doi:10.14720/aas.2024.120.4.19524 Original research article / izvirni znanstveni članek

Utilization of nitrogen-fixing endophytic bacteria to improve tuber yield and nitrogenous nutrient uptake of Cassava plant (*Manihot esculenta* Crantz)

Quang Trung DO [1, 2], Manh Ha NGUYEN [3]

Received August 13, 2024; accepted October 26, 2024
Delo je prispelo 13 avgust 2024, sprejeto 26. oktober 2024

Utilization of nitrogen-fixing endophytic bacteria to improve tuber yield and nitrogenous nutrient uptake of Cassava plant (*Manihot esculenta* Crantz)

Abstract: Cassava (*Manihot esculenta* Cratz) yield remains low due to various environmental stressors, yet sustainable strategies for enhancing productivity, especially through microbial interventions, are underexplored in current literature. This study investigated the potential of endophytic bacteria isolated from native cassava (*M. esculenta* KM98-7) to enhance plant growth and yield under greenhouse and field conditions. Eleven bacterial strains (TL1 to TL11) were isolated and assessed for nitrogen fixation, phosphate solubilization, and indole acetic acid (IAA) synthesis. TL4 and TL8 exhibited superior capabilities, with TL8 producing the highest levels of $NH4^+$ (14.95 mg l^{-1}) and IAA (46.57 mg l^{-1}). Molecular identification revealed that TL4 and TL8 were closely related to *Burkholderia cenocepacia* Vandamme et al. 2003 and *Prestia aryabhattai* (Shivaji et al. 2009) Gupta et al. 2020. Greenhouse trials showed that inoculation with TL8 significantly increased plant height, leaf number, and tuber yield, comparable to 90 kg urea ha^{-1} application. Field experiments confirmed these findings, with the 60 kg urea ha^{-1} + TL8 treatment achieving similar yields to 90 kg urea ha^{-1} without bacterial inoculation. This study demonstrates that integrating nitrogen-fixing bacterial inoculants, particularly strain TL8, with reduced nitrogen fertilization can maintain high cassava productivity while promoting sustainable agricultural practices.

Key words: biofertilizers, crop productivity, endophytic bacteria, plant-growth promotion, sustainable agriculture

Uporaba endofitskih bakterij, ki vežejo dušik za izboljšanje pridelka in privzema hranil manioke (*Manihot esculenta* Crantz)

Izvleček: Pridelek manioke (*Manihot esculenta* Cratz) ostaja majhen zaradi različnih okoljskih stresnih dejavnikov a kljub obstoječim trajnostnim strategijam za povečanje pridelka, še posebej z mikrobi, ostajajo te neuporabljene glede na obstoječe vire. V raziskavi je bil preučevan potencial endofitskih bakterij izoliranih iz lokalne manioke (*M. esculenta* KM98-7) na vzpodbujanje rasti in povečanje pridelka v rastlinjaku in poljskih razmerah. Enajst sevov bakterij (TL1 do TL11) je bilo izoliranih in ocenjenih na vezavo dušika, raztaplanje fosfata, in sintezo indol acetne kisline (IAA). Seva TL4 in TL8 sta pokazala najboljšo sposobnost, pri čemer je sev TL8 proizvedel največ $NH4^+$ (14,95 mg l^{-1}) in IAA (46,57 mg l^{-1}). Molekularno preverjanje je odkrilo, da sta bila seva TL4 in TL8 zelo sorodna vrstama *Burkholderia cenocepacia* Vandamme et al. 2003 in *Prestia aryabhattai* (Shivaji et al. 2009) Gupta et al. 2020. Poskus v rastlinajku je pokazal, da je inokulacija s sevom TL8 značilno povečala višino rastlin, število listov in pridelek gomoljev, ki je bil primerljiv uporabi 90 kg urea ha^{-1}. Poljski poskus je potrdil ta odkritja, kjer je obravnavanje s 60 kg urea ha^{-1} + TL8 doseglo podoben pridelek kot uporaba 90 kg urea ha^{-1} brez bakterijske inokulacije. Raziskava kaže, da vključevanje inokulov bakterij, ki vežejo dušik, še posebej seva TL8, z zmanjšanjem gnojenja z dušikovimi gnojili, lahko ohranja velike pridelke manioke in hkrati pospešuje trajnostno kmetijsko pridelavo.

Ključne besede: biognojila, pridelek gojenih rastlin, endofitke bakterije, pospeševanje rasti rastlin, trajnostno kmetijstvo

1 Faculty of Pharmacy, Dai Nam University, Xom, Phu Lam, Ha Dong, Ha Noi, Vietnam
2 Corresponding author: trungcnsinh@gmail.com
3 Forest Protection Research Center, Vietnamese Academy of Forest Sciences, Duc Thang, Bac Tu Liem, Hanoi, Vietnam

1 NTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a crucial staple food crop in tropical regions, particularly in sub-Saharan Africa, Latin America, and Southeast Asia, providing a primary source of carbohydrates for millions of people. However, achieving high yields, such as 30 tons of cassava tubers per hectare, requires substantial nutrient removal from the soil - approximately 180-200 kg N ha^{-1}, 15-22 kg P_2O_5 ha^{-1}, and 140-160 kg K_2O ha^{-1} (Susan et al., 2010). Consequently, cassava productivity is often constrained by poor soil conditions, such as alum soil, characterized by high acidity and low nutrient availability (Omondi et al., 2018). Traditional agricultural practices aimed at improving soil fertility frequently fall short, necessitating alternative, sustainable solutions to enhance cassava growth and yield.

One promising approach is the use of plant endophytic bacteria, which inhabit plant tissues without causing harm and can confer various benefits to their host plants. These bacteria promote plant growth through mechanisms such as nitrogen fixation, phosphate solubilization, production of growth hormones, and induction of systemic resistance to pathogens (Ferreira et al., 2021; Do et al., 2023; Ferreira et al., 2024). The potential of these bacteria to improve plant health and productivity under stress conditions has been increasingly recognized (Do et al., 2023).

Recent studies have highlighted the positive impacts of endophytic bacterial inoculation on various crops under different stress conditions. For instance, inoculation with *Bacillus* sp. has been shown to enhance the growth and yield of rice in the presence of the bacterium *Xanthomonas oryzae* pv. *Oryzae* (Xoo) that caused bacterial leaf blight disease (Do et al., 2023). Similarly, *Pseudomonas fluorescens* (Flügge 1886) Migula, 1895 strains have been reported to improve the growth of wheat under drought stress by enhancing root development and water uptake (Vurukonda et al., 2016). These findings suggest that endophytic bacteria could be a valuable tool in mitigating abiotic stresses and improving crop productivity on challenging soils.

In the context of cassava, research on the role of endophytic bacteria is still emerging. Recent studies (Ferreira et al., 2021; Feng et al., 2023; Ferreira et al., 2024) demonstrated that endophytic bacteria isolated from cassava plants could promote plant growth and enhance resistance against phytopathogens such as *Phytopythium* sp., a causal agent of soft root rot in cassava, and *Xanthomonas phaseoli* pv. *Manihotis* (Xpm) which causes cassava bacterial blight. Furthermore, the study by Zhang et al., (2022) indicated that cassava-associated endophytes could improve nutrient uptake, leading to better growth and higher yields.

Given these promising findings, the present study aims to investigate the effect of plant endophytic bacterial inoculation on cassava growth and yield. By understanding how these bacteria interact with cassava plants and influence their growth under adverse soil conditions, we can develop sustainable agricultural practices that leverage natural microbial relationships to enhance crop productivity and resilience.

2 MATERIALS AND METHODS

2.1 ISOLATION OF ENDOPHYTIC BACTERIA FROM THE CASSAVA PLANT

A total of 50 cassava (*Manihot esculenta* KM98-7) plant samples, including roots, stems, and leaves, were collected from Tuy Lai commune in My Duc district, Ha Noi, Vietnam. These samples were cleaned and cut into 1 cm pieces. Surface sterilization involved immersion in 70 % ethanol with gentle shaking for 3 minutes, followed by rinsing with sterile distilled water. The samples were then treated with 0.1 % $HgCl_2$ for 1 minute with shaking, rinsed again, treated with 3 % hydrogen peroxide for 3 minutes, and thoroughly rinsed with sterile distilled water four times. To ensure no residual microorganisms, 100 µl of the final rinse water was plated onto Luria-Bertani (LB) medium. Successful sterilization was confirmed by the absence of colonies after 24-48 hours of incubation.

The sterilized samples were ground using a sterile mortar and pestle with 1 ml of sterile distilled water. A 100 µl aliquot of the homogenized sample was inoculated into test tubes containing 3 ml of semi-solid LB medium and incubated at 30 °C for 24-48 hours. The presence of endophytic microorganisms was indicated by a thin film on the medium surface, which was transferred to a solid LB medium and incubated at 30 °C for 24-48 hours. Pure cultures were obtained through subculturing and preserved on slant LB agar tubes at 4 °C.

2.2 CHARACTERIZATION OF ENDOPHYTIC BACTERIA FROM THE CASSAVA PLANT

2.2.1 Nitrogen fixation activity assay

All bacterial strains were screened for nitrogen fixa-

tion abilities by culturing in 50 ml nitrogen-free Burk's liquid medium at room temperature for 3 days with shaking at 160 rpm, followed by centrifugation at 10,000 rpm for 10 minutes. The NH_4^+ content in the supernatant was quantified using Nessler's reagent colorimetric method at 420 nm with NH_4Cl as the standard (Franche et al., 2009). Specifically, 0.1 ml of sodium potassium tartrate solution and 0.1 ml of Nessler's reagent were added to 5 ml of the supernatant. After mixing and allowing it to sit for 20 minutes, the absorbance was recorded at 420 nm. A calibration curve for concentration versus absorbance was created using a series of standard NH_4Cl solutions with varying concentrations. The resulting curve demonstrated a strong linear correlation between absorbance and NH_3 concentration.

2.2.2 IAA production

Indole-3-acetic acid (IAA) synthesis by bacteria was determined using the Salkowski method (Do et al., 2023). Bacteria were cultured in 25 ml of LB medium supplemented with 0.1 g l^{-1} tryptophan, incubated at 30 °C for 48 hours with shaking at 120 rpm. The IAA content in the supernatant was quantified using the Salkowski reagent with colorimetric measurement at 530 nm and IAA as the standard.

2.2.3 Phosphate solubilization

Phosphorus solubilization capability was assessed by culturing bacteria in 100 ml National Botanical Research Institute's phosphate growth (NBRIP) liquid medium at room temperature for 7 days with agitation at 120 rpm (Yanlei and Xiaoping, 2018). The supernatant was collected by centrifugation at 10,000 rpm for 15 minutes at 4 °C. Solubilized phosphorus was quantified using the molybdenum blue method with ammonium molybdate at 880 nm and KH_2PO_4 as the standard.

2.2.4 Molecular identification of bacterial isolate

The molecular method was used to identify the species of strain TL8. Bacteria were grown overnight in LB broth at 30 °C with shaking (120 rpm). Genomic DNA was extracted using the NucleoSpin® Tissue extraction kit (Macherey-Nagel, Germany). The 16S rRNA gene was amplified via PCR with the primer pair 27F and 1492R. PCR conditions were: initial denaturation at 94 °C for 2 minutes, followed by 35 cycles of 94 °C for 30 seconds, 55 °C for 20 seconds, 72 °C for 1 minute, final extension at 72 °C for 5 minutes, and hold at 4 °C. PCR products were cleaned and sequenced by 1st BASE Company (Malaysia). The 16S rRNA gene sequence of strain TL8 was compared to sequences in the GenBank database.

2.3 EVALUATION OF ENDOPHYTIC BACTERIA ON CASSAVA PLANT GROWTH UNDER GREENHOUSE CONDITIONS

The greenhouse experiment was conducted in the greenhouse of the Vietnam National University of Forestry (VNUF) during the winter-spring cropping season of 2022-2023 (November 2022 - May 2023) using cassava cuttings (15-20 cm).

Soil from Tuy Lai commune, My Duc district, Ha Noi, Vietnam, was used. The soil analysis criteria include pH and EC, extracted with distilled water at a ratio of 1:2.5 (soil: water). pH is measured with a pH meter, and EC is measured with an EC meter. Available phosphorus (using the Bray II method) is determined by extracting soil with 0.1N HCl + 0.03N NH_4F at a soil-to-water ratio of 1:7, then measured on a spectrophotometer at a wavelength of 880 nm. Exchangeable potassium is extracted using 0.1M $BaCl_2$ and measured with an atomic absorption spectrometer. Soil texture composition is determined using the Robinson pipette method. The properties of soil were pH_{KCl} 4.69, EC = 1.91 mS cm^{-1}, total N 0.11%, organic matter 3.02 %, soluble P_2O_5 = 20.12 mg P kg^{-1}, soluble K_2O = 0.27 meq $100g^{-1}$.

The bacterial strains with strong abilities to fix nitrogen, solubilize phosphate, and produce IAA were chosen. The selected bacteria strains were cultured in LB media at 30 °C for 24 hrs. The culture was centrifuged at 8000 rpm for 10 minutes at 4 °C, and the cells were resuspended in sterilized water to OD600 = 1.0. Cassava cuttings (15-20 cm) were disinfected and immersed in bacterial suspension for 1 hour before planting in pots (0.3 m × 0.4 m) filled with prepared soil (Zhang et al., 2022).

The experiment followed a two-factor completely

Table 1: Summary of treatment combination under greenhouse conditions

Urea fertilizer	Without bacterial inoculation	With bacterial inoculation of strain TL4	With bacterial inoculation of strain TL8
No urea fertilizer	T1	T5	T9
30 kg urea ha^{-1}	T2	T6	T10
60 kg urea ha^{-1}	T3	T7	T11
90 kg urea ha^{-1}	T4	T8	T12

Table 2: Period and dosage of fertilizer for experiments

Fertilization period	Describe
On the day of planting	Apply whole phosphate fertilizer
1st application (25 DAP)	Apply 1/3 urea fertilizer + 1/3 potassium fertilizer
2nd application (50 DAP)	Apply 1/3 urea fertilizer + 1/3 potassium fertilizer
3rd application (80 DAP)	Apply all the remaining urea and potassium fertilizer

DAP: day after planting

randomized block design: factor A (urea fertilizer levels: 0, 30, 60, and 90 kg ha^{-1}) and factor B (bacterial strains: with and without bacteria) with three replications (Table 1).

Fertilizers used were urea (46 % N), Lam Thao superphosphate (16 % P2O5), and Kali Phu My MOP (60 % Growth parameters (plant height, number of leaves, diameter of base, trunk, and stem) were measured at 90 days after planting (DAP), and tuber yield, number of tubers, tuber length, and tuber diameter were measured at harvest.

The Kjeldahl method was applied to determine the total nitrogen content in cassava plants' leaves, roots, and stems from T1 (without nitrogen fertilizer and bacterial TL8 inoculation) and T9 (with bacterial TL8 inoculation only) experiments. Approximately 0.5 g of dried and finely ground plant tissue from each part was placed in a digestion flask. To each sample, 10 ml of concentrated H_2SO_4 was added along with 0.5 g of a catalyst mixture consisting of K_2SO_4 and selenium (0.005 g). The digestion process was done by heating the mixture until the solution became clear, indicating the complete breakdown of organic matter. After digestion, the solution was cooled and diluted with 50 ml of distilled water. To neutralize the acid and convert ammonium ions (NH_4^+) to ammonia (NH_3), 40 ml of 10 mol l^{-1} NaOH was added to the mixture. The ammonia was then distilled into a receiving flask containing 25 ml of 2 % boric acid solution, with the distillation process continuing until approximately 100 ml of distillate had been collected. The collected distillate was titrated with 0.1 mol l^{-1} HCl until the endpoint was reached, as indicated by a color change of the pH indicator. The volume of HCl used in the titration was recorded, and the total nitrogen content was calculated based on the titration results. This procedure allowed for the accurate quantification of total nitrogen in the different plant tissues according to the Kjeldahl method described by Bremner (1996).

2.4 EVALUATION OF PROMISING ENDOPHYTIC BACTERIA ON CASSAVA PLANT GROWTH UNDER FIELD CONDITIONS

The field experiment was conducted during the summer-autumn crop of 2023 at Tuy Lai commune, My Duc district, Ha Noi, Vietnam. The soil was prepared by plowing to a depth of 15-20 cm and arranging into beds (100 cm width, 50 cm height, 10 m length) with 30 cm spacing between beds. Cuttings were planted in single rows per bed, with 80 cm spacing between cuttings.

The experiment followed a completely randomized block design with one factor, comprising 8 different treatments, each replicated three times (summarized in Table 3). Fertilizer application and growth monitoring were conducted as in the greenhouse experiment (see Table 2).

2.5 DATA ANALYSIS

Data were statistically analyzed using Excel and IRRISTAT software. Tukey's Honestly Significant Difference (HSD) tests were used for pairwise comparisons, maintaining a 95 % confidence level.

Table 3: Summary of treatment combination under field conditions

Treatments	Describe
F1	Without Urea fertilizer and bacterial inoculation
F2	With 30 kg urea ha^{-1}
F3	With 60 kg urea ha^{-1}
F4	With 90 kg urea ha^{-1}
F5	Without urea fertilizer + with bacterial inoculation of TL8
F6	With 30 kg urea ha^{-1}+ with bacterial inoculation of TL8
F7	With 60 kg urea ha^{-1}+ with bacterial inoculation of TL8
F8	With 90 kg urea ha^{-1}+ with bacterial inoculation of TL8

3 RESULTS AND DISCUSSION

3.1 ISOLATION AND CHARACTERIZATION OF ENDOPHYTIC BACTERIA FROM CASSAVA

Eleven strains of endophytic bacteria (TL1 to TL11) were isolated from root, stem, and leaf samples of native cassava (Table 4). Two strains were obtained from stem samples, four from leaf samples, and six from root samples. The isolated bacterial strains predominantly displayed round colonies with small sizes ranging from 0.2 to 0.6 cm, appearing glossy, convex, and translucent white.

Research into endophytic bacteria from cassava plants has demonstrated their significant potential in promoting plant growth and health through various mechanisms, such as nitrogen fixation, phosphate solubilization, and IAA production (Ferreira et al., 2021; Feng et al., 2023; Ferreira et al., 2024).

In terms of nitrogen fixation, only four isolated bacterial strains produced NH_4^+, with strain TL8 showing the highest production at 14.95 mg l^{-1}. This NH_4^+ production was higher than that reported by Zhang et al., (2022), where strain A02 produced 13.38 mg l^{-1}. Therefore, strain TL8 might play a critical role in providing nitrogenous compounds for plant development.

Phosphate solubilization is another critical trait observed in cassava-associated endophytic bacteria. Many isolates could solubilize inorganic phosphate, enhancing phosphorus availability in the soil, which is essential for plant growth. This trait is particularly beneficial for cassava, often cultivated in low-fertility soils with limited phosphorus availability (Omondi et al., 2018; Zhang et al., 2022; Feng et al., 2023; Ferreira et al., 2024). Strains TL4 (15.51 mg l^{-1}) and TL8 (14.27 mg l^{-1}) showed the highest phosphorus solubilization. According to Zhang et al. (2022), strain A02 produced 101.23 mg l^{-1} of phosphorus solubilization in 8 days after incubation. The data suggest that strains TL4 and TL8 present significant promise for the biofertilizer industry.

Additionally, the ability of endophytic bacteria to produce IAA, a plant hormone regulating growth and development, has been well-documented (Zhang et al., 2022; Feng et al., 2023; Ferreira et al., 2024). Many isolates, especially from cassava roots, showed positive results for IAA production (Ferreira et al., 2021; Zhang et al., 2022). IAA stimulates root elongation and improves nutrient uptake, supporting overall plant growth and resilience. Six endophytic bacterial strains in our study synthesized IAA, with the highest synthesis by TL8 (46.57 mg l^{-1}), followed by TL7 (30.51 mg l^{-1}) and TL3 (21.15 mg l^{-1}). The lowest IAA synthesis was by TL1 at 11.23 mg l^{-1}. Ferreira et al. (2021) reported strain A02 produced 1.56 mg l^{-1} of IAA in 2 days. These results indicate that strain TL8 could potentially be applied to produce IAA, promoting plant growth.

Based on their superior abilities in nitrogen fixation, phosphorus solubilization, and IAA synthesis, strains TL4 and TL8 were selected for further study.

Table 4: Characteristics of endophytic bacteria isolated from the cassava plant

Isolates	Source	Amount of NH_4^+ (mg l^{-1})	Amount of soluble PO_4^{3-} (mg l^{-1})	Amount of IAA (mg l^{-1})
TL1	Stem	-	-	11.23 ± 0.08 ^{d}
TL2		-	6.43 ± 0.12 ^{cd}	-
TL3	Leaf	-	-	21.15±0.04 ^{c}
TL4		4.31 ± 0.11 ^{b}	15.51 ± 0.09 ^{a}	15.12 ± 0.05 ^{d}
TL5		-	-	-
TL6	Root	3.24 ± 0.03 ^{c}	8.71 ± 0.12 ^{c}	-
TL7		-	3.09 ± 0.13 ^{e}	30.51 ± 0.09 ^{b}
TL8		14.95 ± 0.21 ^{a}	14.27 ± 0.21 ^{b}	46.57 ± 0.11 ^{a}
TL9		-	-	-
TL10		3.21 ± 0.04 ^{c}	3.12 ± 0.11 ^{e}	14.23 ± 0.07 ^{d}
TL11		-	5.25 ± 0.21 ^{d}	-

Data present means ± SD (n = 3). Values in the same column with the same letter(s) are not significantly different as determined by the least significant difference (HSD) test ($p < 0.05$).

Molecular identification indicated that TL4 and TL8 were closely related to *Burkholderia cenocepacia* and *Priestia aryabhattai*, with percentage identities of 98.85 % and 98.74 %, respectively. The sequences were deposited in GenBank with accession numbers PQ113673 and PQ119839. These endophytic bacterial strains were further studied under greenhouse and field conditions to highlight their potential as biofertilizers in sustainable agriculture practices.

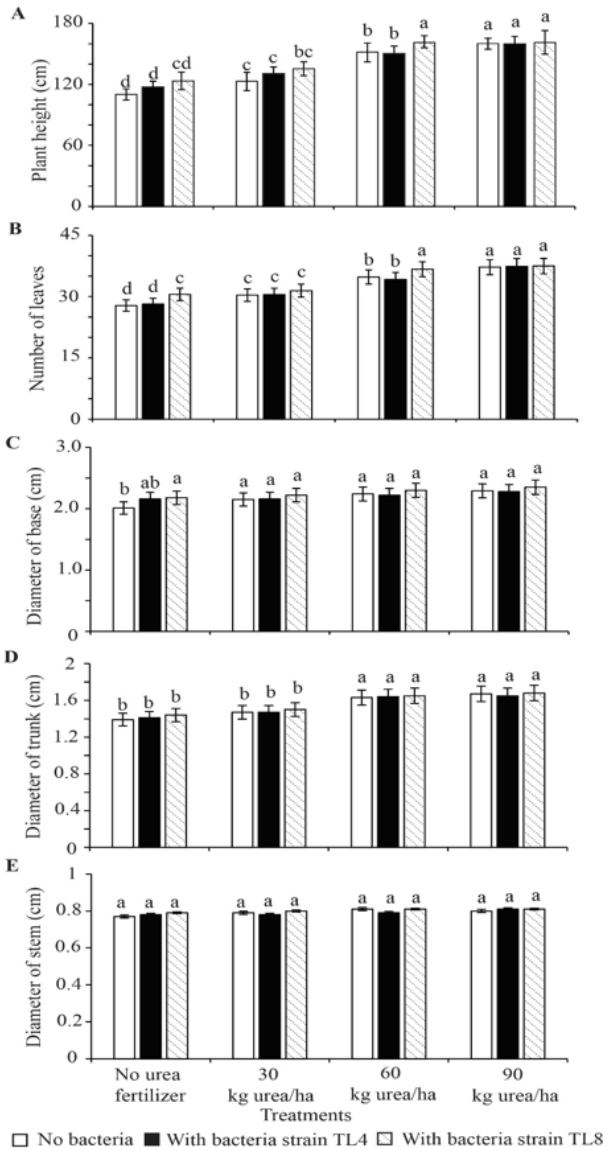

Figure 1: Effects of endophytic bacterial strains TL4 and TL8 combined with the doses of nitrogen fertilizer on (A) cassava plant height; (B) number of leaves; (C-E) Diameter of base, trunk, and stem at 90 DAP under greenhouse conditions. Plotted data present means ± SD (n = 3), the different letter(s) indicate significant differences as determined by the least significant difference (HSD) test ($p < 0.05$).

3.2 EFFECTS OF ENDOPHYTIC BACTERIAL STRAIN TL4 AND TL8 COMBINED WITH NITROGEN FERTILIZER DOSES ON THE GROWTH AND YIELD OF CASSAVA UNDER GREENHOUSE CONDITIONS

The results of this study underscore the significant role that nitrogen fertilization and bacterial inoculation play in the growth and yield of cassava under greenhouse conditions. At 90 days after planting (DAP), statistically significant differences in plant height were observed between the nitrogen fertilization and bacterial inoculation experiments (Figure 1A). Plant height in the nitrogen fertilization experiments ranged from 110.13 to 153.19 cm, with the lowest heights in the non-nitrogen-fertilized plants. Among the bacterial inoculation treatments, plant height ranged from 122.34 to 151.26 cm, with the highest in plants inoculated with strain TL8 (151.26 cm). Additionally, the number of leaves (Figure 1B), base diameter (Figure 1C), trunk diameter (Figure 1D), and stem diameter (Figure 1E) showed statistically significant differences among the nitrogen fertilization treatments. The lowest number of leaves, base diameter, trunk diameter, and stem diameter were observed in the non-nitrogen fertilized plants. This is because nitrogen significantly influences cassava growth and development, promoting robust leaf, stem, and root development compared to nitrogen-deficient plants (Rafikova et al., 2016; Xing et al., 2016).

Moreover, the concentrations of total N in cassava plants' leaves, stems, and roots inoculated with endophytic bacteria TL8 were increased compared to the control. Total N in the stems and roots did not statistically differ between experiments; however, total N in the leaves differed, and the inoculation of TL8 had the highest concentrations (Table 5). These results are in agreement with the results of previous reports (Szilagyi-Zecchin et al., 2014; Li et al., 2017) which demonstrated that cassava stems inoculated with endophytic bacteria led to a higher amount of nitrogen content in the leaves. Nitrogen uptake is crucial for cassava productivity as it enhances photosynthesis, supports protein and enzyme synthesis, and maintains a balanced carbon-to-nitrogen ratio. Adequate nitrogen improves chlorophyll content, promotes root development, and leads to greater tuber biomass and starch accumulation (Feng et al., 2023). Studies confirm that nitrogen-deficient cassava shows reduced growth and yield, while optimal nitrogen fertili-

Table 5: Effect of endophytic bacteria TL8 on the nitrogen content of cassava plant in a greenhouse experiment

Treatment	Nitrogen content (mg g^{-1})		
	Leaves	Stems	Roots
With bacterial TL8 inoculation only	46.87 ± 0.43a	13.58 ± 0.51a	13.08 ± 0.61a
Without nitrogen fertilizer and bacterial TL8 inoculation	35.96 ± 0.74b	13.04 ± 0.46a	12.73 ± 0.58a

Data present means ±SD (n = 3). Values in the same column with the same letter(s) are not significantly different as determined by the least significant difference (HSD) test ($p < 0.05$).

zation improves nutrient use efficiency, boosting productivity sustainably (Feng et al., 2023).

Bacterial inoculation with strain TL8 led to the highest plant height (151.26 cm), suggesting superior nitrogen-fixation capabilities compared to strain TL4. This finding aligns with studies highlighting the potential of specific endophytic bacterial strains to enhance plant growth by improving nitrogen availability (Zhang et al., 2022). The observed statistical differences in the number of leaves, base diameter, and stem diameter further support the importance of adequate nitrogen supply (Feng et al., 2023).

Tuber characteristics, including the number of tubers per pot (Figure 2A), tuber diameter (Figure 2B), and tuber length (Figure 2C), differed statistically among the nitrogen fertilization treatments. The combination of 60 kg urea ha^{-1} with bacterial inoculation showed significant differences compared to the no-nitrogen treatment and the 30 kg urea ha^{-1} treatment with bacterial inoculation, but not from the 90 kg urea ha^{-1} treatment combined with bacterial inoculation. Among the bacterial inocula-

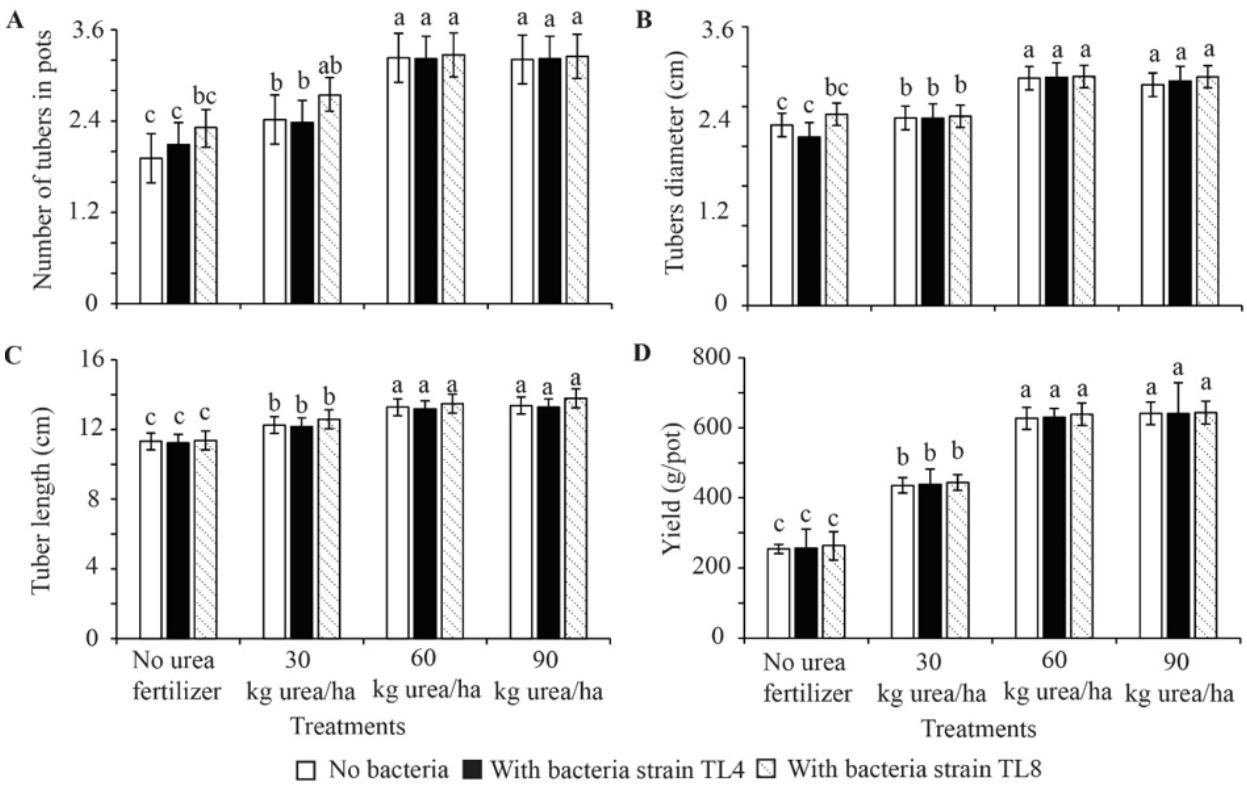

Figure 2: Effects of endophytic bacterial strains TL4 and TL8 combined with the doses of nitrogen fertilizer on (A) the number of tubers per 10 m2; (B) Length of tubers; (C) Diameter of tuber; and (D) Yield at harvest under greenhouse conditions. Plotted data present means ± SD (n = 3), the different letter(s) indicate significant differences as determined by the least significant difference (HSD) test ($p < 0.05$).

tion treatments, significant differences were observed for the number of tubers per pot, tuber diameter, and tuber length, with the highest values for these parameters in the TL8 inoculation.

Tuber yield per pot also differed significantly between the nitrogen fertilization and bacterial inoculation treatments (Figure 2D), with yields ranging from 254.37 to 640.94 g pot^{-1}. The lowest yield was observed without nitrogen fertilization (254.37 g pot^{-1}), and the lowest yield among bacterial inoculation treatments was without bacterial inoculation (441.69 g pot^{-1}). Providing sufficient nitrogen enhances cassava growth and increases tuber yield (Uwah et al., 2013; Zhang et al., 2022). Among the two experimental bacterial strains, TL8 increased the number of tubers, tuber diameter, and tuber length, enhancing cassava yield compared to TL4. This superior performance may be attributed to TL8's higher nitrogen-fixation activity, IAA production, and phosphate solubilization, which enhance nutrient availability and uptake by the plant (Biswas et al., 2022; Argotte-Ibarra et al., 2022; Do et al., 2023).

Overall, this study demonstrates that nitrogen fertilization and bacterial inoculation, particularly with strain TL8, significantly improve cassava growth and yield under greenhouse conditions. Further research exploring the long-term effects of these treatments with strain TL8 under field conditions is necessary to refine cassava cultivation practices.

3.3 EFFICACY OF BACTERIA TL8 ON THE GROWTH AND YIELD OF CASSAVA UNDER FIELD CONDITIONS

At 90 DAP, plant heights across the experiments were statistically different (Figure 3A), ranging from 108 to 153 cm. The application of 60 kg urea ha^{-1} combined with TL8 inoculation (F6) produced plant heights similar to those achieved with 90 kg urea ha^{-1} without bacterial inoculation (F7). The lowest plant height was observed in treatments without nitrogen fertilization and bacterial inoculation (F1). The number of leaves also showed statistically significant differences (Figure 3B), with the lowest number (30.28 leaves) in the non-nitrogen fertilization experiments (F1). These data indicate the potential application of strain TL8 to enhance the nitrogen efficiency of cassava plants under field conditions.

Base and trunk diameters were significantly different (Figures 3C and 3D), with the highest diameters found in the treatments with 60 kg urea ha^{-1} + strain TL8 (F6), 90 kg urea ha^{-1} + no bacterial inoculation (F7), and 90 kg urea ha^{-1} + strain TL8 (F8). This aligns with previous research demonstrating that adequate nitrogen supply, especially when combined with beneficial bacterial inoculants, can significantly enhance structural growth parameters (Biswas et al., 2022; Zhang et al., 2022, Aasfar et al., 2024).

The diameter of the stem across the experiments did not show any statistically significant differences, ranging from 0.79 to 0.88 cm (Figure 3E). This consistency suggests that while nitrogen and bacterial treatments influence overall plant height and biomass, they might not significantly alter certain morphological traits under the given experimental conditions. Similar findings have been reported in studies on other crops, where the impact of nitrogen-fixing bacteria was more pronounced on overall growth and yield metrics rather than specific morphological characteristics (Aasfar et al., 2024). Additionally, applying 60 kg urea ha^{-1} combined with TL8 bacterial inoculation (F6) resulted in cassava growth not statistically different from that achieved with 90 kg urea ha^{-1} without bacterial inoculation (F7). This is particularly relevant for sustainable agriculture, where reducing the dependency on chemical fertilizers can have significant environmental benefits (Do et al., 2023; Aasfar et al., 2024). The ability of TL8 to enhance nitrogen efficiency could lead to more sustainable cassava production practices, aligning with global efforts to minimize agricultural inputs while maintaining high yields.

Further supporting these findings, previous research has shown that the application of nitrogen-fixing bacteria can lead to increased plant height and dry weight in various crops, highlighting their role in improving nitrogen availability and utilization (Xu et al., 2018; Do et al., 2023; Aasfar et al., 2024). These studies suggest that the beneficial effects of bacterial inoculants are not limited to cassava but extend to other rooted crops as well.

The number of tubers differed significantly among the experiments (Figure 4A), ranging from 31.35 to 58.17 tubers. The lowest number of tubers (31.35) was observed in the no-nitrogen fertilization treatment (F1). This finding aligns with the general understanding that nitrogen is a key nutrient for promoting tuber growth and overall plant productivity (Zhang et al., 2022; Aasfar et al., 2024).

Tuber length also showed statistically significant differences, with the highest lengths recorded in the treatments including F6 (60 kg urea ha^{-1} + TL8 inoculation), F7 (90 kg urea ha^{-1} without bacterial inoculation), and F8 (90 kg urea ha^{-1} + TL8 inoculation) (Figure 4B). Similarly, tuber diameter varied significantly at the 5 % level, ranging from 4.55 to 5.48 cm, with the smallest diameter (4.55 cm) in the F1 treatment (Figure 4C). These results highlight the beneficial effects of adequate nitrogen supply, whether through fertilization or bacterial inoculation, on tuber size and quality. Moreover, the results indicated that applying 60 kg urea ha^{-1} combined with

strain TL8 (F6) achieved similar numbers, lengths, and diameters of tubers as the 90 kg urea ha^{-1} without bacterial inoculation (F7). This suggests that the nitrogen-fixing ability of strain TL8 contributed additional nitrogen to the cassava plants, allowing the F6 treatment to match the performance of the F7 treatment. Previous research supports these findings, showing that nitrogen-fixing microbial fertilizers can enhance plant growth and yield (Zhang et al., 2022; Aasfar et al., 2024).

Combining nitrogen fertilization with bacterial inoculation significantly impacts cassava tuber yield, demonstrating the potential for optimizing fertilizer use while maintaining high productivity. Figure 4D indicates that tuber yield did not differ significantly between the treatments of F7 (18.49 t ha^{-1}), F6 (18.42 t ha^{-1}), and F8 (19.15 t ha^{-1}). However, these treatments showed a 1.12% higher yield compared to the no-nitrogen treatments (F1 at 9.83 t ha^{-1} and F2 at 10.86 t ha^{-1}), as well as the F4 (15.93 t ha^{-1}), F3 (13.69 t ha^{-1}), and F5 (15.79 t ha^{-1}) treatments. These results suggest that using 60 kg urea ha^{-1} combined with strain TL8 achieved similar yields to the application of 90 kg urea ha^{-1} without bacterial inoculation, indicat-

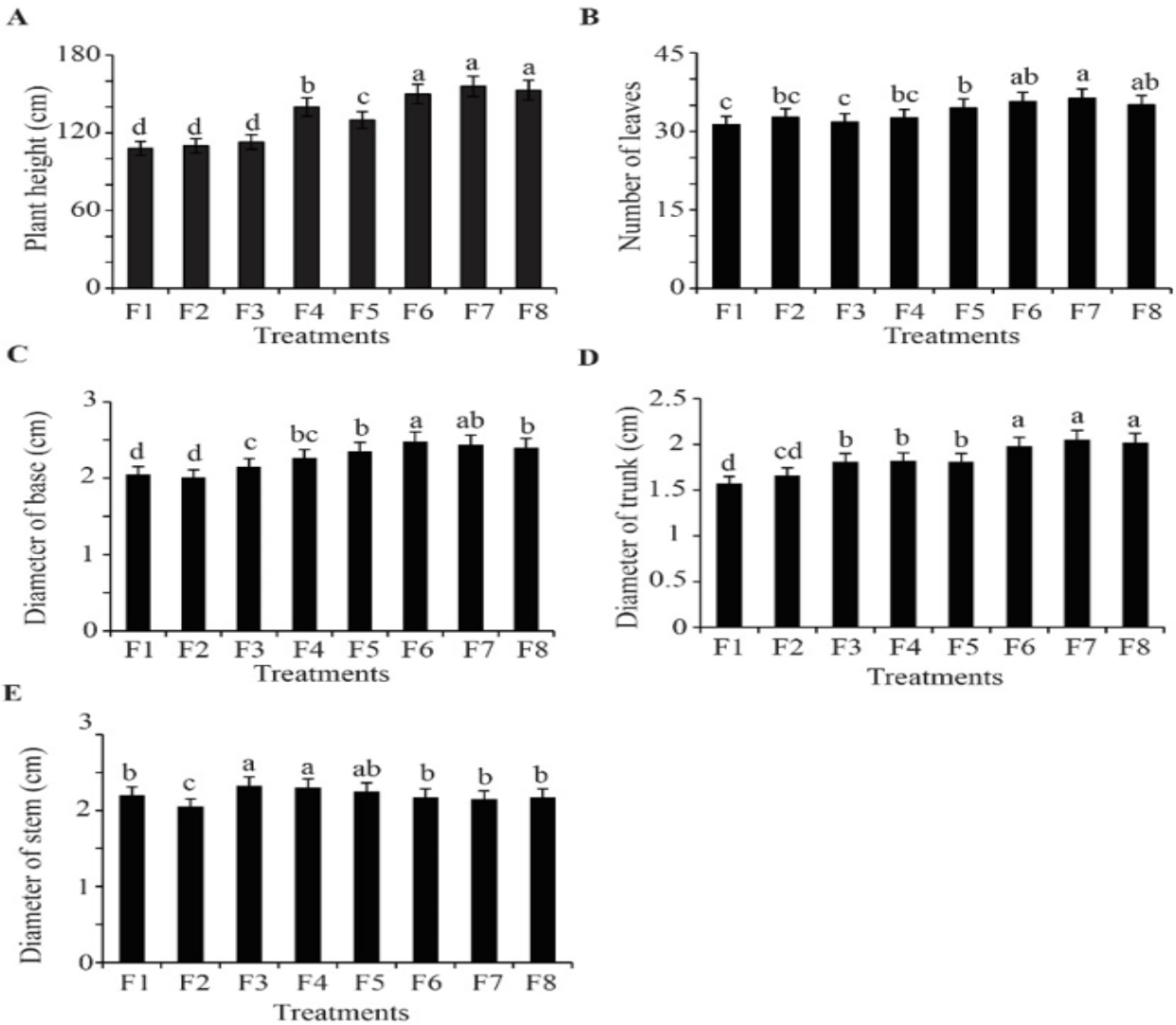

Figure 3: Effects of bacterial strain TL8 combined with the doses of nitrogen fertilizer on (A) cassava plant height; (B) number of leaves; (C-E) Diameter of base, trunk, and stem at 90 DAP under field conditions. F1: without urea fertilizer and bacterial inoculation; F2: without urea fertilizer and with bacterial strain TL8; F3: with 30 kg urea ha^{-1} and without bacterial strain TL8; F4: with 30 kg urea ha^{-1}and with bacterial strain TL8; F5: with 60 kg urea ha^{-1} and without bacterial strain TL8; F6: with 60 kg urea ha^{-1} and with bacterial strain TL8; F7: with 90 kg urea ha^{-1} and without bacterial strain TL8; F8: with 90 kg urea ha^{-1} and with bacterial strain TL8. Plotted data present means ± SD (n = 3), the different letter(s) indicate significant differences as determined by the least significant difference (HSD) test ($p < 0.05$).

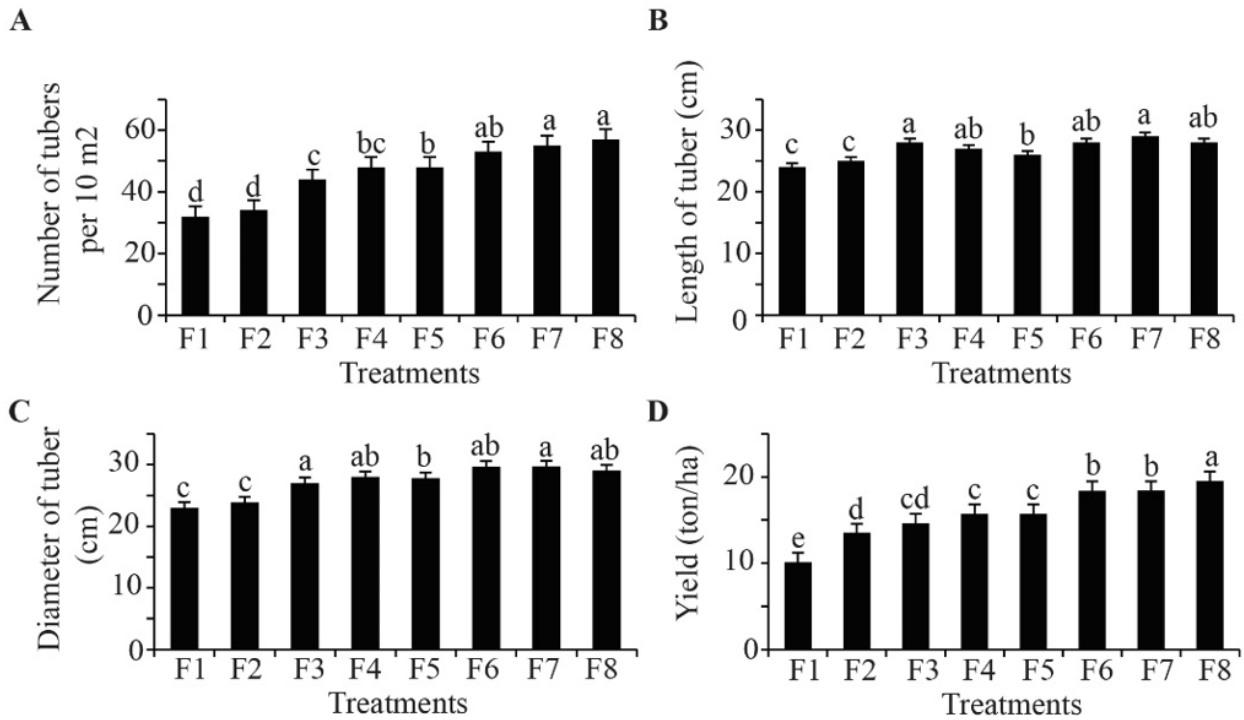

Figure 4: Effects of bacterial strain TL8 combined with nitrogen fertilizer doses on (A) the number of tubers per 10 m2; (B) Length of tubers; (C) Diameter of tuber; and (D) Yield at harvest under field conditions. F1: without urea fertilizer and bacterial inoculation; F2: without urea fertilizer and with bacterial strain TL8; F3: with 30 kg urea ha^{-1} and without bacterial strain TL8; F4: with 30 kg urea $ha^{-1}$and with bacterial strain TL8; F5: with 60 kg urea ha^{-1} and without bacterial strain TL8; F6: with 60 kg urea ha^{-1} and with bacterial strain TL8; F7: with 90 kg urea ha^{-1} and without bacterial strain TL8; F8: with 90 kg urea ha^{-1} and with bacterial strain TL8. Plotted data present means ± SD (n = 3), the different letter(s) indicate significant differences as determined by the least significant difference (HSD) test ($p < 0.05$).

ing that a reduction of 30 kg urea ha^{-1} could be feasible for cassava cultivation. This finding aligns with studies by Zhang et al. (2022) and Aasfar et al. (2024), which suggest that nitrogen-fixing bacteria can reduce the reliance on nitrogen fertilizers, emphasizing their secondary role when effective bacterial inoculation is applied.

The observed results underscore the benefits of integrating nitrogen-fixing bacterial inoculants into fertilization practices. By reducing the reliance on chemical fertilizers, farmers can achieve cost savings and minimize environmental impacts, such as nitrogen leaching and soil degradation. This approach aligns with sustainable agricultural practices and supports the shift toward more eco-friendly farming methods (Zhang et al., 2022).

4 CONCLUSIONS

The integration of nitrogen-fixing endophytic bacteria, specifically strain TL8, with nitrogen fertilization demonstrates significant potential for enhancing cassava growth and yield. The study's findings highlight the superior performance of TL8 in nitrogen fixation, phosphate solubilization, and IAA synthesis, contributing to improved plant height, leaf number, and tuber yield under both greenhouse and field conditions. The ability of TL8 to achieve similar yields with reduced nitrogen fertilization (60 kg urea ha^{-1}) as conventional higher fertilization rates (90 kg urea ha^{-1}) underscores its role in promoting sustainable agricultural practices. This approach not only enhances crop productivity but also reduces the reliance on chemical fertilizers, offering environmental and economic benefits. Further research is warranted to explore the long-term impacts and optimize the application of these bacterial inoculants in diverse agricultural settings.

5 REFERENCES

Argotte-Ibarra, L., Barreiro-Quino, O.F., Carlos, A.R., José, A.H.M., Hans, T.C.S. (2022). Analysis of the solubility of phosphate rock from Aipe (Colombia) via formation of 2Na-EDTA complex. *Chemosphere*, *286*, 131786. doi:10.1016/j.chemosphere.2021.131786

Aasfar, A., Meftah Kadmiri, I., Azaroual, S.E., Lemriss, S., Mernissi, N.E., Bargaz, A., Zeroual, Y., Hilali, A. (2024). Agronomic advantage of bacterial biological nitrogen fixation on wheat plant growth under contrasting nitrogen and phosphorus regimes. *Frontiers in Plant Science*, *15*, 1388775. doi:10.3389/fpls.2024.1388775

Biswas, J.K., Anurupa, B., Mahendra, R., Ravi, N., Bhabananda, B., Meththika, V., Madhab, C.D., Santosh, K.S., Erik, M. (2018). Potential application of selected metal resistant phosphate solubilizing bacteria isolated from the gut of earthworm (*Metaphire posthuma*) in plant growth promotion. *Geoderma*, *330*, 117–124. doi:10.1016/j.geoderma.2018.05.034

Bremner, J.M. (1996). Nitrogen-total. In: Sparks DL, editor. *Methods of soil analysis. Chemical methods.* Madison: oil Science Society of America, p.1085^{-1}121. doi:10.2136/sssabookser5.3.c37

Do, T.Q., Nguyen, T.T., Dinh, V.M. (2023). Application of endophytic bacterium *Bacillus velezensis* BTR11 to control bacterial leaf blight disease and promote rice growth. *Egyptian Journal of Biological Pest Control*, *33*, 97. doi: 10.1186/s41938-023-00740-w

Feng, Y., Zhang, Y., Shah, O.U., Luo, K., Chen, Y. (2023). Isolation and identification of endophytic bacteria *Bacillu* sp. ME9 that exhibits biocontrol activity against *Xanthomonas phaseoli* pv. *manihotis*. *Biology*, *12*(9), 1231. doi:10.3390/biology12091231

Ferreira, S.C., Nakasone, A.K., Nascimento, S.M.C., Oliveira, D.A., Siqueira, A.S., Cunha, E.F.M., de Souza, C.R.B. (2021). Isolation and characterization of cassava root endophytic bacteria with the ability to promote plant growth and control the *in vitro* and *in vivo* growth of *Phytopythium* sp. *Physiological and Molecular Plant Pathology*, *116*, 101709. doi: 10.1016/j.pmpp.2021.101709

Ferreira, S.C., Nakasone, A.K., Cunha, E.F.M., Serrão, C.P., Souza, C.R.B. (2024). *Klebsiella* endophytic bacteria control cassava bacterial blight in the eastern Amazon. *Acta Amazonica*, *54*, e54ag23160. doi:10.1590/1809-4392202301601

Franche, C., Lindström, K., Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant Soil*, *321*, 35–59. doi:10.1007/s11104-008-9833-8

Li, H.B., Singh, R.K., Singh, P., Qi-Qi, S., Yong-Xiu, X., Li-Tao, Y., Yang-Rui, L. (2017). Genetic diversity of nitrogen-fixing and plant growth promoting *Pseudomonas* species isolated from sugarcane rhizosphere. *Frontiers in Microbiology*, *8*. doi:10.3389/fmicb.2017.01268

Omondi, J.O., Lazarovitch, N., Rachmilevitch, S., Boahen, S., Ntawuruhunga, P., Sokolowski, E., Yermiyahu, U. (2018). Nutrient use efficiency and harvest index of cassava decline as fertigation solution concentration increases. *Journal of Plant Nutrition and Soil Science*, *181*(5), 644-654. doi:10.1002/jpln.201700455

Rafikova, G.F., Korshunova, T., Yu, M.L.F., Chetverikov, S.P., Loginov, O.N. (2016). A new bacterial strain, *Pseudomonas koreensis* IB-4, as a promising agent for plant pathogen biological control. *Microbiology*, *85*, 333–341. doi:10.1134/S0026261716030115

Szilagyi-Zecchin, V.J., Ikeda, A.C., Hungria, M., Adamoski, D., Kava-Cordeiro, V.K., Glienke, C., Galli-Terasawa, L.V. (2014). Identification and characterization of endophytic bacteria from corn (*Zea mays* L.) roots with biotechnological potential in agriculture. *AMB Express*, *4*, 1–9. doi:10.1186/s13568-014-0026-y

Susan, K., Suja, G., Sheela, M.N., Ravindran, C.S. (2010). Potassium: The key nutrient for cassava production, tuber quality and soil productivity – An Overview. *Journal of Root Crops*, *36*, 132144.

Uwah, D.F., Effa, E.B., Ekpenyong, L.E., Akpan, I.E. (2013). Cassava (*Manihot esculenta* Crantz) performance as influenced by nitrogen and potassium fertilizers in Uyo, Nigeria. *Journal of Animal and Plant Sciences*, *23*(2), 550-555.

Vurukonda, S.S., Vardharajula, S., Shrivastava, M., SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research*, *184*, 13-24. doi:10.1016/j.micres.2015.12.003.

Xing, Y.X., Wei, C.Y., Mo, Y., Yang, L.T., Huang, S.L., Li, Y.R. (2016). Nitrogen-fixing and plant growth-promoting ability of two endophytic bacterial strains isolated from sugarcane stalks. *Sugar Tech*, *18*, 373–379. doi:10.1007/s12355-015-0397-7

Xu, J., Kloepper, J.W., Huang, P., McInroy, J.A., Hu, C.H. (2018). Isolation and characterization of N-2-fixing bacteria from giant reed and switchgrass for plant growth promotion and nutrient uptake. *Journal of Basic Microbiology*, *58*(5), 459-471. doi:10.1002/jobm.201700535

Yanlei, Z., Xiaoping, S. (2018). Evaluation of the plant-growth-promoting abilities of endophytic bacteria from the psammophyte *Ammodendron bifolium*. *Canadian Journal of Microbiology*, *64*(4), 253-264. doi:10.1139/cjm-2017-0529

Zhang, X., Tong, J., Dong, M., Akhtar, K., He, B. (2022). Isolation, identification and characterization of nitrogen fixing endophytic bacteria and their effects on cassava production. *PeerJ*, *10*, e12677. doi:10.7717/peerj.12677