil colloids [40]. Such improvements can be attributed to microbial-mediated organic matter turnover and biological nitrogen fixation, which augment soil nitrogen availability [22,23]. Bacillus species are recognized for their versatile metabolic capacities, supporting both nitrogen fixation and carbon cycling including the enhancement of soil structure [52]. Furthermore, the bacterium appears to increase AP and EK through organic acid secretion and enzymatic activity, consistent with reports of Bacillus spp. functioning as plant growth-promoting rhizobacteria [3,40]. Overall, the present study demonstrated that the co-application of strain CM2 with reduced nitrogen fertilization resulted in the maintenance and enhancement of key soil fertility indicators. This reflects that strain CM2 has the potential to reduce chemical inputs while sustaining soil health and crop productivity. These results support the emerging role of microbial inoculants as eco-friendly alternatives to excessive fertilizer use in contemporary agricultural systems [53].

Table 2. Influence of B. megaterium strain CM2 and varying nitrogen levels on soil chemical characteristics

Treatments	pH	CEC (cmol$^+$ kg^{-1})	SOM (%)	TN (%)	AP (mg kg^{-1})	EK (mg kg^{-1})
BM1	6.06±0.05e	10.4±0.33d	2.42±0.02c	0.24±0.03c	111±0.82d	133±0.03c
BM2	6.32±0.02d	11.9±0.74c	2.63±0.93c	0.27±0.02bc	205±4.08b	150±0.13b
BM3	6.78±0.07b	13.2±0.16b	2.72±0.60b	0.28±0.02ab	186±0.41c	133±0.03c
BM4	6.99±0.07a	14.8±0.65a	2.99±2.46a	0.31±0,01a	218±0.07a	168±0.01a
BM5	6.57±0.06c	12.8±0.65bc	2.64±1.24c	0.28±0.03ab	205±0.07b	130±0.13c
P_{value}	0.000	0.000	0.000	0.012	0.000	0.000
CV (%)	15.52	23.4	8.32	14.7	11.4	12.0

P > 0.05: no significant differences; P ≤ 0.05 and P ≤ 0.01: significant differences at the 5% and 1% levels, respectively; CV: coefficient of variation; data presented as mean ± SD (n = 4); values within the same column sharing the same letter are not significantly different

3.3. Leaf number, chlorophyll and plant height of baby corn at 15 and 30 DAS

As demonstrated in Table 3, the co-application of strain CM2 with reduced nitrogen rates exhibited a substantial enhancement in the parameters of baby corn growth at 30 DAS in comparison with uninoculated control. In contrast, no significant differences were observed at 15 days after sowing (DAS), yet substantial variations emerged by 30 DAS. The leaf number increased from 6.34 (BM1) to 8.38 (BM4), representing a 32.2% rise, indicating accelerated vegetative development under strain CM2 inoculation. Similarly, chlorophyll content exhibited an increase from 39.2 (BM1) to 45.7 (BM4), corresponding to a 16.6% enhancement, suggesting enhanced nitrogen assimilation and photosynthetic capacity. The similar pattern was observed in plant height, with strain CM2-treated plants (BM4 = 44.6 cm) being 19.3% taller than the control (37.4 cm). Intermediate treatments (BM2–BM3) also demonstrated notable gains, whereas BM5 exhibited moderate improvements. These responses suggest that strain CM2 effectively enhanced nutrient uptake and photosynthetic efficiency, likely through biological nitrogen fixation, phytohormone synthesis, and enhanced nutrient availability [55,36]. Comparable results have been reported in maize and legumes inoculated with Bacillus spp., showing increased chlorophyll and biomass under reduced nitrogen inputs [55]. Hence, strain CM2 inoculation resulted in sustained and enhanced growth performance despite reduced fertilizer levels, thereby confirming its potential as a sustainable biofertilizer for reducing nitrogen dependency.

Table 3. Effects of B. megaterium CM2 and reduced nitrogen levels on leaf number, chlorophyll, and plant height of baby corn at 15 and 30 DAS

Treatment	Leaf number (leaves)		Chlorophyll		Plant height (cm)	
	Days after sowing (DAS)					
	15	30	15	30	15	30
BM1	3.94 ±0.03c	6.34±0.03e	39.7±0.16	39.2±0.16e	18.9±0.08d	37.4±0.33e
BM2	3.99±0.07bc	7.06 ±0.05d	39.8±0.16	44.2±0.16c	19.2±0.16c	43.8±0.163c
BM3	4.05±0.04ab	7.94 ±0.03c	40.0±0.82	45.2±0.16b	19.6±0.16b	44.2±0.163b
BM4	4.08±0.07a	8.38±0.02a	40.1±0.08	45.7±0.16a	20.2±0.16a	44.6±0.163a
BM5	3.95±0.04c	8.26±0.05b	39.8±0.16	41.7±0.16d	19.1±0.08cd	39.9±0.08d
$\mathbf{P_{value}}$	0.007	0.000	0.593	0.000	0.000	0.000
CV (%)	14.4	11.9	12.3	6.74	5.15	7.54

P > 0.05: no significant differences; P ≤ 0.05 and P ≤ 0.01: significant differences at the 5% and 1% levels, respectively; CV: coefficient of variation; data presented as mean ± SD (n = 4); values within the same column sharing the same letter are not significantly different

3.4. Productive traits and cob yield

As illustrated by the findings presented in Table 4, the combined application of B. megaterium CM2 and reduced nitrogen fertilization exerted a different impact on the reproductive traits of baby corn. Although silking time showed a slight variation among treatments, the earliest silking was observed in BM2 (48.9 days), whereas the latest occurred in BM5 (50.1 days). This suggests that excessive N reduction without optimal microbial support may delay reproductive development. The length of cobs was found to be at its maximum in BM2 (11.6 cm), representing a 12.6% increase over the shortest cob recorded in BM5 (9.83 cm); while other treatments demonstrated intermediate values. The treatment effects were found to be more pronounced for cob diameter and cob weight. The diameter of the largest cob diameter was achieved under BM2 (1.87 cm), exceeding the control (BM1 = 1.81 cm) by approximately 3.3%. The diameter of smallest diameter occurred in BM5 (1.70 cm). Similarly, the weight of cob exhibited a positive response to B. megaterium CM2 inoculation, with BM2 and BM4 producing the heaviest cobs (15.0 and 14.9 g cob^{-1}, respectively). These values obtained were 21–22% higher than those achieved by the lowest-performing treatment, BM1 (12.4 g cob^{-1}) and 15–17% greater than those obtained by BM5, which received the highest N reduction. The superior performance of BM2 and BM4 suggests that B. megaterium CM2 inoculation effectively enhanced nutrient uptake and assimilate partitioning during the reproductive stage, particularly under moderate nitrogen reduction. These effects are likely associated with improved biological nitrogen fixation, phytohormone biosynthesis, and enhanced metabolic activity mediated by Bacillus spp. [56,36]. Similar improvements in cob development and yield components under reduced nitrogen input have been reported for maize systems inoculated with beneficial Bacillus strains, highlighting improved nitrogen-use efficiency and enzymatic activation [55,23]. Overall, the results confirm that the integration of B. megaterium CM2 with moderate N reduction (BM2–BM4) has the capacity to maintain or enhance yield-related traits while reducing reliance on chemical nitrogen fertilizers.

Table 4. Influence of B. megaterium CM2 and reduced N application on silking time, length, diameter, and weight of baby corn cobs

Treaments	Silking (Day)	Cob length (cm)	Cob diameter (cm)	Cob weight (g cob^{-1})
BM1	49.3±0.24c	10.3±0.25b	1.81±0.01ab	12.4±0.33c
BM2	48.9±0.74c	11.6±0.49a	1.87±0.02a	15.0±0.82a
BM3	49.9±0.08ab	10.4±0.32b	1.77±0.06bc	13.1±0.08b
BM4	49.4±0.32bc	10.6±0.16b	1.83±0.03ab	14.9±0.08a
BM5	50.1±0.08a	9.83±0.02c	1.70±0.08c	12.8±0.16bc
P_{value}	0.003	0.000	0.001	0.000
CV (%)	12.8	5.75	13.6	19.9

P > 0.05: no significant differences; P ≤ 0.05 and P ≤ 0.01: significant differences at the 5% and 1% levels, respectively; CV: coefficient of variation; data presented as mean ± SD (n = 4); values within the same column sharing the same letter are not significantly different

Results from Table 4 demonstrate that the combined application of B. megaterium CM2 and reduced nitrogen fertilization differentially affected reproductive traits of baby corn. While silking time varied slightly among treatments, the earliest silking was observed in BM2 (48.9 days), whereas the latest occurred in BM5 (50.1 days), indicating that excessive N reduction without optimal microbial support may delay reproductive development. Cob length was maximized in BM2 (11.6 cm), representing a 12.6% increase over the shortest cob recorded in BM5 (9.83 cm), whereas other treatments showed intermediate values. More pronounced treatment effects were evident for cob diameter and cob weight. The largest cob diameter was achieved under BM2 (1.87 cm), exceeding the control (BM1 = 1.81 cm) by approximately 3.3%, while the smallest diameter occurred in BM5 (1.70 cm). Similarly, cob weight responded strongly to B. megaterium CM2 inoculation, with BM2 and BM4 producing the heaviest cobs (15.0 and 14.9 g cob^{-1}, respectively). These values were 21–22% higher than the lowest-performing treatment BM1 (12.4 g cob^{-1}) and 15–17% greater than BM5, which received the highest N reduction. The superior performance of BM2 and BM4 suggests that B. megaterium CM2 inoculation effectively enhanced nutrient uptake and assimilate partitioning during the reproductive stage, particularly under moderate nitrogen reduction. These effects are likely associated with improved biological nitrogen fixation, phytohormone biosynthesis, and enhanced metabolic activity mediated by Bacillus spp. [56,36]. Similar improvements in cob development and yield components under reduced nitrogen input have been reported for maize systems inoculated with beneficial Bacillus strains, highlighting improved nitrogen-use efficiency and enzymatic activation [55,23]. Overall, the results confirm that integrating B. megaterium CM2 with moderate N reduction (BM2–BM4) can maintain or enhance yield-related traits while reducing reliance on chemical nitrogen fertilizers.

Table 5. Impact of B. megaterium CM2 and reduced nitrogen input on grade-1 and grade-2 cob numbers and yield yield ratios in baby corn

Treatment	Total number of cobs	Cob number of grade 1	Cob number of grade 2	Cob yield ratios
		(cobs plant^{-1})		(%)
BM1	2.46±0.05ab	1.54±0.03b	0.924±0.00bc	25.8±0.16c
BM2	2.69±0.07a	1.89±0.07a	0.871±0.00c	28.9±0.08a
BM3	2.64±0.03ab	1.88±0.07a	0.90±0.08c	28.6±0.16b
BM4	2.17±0.06bc	1.14±0.03c	1.04±0.03a	24.4±0.32d
BM5	2.15±0.04c	0.97±0.06d	1.00±0.08ab	23.8±0.16e
P_{value}	0.000	0.000	0.002	0.000
CV (%)	12.6	22.2	14.3	18.4

$P > 0.05$: no significant differences; $P \leq 0.05$ and $P \leq 0.01$: significant differences at the 5% and 1% levels, respectively; CV: coefficient of variation; data presented as mean ± SD (n = 4); values within the same column sharing the same letter are not significantly different

The combined application of B. megaterium CM2 with reduced nitrogen fertilizer rates, as clearly demonstrated in Table 5, exerted a pronounced influence on pivotal yield attributes associated with baby corn, including the total number of cobs per plant, the number of grade 1 and grade 2 cobs, and the ratio of marketable produce. Among all treatments, BM2 and BM3 consistently outperformed the others, indicating their superior effectiveness in enhancing the productivity of baby corn. Specifically, BM2 and BM3 produced the highest total cob numbers, reaching 2.69 and 2.64 cobs plant^{-1}, respectively, with no statistically significant difference between them. These values obtained in this study were found to be significantly higher than those recorded in the uninoculated control (BM1) and in treatments involving more severe N reduction (BM4 and BM5). A similar trend was observed for grade 1 cobs, which represent marketable-quality yield. BM2 and BM3 achieved the greatest numbers of grade 1 cobs (1.89 and 1.88 cobs plant^{-1}, respectively), thus highlighting the strong positive interaction between CM2 inoculation and moderate N reduction. In contrast, BM5 exhibited the poorest performance, producing only 0.97 grade 1 cobs plant^{-1}, which was significantly lower than BM4 (1.14 cobs plant^{-1}) and all other treatments. The cob yield ratio further reinforced the superiority of BM2 and BM3. The treatments attained the highest ratios of marketable cobs (28.9% and 28.6%, respectively), and were statistically comparable to each other, but significantly higher than those observed under BM1, BM4, and especially BM5. The lowest cob yield ratio recorded in BM5 (23.8%) suggests that excessive N reduction, even in the presence of B. megaterium CM2 inoculation, negatively affected the efficiency of marketable cob production. Interestingly, no significant differences were detected among the treatments with respect to the number of grade 2 cobs. This finding suggests that B. megaterium CM2 inoculation, in combination with optimized N management, primarily enhanced the formation of high-quality (grade 1) cobs rather than increasing the proportion of lower-grade produce. Therefore, improvements in total yield under BM2 and BM3 were driven mainly by an increase in marketable cob quality rather than a shift in cob grading distribution.

The comparative analysis across treatments identified BM2 and BM3 as the most effective strategies for maximizing baby corn productivity and marketable yield. The findings clearly demonstrate that integrating B. megaterium CM2 inoculation with moderate N fertilizer reduction has the potential to optimize the yield performance, whereas excessive N reduction (BM4 and BM5) compromises cob number and marketable yield fraction. These findings are consistent with recent reports indicating that biofertilizer-based N management enhances corn yield and quality while sustaining soil fertility and reducing chemical N inputs [57,49].

Table 6. Influence of B. megaterium CM2 and reduced N rates on total yield, cob quality, silk yield, and plant biomass of baby corn

Treatment	Total yield of cobs (t ha^{-1})	Percentage of grade-1 cobs (%)	Silk yield (t ha^{-1})	Plant biomass (t ha^{-1})
BM1	2.64±0.033b	51.6±0.163c	1.29±0.008	33.6±0.327c
BM2	2.98±0.034a	61.4±0.327ab	1.30±0.082	37.4±0.327a
BM3	2.98±0.163a	65.8±0.163a	1.31±0.008	37.5±0.408a
BM4	2.35±0.040c	65.7±0.245a	1.36±0.032	37.8±0.163a
BM5	2.33±0.025c	56.5±0.408b	1.28±0.016	36.8±0.163b
P_{value}	0.000	0.000	0.05	0.000
CV (%)	13.3	16.5	13.3	14.5

$P > 0.05$: no significant differences; $P \leq 0.05$ and $P \leq 0.01$: significant differences at the 5% and 1% levels, respectively; CV: coefficient of variation; data presented as mean ± SD (n = 4); values within the same column sharing the same letter are not significantly different

Table 6 demonstrates the impact of B. megaterium CM2 inoculation at varying N fertilizer reduction levels on baby corn yield and quality attributes. The data revealed that B. megaterium CM2 inoculation significantly enhanced total cob yield, the proportion of grade-1 cobs, and plant biomass ($p \leq 0.01$), whereas silk yield remained statistically unchanged across treatments. The highest total cob yields were observed in BM2 and BM3 (2.98 t ha^{-1}), corresponding to a 12.9% increase over the uninoculated control (BM1 = 2.64 t ha^{-1}).

Similarly, the proportion of grade-1 cobs exhibited a substantial increase from 51.6% in BM1 to 65.8% in BM3, representing a 27.6% enhancement in marketable yield. The present study also demonstrated that plant biomass was elevated, ranging from 33.6 t ha^{-1} in BM1 to 37.8 t ha^{-1} in BM4, indicating the enhanced nutrient assimilation and photosynthetic capacity.

These findings of this study indicate that CM2 inoculation effectively mitigated the reduction in N fertilizer by enhancing N fixation, promoting phytohormone production, and facilitating nutrient uptake [58]. Recent studies further support that Bacillus-based biofertilizers can improve cob filling, grain quality, and biomass accumulation under low-N conditions [59]. Overall, BM2 and BM3 demonstrated the most favorable outcomes, highlighting the potential of B. megaterium CM2 as a sustainable microbial inoculant capable of increasing baby corn yield and quality, while reducing reliance on chemical nitrogen inputs.

4. Conclusion

The integration of B. megaterium CM2 with reduced nitrogen fertilizer substantially enhanced soil fertility and baby corn productivity in the Mekong Delta. Furthermore, B. megaterium CM2 inoculation enhanced soil pH buffering, nutrient retention, and organic matter accumulation, thus demonstrating strong contributions to N and P cycling. Agronomically, inoculated plants exhibited higher chlorophyll content, greater vegetative growth, and superior yield attributes, particularly at nitrogen rates between 50 and 75% of the recommended level. Treatments BM2 and BM3 achieved the best overall performance, increasing total edible cob yield by 12–13% and marketable cobs by nearly 28% in comparison to the control. These findings of this study demonstrate that B. megaterium CM2 effectively compensates for reduced N fertilizer inputs through biological nitrogen fixation and plant growth promotion, thereby sustaining productivity while minimizing chemical fertilizer dependence. The present study provides empirical evidence supporting B. megaterium CM2 as a sustainable biofertilizer suitable for tropical agricultural systems. The wider adoption of such microbial technologies has the potential to enhance nutrient-use efficiency, reduce production costs, and contribute to environmentally responsible crop management strategies aligned with the United Nations Sustainable Development Goals (SDGs 2, 12, and 13).

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