








Enhancing tomato (*Solanum lycopersicum* (L.) Mill. agroproductivity: A scientific exploration of native *Rhizobium* biofertilizers

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ABSTRACT

Objective: To evaluate the effect of applying native bacterial strains as biofertilizers on the growth and yield of tomato (*Solanum lycopersicum*) cultivated under conventional agricultural practices through a field experiment.

Design/methodology/approach: Soil fertility was assessed by analyzing physicochemical parameters, including pH, electrical conductivity, cation exchange capacity (CEC), total carbon, nitrogen, phosphorus, and the C/N ratio, using standardized laboratory methods. Additionally, the ability of native *Rhizobium* strains to act as Plant Growth-Promoting Bacteria (PGPB) was evaluated through nitrogen fixation, phosphate solubilization, auxin synthesis, siderophore production, and ACC deaminase activity tests. The biofertilization trial involved three treatments with different native *Rhizobium* strains, a chemical fertilizer control (Triple 17), and a non-inoculated control. Growth, chlorophyll content, and yield parameters were statistically analyzed using one-way ANOVA and Tukey's test ($p < 0.05$).

Results: The soil exhibited slight clay characteristics, variable pH, and cation exchange capacity, with low nitrogen (N) and phosphorus (P) content. Native *Rhizobium* strains demonstrated significant potential as PGPB, showing nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) synthesis, and siderophore production. Biofertilization with these strains significantly improved ($p < 0.05$) tomato plant growth, chlorophyll content, and fruit quality compared to controls.

Limitations on study/implications: Unusual climatic variations, limited irrigation access, and subpar phytotechnical management affect tomato crop yields due to significant genetic variability. Evaluating biofertilization in various production cycles is crucial.

Findings/conclusions: Native *Rhizobium* biofertilizers enhance tomato growth and quality, addressing agroproductivity challenges.

Keywords: Agroproductivity, biofertilizers, *Rhizobium*, tomato cultivation.

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INTRODUCTION

The exponential growth of the human population has intensified the demand for food, leading to a widespread use of agrochemicals in agriculture (Gupta *et al.*, 2015). To



address this challenge, sustainable agricultural practices integrate biological sciences for safe and environmentally friendly approaches. Biofertilizers have gained importance for efficiently promoting plant growth, enhancing soil fertility, and reducing environmental contamination, thereby contributing to innovative and sustainable agriculture (Singh *et al.*, 2021).

Biofertilizers, particularly plant growth-promoting bacteria (PGPB), offer a promising approach to improving agricultural resilience and improve food production. PGPB, like *Rhizobium* species, establish symbiotic relationships, stimulate plant growth, improve nutrient absorption, and strengthen disease resistance. Their ability to fix atmospheric nitrogen provides an eco-friendly alternative to synthetic nitrogen fertilizers, mitigating environmental impact (Soumare *et al.*, 2020). Notably, *Rhizobium* species are versatile, colonizing the rhizosphere of non-leguminous plants, making them a practical and safe biofertilizer option for various crops, especially those consumed raw (Flores-Félix *et al.*, 2019; Gen-Jiménez *et al.*, 2023).

In southern Mexico, agriculture plays a fundamental role as a primary source of regional and national food security. This research focuses on the application of PGPB in the cultivation of tomatoes (*Solanum lycopersicum*) in Chiapas, Mexico. Tomatoes are of paramount importance as a versatile and widely consumed vegetable, and their cultivation holds significant economic value. Some recent studies have explored the application of *Rhizobium* bacteria as biofertilizers for tomato crops, demonstrating their potential to improve yield and quality (Gen-Jiménez *et al.*, 2023). Therefore, the objective of this study was to evaluate the impact of applying native bacterial strains as biofertilizers on the growth and yield of tomatoes (*S. lycopersicum*) cultivated using conventional agricultural practices.

MATERIALS AND METHODS

Experimental site

The biofertilization trials were conducted in a commercial tomato cultivation (*Solanum lycopersicum*) located in the “El Diamante” locality in the municipality of Ocozocoautla, Chiapas (16° 59' 23" N and 93° 44' 32" W) at an average altitude of 640 meters above sea level (Figure 1).

Soil characterization

Soil samples were collected from five randomly selected locations within the experimental tomato plot, both before and after biofertilization. Soil texture was determined using granulometric analysis (Bouyoucos, 1962), while pH and electrical conductivity (EC) were measured with a digital pH meter. Cation exchange capacity (CEC) was evaluated according to the specifications outlined in the Official Mexican Standard NOM-021. Total carbon and total nitrogen were quantified using a FLASH 2000[®] auto-analyzer, and total phosphorus was assessed through the HNO₃/HClO₄ solubilization method (Rincón-Molina *et al.*, 2020). Additionally, the C/N ratio was calculated. An additional soil sample from a non-biofertilized control was also collected.

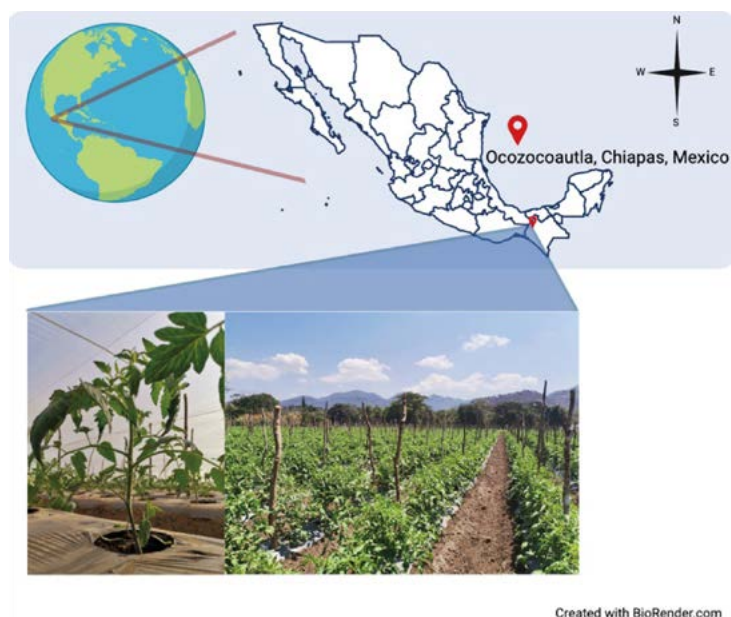


Figure 1. Location of the experimental plot of tomato cultivation.

Bacterial strains

The native nitrogen-fixing strains *Rhizobium calliandrae* LBP2-1, *Rhizobium mayense* NSJP1-1^T, and *Rhizobium jaguaris* SJP1-2 were used in this experiment (Rincón-Rosales et al., 2013).

Evaluation of the ability of bacterial strains as PGPB

Nitrogen fixation

The acetylene reduction assay (ARA) involved culturing bacteria in a nitrogen-free medium. After a 72-h incubation at 28 °C, cultures were exposed to 1% acetylene. Acetylene, generated by dissolving calcium carbide in tap water, was injected at a final concentration of 1% (v/v), replacing an equivalent air volume. ARA was assessed using a Varian 3300 gas chromatograph with a flame ionization detector (Navarro-Noya et al., 2012).

Phosphate solubilization

Isolates, cultured individually in yeast-malt extract broth (YM medium) overnight, were adjusted to OD₆₀₀ nm of 1.0. They were then inoculated into NBRIP medium with insoluble tricalcium phosphate (Ca₃(PO₄)₂) at pH 7.0, phosphate-solubilizing bacteria were identified by clear zones around colonies after 5 days at 30 °C. The phosphate solubilization index (PSI) was calculated following Liu et al. (2015).

Auxin synthesis

Indole-3-acetic acid (IAA) production was measured using the colorimetric Salkowski reagent method (Brick et al., 1991). Bacterial isolates were cultured in YMB medium with L-tryptophan, incubated for 7 days at 28 °C. After centrifugation, supernatant was mixed

with Salkowski reagent, incubated in the dark at 28 °C for 30 min, and absorbance was measured at 530 nm (O'Hara *et al.*, 1989). Auxin concentration was determined using a standard IAA curve.

Siderophores production

Bacterial isolates were cultured on chrome azurol-S (CAS) agar medium. Colonies exhibiting an orange halo after a 3-day incubation period at 28 °C were identified as positive for siderophore production. The diameter of the orange halo was measured, and the result was quantified as Siderophore Induced Droplet Formation (SID), following the methodology established by Alexander and Zuberer (1991).

ACC deaminase

The deaminase ACC activity was determined according to Glick (1995). A bacterial inoculum of 5 μ l of 10^9 cel/mL ($OD_{600\text{nm}}=0.2$) of each of the isolates was inoculated in culture medium containing: 0.25 g K_2HPO_4 ; 0.05 g $MgSO_4 \cdot 7H_2O$; 0.025 g $FeSO_4 \cdot 7H_2O$; 0.25 g $CaCO_3$; 0.05 g NaCl; 0.0012 g $NaMoO_4 \cdot 2H_2O$; 2.5 g glucose; 3.75 g agar; 240 ml distilled water, and 0.03% of ACC as the sole source of nitrogen. The Petri dishes were incubated at 30 °C for 4 d. Colonies were subcultured in fresh medium containing ACC and incubated under the same conditions to confirm growth.

Biofertilization trials in tomato crop (*Solanum lycopersicum*)

The biofertilization trial on “bola” variety tomatoes involved three treatments with native *Rhizobium* strains (T1=*R. calliandrae*, T2=*R. jaguaris*, T3=*R. mayense*), Triple 17 as positive control, and non-inoculated plants as negative control. Inoculation, initially via spray at 1×10^6 CFU/mL during transplant and a second application 25 days later, was performed on 25 plants per treatment in mulched furrows. Chemical treatments followed standard agronomic practices. After 120 days, various parameters were assessed, including plant height, total and root weight, chlorophyll content, and nitrogen and phosphorus levels, along with fruit number and weight.

Statistical analysis of experimental data

The data collected from the biofertilization trials consisted of 75 samples in total, with 25 samples per treatment, including triplicates. Given the sample size and replication structure, it was assumed that the data followed a normal distribution based on the central limit theorem, which states that with a sufficiently large sample size ($n \geq 50$), the distribution of the sample mean approximates normality.

The data were subjected to a one-way analysis of variance (ANOVA) at a significance level of alpha (α)=0.05 using the statistical software Statgraphics Centurion XV.2 for all studied variables. When the ANOVA indicated significant differences among treatments, Tukey's honest significant difference (HSD) test was used for multiple pairwise comparisons ($p < 0.05$) to identify specific differences between treatment means.

RESULTS AND DISCUSSION

Biofertilized soils displayed higher pH, Cation Exchange Capacity (CEC), Total Carbon (C), Total Nitrogen (N), and Total Phosphorus (P) but lower Electrical Conductivity (EC), indicating reduced salinity (Table 1). These physicochemical improvements are crucial, as increased CEC enhances the soil's ability to retain essential nutrients, directly benefiting plant growth (Dey et al., 2023). The increase in pH and reduction in EC observed after biofertilization align with findings reported by Gogoi *et al.*, 2004 and Helmy *et al.*, 2013, where biofertilizers mitigated soil acidity and salinity, improving overall soil health. CEC in biofertilized soils measured 13.7 cmol/kg, and though C and P levels were adequate, total N content was relatively low. Both biofertilized and non-biofertilized soils exhibited a low C:N ratio, indicating slow mineralization and nitrogen release. The stability of the C:N ratio suggests that native *Rhizobium* strains may enhance nitrogen retention rather than promote rapid mineralization, which can be beneficial for sustaining long-term soil fertility (Chen *et al.*, 2024). These findings highlight biofertilization's positive impact on enhancing soil fertility and physicochemical properties, potentially improving tomato crop yields and overall agricultural sustainability.

It is important to emphasize that the observed reduction in electrical conductivity not only reflects decreased salinity but also implies a lower risk of ion toxicity to plants, particularly sodium and chloride ions, which are detrimental under arid or semiarid conditions (Seifi *et al.*, 2017; Geilfus, 2018). Moreover, the improved availability of total phosphorus suggests enhanced microbial activity, as *Rhizobium* strains are known to release organic acids that mobilize insoluble phosphate reserves in the rhizosphere (Etesami, 2023; Fadeh *et al.*, 2023). This dynamic is critical in tropical soils, where phosphorus is often immobilized in forms unavailable to plants. Native *Rhizobium* strains used as biofertilizers exhibited diverse Plant Growth-Promoting Bacteria (PGPB) qualities (Table 2). Notably, *R. jaguaris* demonstrated high acetylene reduction capacity (ARA), indicating strong nitrogen fixation. *R. calliandrae* showed the highest phosphate solubilization index (PSI=1.41), indicating proficiency in solubilizing both dicalcium and tricalcium phosphate (Figure 2). Such phosphorus solubilization is crucial for crop productivity, as phosphorus availability is often limited in agricultural soils (Ringeval *et al.*, 2017). Additionally, all three strains synthesized auxins, particularly *R. mayense* with notable indole-3-acetic acid (IAA) production at 34.8 mg/L. This auxin production is linked to improved root architecture, which enhances water and nutrient uptake, as demonstrated by Batista *et al.*, 2021.

Table 1. Physicochemical characteristics of soil in tomato crops enhanced by native *Rhizobium* Biofertilization.

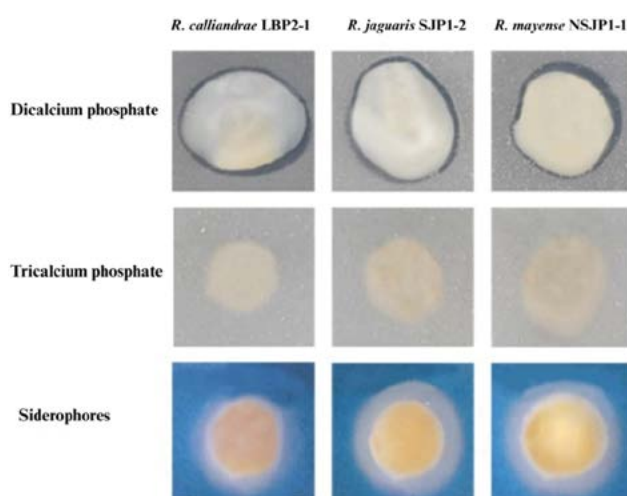
Soil samples	pH	EC ^a (dSm ⁻¹)	CEC ^b Cmol kg ⁻¹	Total C (%)	Total N (%)	Total P (%)	C:N ratio
Soil before biofertilizer	5.8±(0.04) ^c	0.87±(0.06)	10.5±(0.4)	1.17±(0.01)	0.65±(0.06)	1.46±(0.04)	1.8 ±(0.3)
Soil after biofertilizer	6.4±(0.07)	0.48±(0.04)	13.7±(0.5)	1.42±(0.03)	0.83±(0.04)	2.25±(0.06)	1.7±(0.5)
Chemical Fertilizer	5.4±(0.02)	2.69±(0.03)	18.0±(0.2)	2.28±(0.02)	0.95±(0.03)	3.22±(0.04)	2.4±(0.4)

^a EC: Electrolytic conductivity; ^b CEC: Cation exchange capacity; ^c Mean of three replicates. In parentheses standard deviation.

Table 2. Multifunctional PGPB qualities of native *Rhizobium* strains.

Strain	ARA ^a (nmol C ₂ H ₄ per culture h ⁻¹)	P-solubilization (PSI) ^b	IAA production (mg/L)	Siderophore production (SID) ^c	ACC- deaminase
<i>Rhizobium calliandrae</i>	425±(1.61) ^d	1.41±(0.62)	25.4±(0.98)	1.18±(0.75)	(+)
<i>Rhizobium jaguaris</i>	645±(1.41)	1.52±(0.48)	28.6±(1.12)	1.68±(0.95)	(+)
<i>Rhizobium mayense</i>	418±(1.35)	1.48±(0.58)	34.8±(1.34)	1.28±(0.67)	(+)

^aARA, Acetylene Reduction Assay; ^bPSI, Phosphate Solubilization Index. ^cSID, Siderophore Induced Droplet Formation. ^dMean values of three replicates. The values in parenthesis are standard deviations. (+)=positive.

**Figure 2.** Halos of phosphate solubilization and production of siderophores by native *Rhizobium*.

These multifactorial traits are consistent with the concept of “multifunctional PGPB”, where the synergistic expression of plant hormone production, nutrient solubilization, and stress alleviation (*e.g.*, through ACC deaminase activity) positions *Rhizobium* as more than just a nitrogen fixer. This redefines their potential for non-legume crops, aligning with Glick (2012) and Dheeman *et al.*, 2022, who emphasized the versatility of PGPB in a broad range of plant hosts. Furthermore, all three strains exhibited siderophore production, with *R. jaguaris* showing the highest percentage of siderophore production (SID=1.68) among the *Rhizobium* species analyzed. These findings align with previous studies demonstrating the capacity of rhizobacteria, including *Rhizobium* spp., to solubilize phosphorus through the secretion of organic acids (Taktek *et al.*, 2015).

Native *Rhizobium* strains’ siderophore production is essential for plant iron uptake, critical in N₂-fixing systems and enzyme synthesis (Datta and Chakrabartty, 2014). Siderophores are low-molecular-weight iron-chelating compounds that bacteria release to scavenge ferric iron (Fe³⁺) from the soil, especially under conditions of limited bioavailable iron, a common scenario in alkaline or calcareous soils. By forming stable Fe³⁺-siderophore complexes, these molecules facilitate iron uptake not only for the bacteria but also for the host plant through rhizosphere exchange mechanisms (Ahmed *et al.*, 2014). In this context,

the siderophore-mediated increase in iron availability can enhance photosynthetic activity, promote enzymatic function, and improve overall plant vigor.

This study emphasizes their diverse role as biofertilizers, enhancing soil fertility and plant growth. Inoculation significantly influenced various parameters in field-grown tomato plants, with biofertilized plants showing increased height and total weight, particularly with *R. calliandrae* and *R. mayense* application. The impact results from native *Rhizobium* strains' inherent abilities in N₂ fixation, phosphate solubilization, and auxin synthesis, vital for plant growth. Additionally, these strains play a crucial role in nutrient uptake, especially for essential elements like nitrogen and phosphorus (Gen-Jimenez *et al.*, 2023).

Rhizobium bacteria crucially influence biochemical and metabolic processes, enhancing crop quality as precursors in various pathways. Biofertilization, especially with native *Rhizobium* strains, significantly affects mineral concentration, carotenoids, and lycopene levels in tomatoes (Flores-Felix *et al.*, 2021). These findings underscore native *Rhizobium* strains' potential to positively impact tomato plants, improving crop yield and quality. Moreover, by modulating phytohormone signaling pathways, particularly auxin and ethylene, these bacteria can influence not only vegetative development but also

Table 3. Effect of biofertilization with native *Rhizobium* bacteria on morphometric and biochemical parameters in tomato cultivation.

Treatments	Plant height (cm)	Plant weight (g)	Chlorophyll Content (%)	Total N (%)	Total P (%)	Fruit (g)	Fruit/plant
<i>R. calliandrae</i>	43.5 a*	264.3 a	39.5 ab	4.06 b	0.75 a	217.1 a	18.2a
<i>R. jaguaris</i>	42.9 a	152.3 c	42.0 a	3.12 c	0.60 a	206.8 ab	15.9b
<i>R. mayense</i>	41.5 a	242.6 a	41.4 a	2.78 c	0.62 ab	196.6 ab	17.9a
Chemical fertilizer	41.5 a	176.8 bc	44.7 a	6.41 a	0.48 b	187.6 ab	15.5b
Negative control	40.9 a	181.0 b	33.1 b	4.28 b	0.57 b	177.7 b	17.2ab
p-value	0.1677	0.0000	0.0002	0.0000	0.0003	0.0432	0.0002
[¶] HSD Tukey (p<0.05)	3.363	25.384	6.681	0.380	0.152	38.038	1.831

*Mean values were obtained from n=25 replicas; [¶]HSD (Honestly Significant Difference). Means followed by the same letter are non-significant (p<0.05).



Figure 3. Effect of biofertilization with native *Rhizobium* bacteria on tomato growth and production.

reproductive processes such as flowering and fruit set. This interaction may explain the observed improvements in fruit yield and quality. The integration of *Rhizobium*-based biofertilization into tomato production systems thus presents a promising strategy not only for enhancing productivity, but also for improving the nutritional value and marketability of the crop in a sustainable manner. These results contribute to a growing body of evidence supporting the use of native, crop-adapted microbial consortia to optimize agroecosystem functioning while reducing dependence on synthetic inputs.

CONCLUSIONS

The application of native *Rhizobium* strains, such as *Rhizobium calliandrae*, *R. mayense*, and *R. jaguaris*, as biofertilizers significantly improved soil quality and positively influenced tomato plant growth. These strains demonstrated growth-promoting traits, including nitrogen fixation and phosphate solubilization, which were associated with increases in plant height and total biomass. The results of this study highlight the promising potential of native *Rhizobium* strains as sustainable biotechnologies for enhancing crop performance while contributing to improved soil health. Future research, including metagenomic analysis and next-generation sequencing, could further elucidate the significance of employing rhizobial biofertilizers in agricultural systems.

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