Inoculation of *Solanum lycopersicum* L. with *Pseudomonas* sp. as plant growth promoters and reduced doses of macronutrients increases the yield and quality of tomato fruits

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Background. Greenhouse tomato cultivation is steadily increasing. However, the production method involves a system of high nutritional demand where synthetic fertilizers are mainly applied, hindering the absorption of nutrients by the plants and provoking excess fertilizer in soil or bodies of water. In addition to the increased cost of fertilizers for global agriculture, it is necessary to seek efficient alternatives promptly. In this context, the use of Plant Growth-Promoting Rhizobacteria (PGPR) represents an advantage in agriculture by allowing the partial reduction of inorganic inputs. This study evaluated the phytostimulant effect of five *Pseudomonas* isolates to contribute to a 50% reduction in the concentration of nitrogen, phosphorus, and calcium applied through hydroponic systems in the greenhouse cultivation of the El Cid F1 tomato variety. The isolates were evaluated individually and in consortium. **Methods**. In preliminary in vitro tests, the *Pseudomonas* sp. isolates C13, C14, and C15, *Pseudomonas fluorescens* C30, and *Pseudomonas putida* ACJ14 isolates, and consortium showed the capacity to fix nitrogen, solubilize phosphate, solubilize calcium, and produce indole-3-acetic acid. Overall, the results of the greenhouse trials allowed for determining the potential of the *Pseudomonas* sp. isolates to stimulate vegetative growth and improve the values of firmness, total soluble solids, titratable acidity, and lycopene concentration in the fruit. **Results**. The *P. putida* ACJ14 and *Pseudomonas* sp. C14 isolates stood out for increasing fruit yield by 54% and 73%, respectively. Additionally, lycopene in tomato fruit was increased by the inoculation of the *P. putida* ACJ14 with a value of 132.9 mg/g of fruit and with the *Pseudomonas* sp. C14 with 130.22 mg/g of fruit. When studying the isolates in a consortium, no significant difference was seen in any of the parameters with respect to the effect caused by each of the isolates. Furthermore, the ability of the isolates to persist in the tomato rhizosphere for up

to 30 days after inoculation into the root system was demonstrated. **Conclusions.** The results suggest that the use of the isolates *Pseudomonas* sp. isolates C13, C14, C15, *P. fluorescens* C30, and *P. putida* ACJ14 as sustainable PGPR alternatives, allowing a 50% reduction of nitrogen, phosphorus, and calcium in tomato fertilization in greenhouses.

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Abstract

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Methods. In preliminary in vitro tests, the *Pseudomonas* sp. isolates C13, C14, and C15, *Pseudomonas fluorescens* C30, and *Pseudomonas putida* ACJ14 isolates, and consortium showed the capacity to fix nitrogen, solubilize phosphate, solubilize calcium, and produce indole-3-acetic acid. Overall, the results of the greenhouse trials allowed for determining the potential of the *Pseudomonas* sp. isolates to stimulate vegetative growth and improve the values of firmness, total soluble solids, titratable acidity, and lycopene concentration in the fruit.
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Introduction

Tomato (*Solanum lycopersicum* L.) are the world's most-produced vegetable (*Parra-Gómez et al., 2016*). Mexico ranks as the eighth largest producer and the leading exporter to the international market, contributing 24.7% of the global supply of tomatoes (*SIAP, 2018*). Therefore, due to its economic impact, its cultivation represents one of Mexican agriculture's most important socioeconomic activities. In addition, it is considered a substantial food with high nutritional value due to its protein, mineral, and fiber content and for being an important source of bioactive compounds with antioxidant activity (*Chaudhary et al., 2018; Perveen et al., 2015*), which attributes to it the potential to be used as a basis in the development of functional foods (*Chaudhary et al., 2018*). It is a critical element in the fight against global malnutrition (*Vats et al., 2020*). It satisfies nutritional requirements in the search to ensure food security for the estimated population by 2050 (*Hunter et al., 2017*).

In recent years, there has been an increase in tomato production in protected agriculture, mainly in greenhouses, due to its productive potential and higher yields compared to other agricultural systems (*FIRA, 2019*). However, in this type of agriculture, nutritional conditions are provided by applying nutrient solutions that contain balanced chemical elements, considering factors such as the phenological stage of the plant, the frequency of application, and environmental conditions. Therefore, the deficiency of even a single nutrient limits its growth and development, affecting yield (*López-Marin, 2017*). In current practice, the applied concentration of agricultural inputs exceeds nutritional requirements to avoid deficiencies; this leads to significant loss of inorganic fertilizers by leaching, volatilization, or accumulation in the soil when they exceed the crops' absorption rate (*Burchi et al., 2018*). This results in disturbances in the global ecosystem, including an imbalance in the functions of rhizospheric microorganisms involved in plant growth, development, and nutrition, limiting their agricultural potential and posing a risk to sustainable productivity (*Lenka et al., 2016*). On the other hand, the irrational use of these products is reflected in a significant increase (between 10 and 25%) in production costs (*Salgado y Núñez, 2012*). In addition, the recent rise in the price of fertilizer due to various factors requires implementing agricultural production techniques focused on the efficient use of resources, with a trend towards sustainable agriculture (*FAO, 2012*).

In this sense, research has focused on using microorganisms that inhabit the rhizosphere to promote plant growth, called Plant Growth Promoting Rhizobacteria (PGPR) (*Subrahmanyam et al., 2020*). PGPR constitutes 2 to 5% of rhizospheric bacteria (*Prasad et al., 2019*). Their

presence in the soil improves germination rate and seed vigor (*Rudolph, Labuschagne and Aveling, 2015*), influences root development (*Verbon and Liberman, 2016*), restores soil quality and fertility in contaminated soils (*Kaur, 2021*), protects against phytopathogenic microorganisms and environmental stress conditions (*Kumar et al., 2019*), and helps reduce the global dependence on destabilizing chemical agroinputs (*Laslo and Mara, 2019*). PGPR are involved in transforming nutrients into biologically assimilable forms or stimulates the development of the root system, leading to an increase in the surface area and proliferation of root hairs, thus improving nutrient acquisition (*Calvo et al., 2016*). Recently, PGPR has been demonstrated as an ecological option that benefits numerous plant species. Various strains of microorganisms have been isolated, and it has been shown that their inoculation in plants influences the concentration of nutrients, positively impacting productivity (*Calvo et al., 2016*), allowing the application of inorganic fertilizer to be optimized in different crops (*Bona et al., 2017*). *Pseudomonas* is one of the most diverse and widely distributed genera due to its high adaptability in various environments (*Poncheewin et al., 2022*). It has an advantage over other bacteria due to its ability to colonize and persist in the rhizosphere (*Zboralski and Filion, 2020*), where it exhibits great metabolic and functional versatility, playing a key role in its activity to promote plant growth or control phytopathogenic microorganisms (*Roquigny et al., 2017*). Various activities have been described within this genus, highlighting the production of siderophores (*Kotasthane et al., 2017*) and antifungals (*Hernández-León et al., 2015*). In addition, their ability to fix atmospheric nitrogen (*Fox et al., 2016*), produce indole-3-acetic acid (IAA) from tryptophan (*Li et al., 2017*), and solubilize phosphates (*Kour et al., 2020*) has been determined. Recently, the presence of antifreeze proteins was reported in three different strains of *Pseudomonas* sp., which are responsible for inhibiting the recrystallization activity of ice. This fact was related to promoting tomato seed germination and root development in plants grown at 14 °C (*Vega-Celedon et al., 2021*), highlighting their adaptability in adverse environments and ability to mitigate abiotic stress.

Moreover, the genus *Pseudomonas* exhibits a wide genetic diversity since only 25 to 35% of the genome of all members of the genus is composed of core genes, while the rest confer metabolic, ecological, and functional variation at the species level and between strains of the same species (*Loper et al., 2012*). In other words, the performance of bacteria as plant growth promoters varies depending on different factors such as plant genotype, climatic, and growing conditions (soil type, temperature, humidity, etc.). In this sense, specific bacterial strains that have shown promising effects under controlled laboratory conditions may fail in real-world situations (*Díaz-Rodríguez et al., 2021*). Hence, their agricultural use as bioinoculants and the market focused on sustainable agriculture remains limited, accounting for only about 1% of the conventional agriculture market (*The Insight Partners, 2022; The Insight Partners, 2024; Mordor Intelligence, 2024a; Mordor Intelligence, 2024b*).

In this context, the present research aimed to generate relevant information on the effect of *Pseudomonas* sp. inoculation on the yield and fruit quality of tomatoes grown with reduced doses of macronutrients and in greenhouse conditions.

Materials & Methods

Evaluation of bacterial activities associated with nutrient availability

The bacteria used were isolated from sugarcane (*Saccharum officinarum)* and agave's (*Agave* sp.) rhizosphere and identified as species of the genus *Pseudomonas*, designated as *Pseudomonas* sp. C13, *Pseudomonas* sp. C14, *Pseudomonas* sp. C15, *P. fluorescens* C30, and *P. putida* ACJ14. The five isolates were selected for their activity related to the transformation of nutrients into biologically assimilable forms based on the following evaluations:

Biological nitrogen fixation

The ability to fix atmospheric nitrogen by isolates was determined using 200 µL of a bacterial suspension previously adjusted to 0.7 OD (approximately $1x10^9$ CFU/mL) was inoculated into 3 mL of 10% Soil Extract Broth and incubated with constant shaking at 100 rpm for 72 h. Samples were centrifuged at 6000 rpm for 15 min, and 10 mL of 2 M KCl were added. They were then shaken for 60 min and allowed to settle for an additional 60 min. The supernatant was then centrifuged at 6000 rpm for 10 min. Nitrogen quantification was performed indirectly by determining the concentration of ammonium ions using the Berthelot colorimetric technique and measuring absorbance in a visible light spectrophotometer at 632.9 nm (*Weatherburn, 1967; Lara-Mantilla, Villalba-Anaya y Oviedo-Zumaqué, 2007*).

Production of indole-3-acetic acid

The indole-3-acetic acid (IAA) quantification was determined by gas chromatography and mass spectrometry (GC-MS). The isolates were cultured in triplicate for 96 h in 500 mL of Tryptic Soy Broth supplemented with 200 µg/mL of tryptophan. The supernatant obtained after centrifugation was separated and adjusted to pH 5 ± 0.02 using 1 N HCl. IAA was extracted with an equal volume of ethyl acetate, evaporated to dryness, and diluted in 3 mL of HPLC-grade ethyl acetate. It was then dried under a nitrogen gas stream, diluted in 2 mL of acetyl chloride in methanol (1:4), sonicated for 15 minutes, and incubated at 75°C for 1 hour. The sample was cooled to 30°C dried under a nitrogen gas stream, and 1 mL of dichloromethane and 1.5 mL of acetic anhydride were added. Finally, the derivatized IAA was dissolved in 100 µL of ethyl acetate, and one µL was injected into a GC-MS. IAA quantification was performed using a calibration curve of the IAA standard, derivatized under the same conditions (*Contreras-Cornejo et al., 2009*).

Phosphate solubilization

Quantification of phosphate solubilized by bacteria in the form of orthophosphates was determined by inoculating 200 µL of the bacterial suspension adjusted to 0.7 OD into 50 mL of NBRIP growth medium (National Botanical Research Institute's Phosphate growth medium) (*Nautiyal, 1999*) and shaking at 150 rpm for 7 days. The sample was centrifuged at 5000 rpm for 15 min, and the supernatant was used for orthophosphate quantification using the molybdenum blue method (Murphy and Riley, 1962). Four mL of the supernatant was taken, and one mL of

the reagent mixture was added. The tubes with the analyte were incubated at room temperature for 10 min, and the quantification was determined by measuring the absorbance at 882 nm (*Rodríguez-Gámez, Aguilera-Rodríguez y Pérez-Silva, 2013*).

Calcium solubilization

The quantification of calcium solubilized from calcium carbonate by bacterial activity was determined by atomic absorption spectroscopy. For sample preparation, the bacterial suspension was adjusted to 0.7 OD and 200 µL was inoculated into 50 mL of modified Pikovskaya liquid medium (*Paguay y Vasco, 2013*), followed by incubation for 7 days. The samples were then vacuum-filtered with a 0.4 µm membrane, and corresponding dilutions were performed for analysis. Free calcium concentrations were calculated using a standard curve with successive dilutions of calcium carbonate.

Effect of *Pseudomonas* sp. on germination and vigor of tomato seeds

The seeds were disinfected by immersion in sterile distilled water, followed by successive washes with 5.2% sodium hypochlorite for 15 minutes, 70% ethanol for 3 minutes, and then again in sterile distilled water. Seeds were inoculated by immersion and shaking at 100 rpm for 1 hour in a bacterial suspension adjusted to 0.7 OD. The germination percentage was determined in triplicate by placing 20 previously disinfected and inoculated seeds in Petri dishes with a cotton layer moistened with sterile distilled water, sealed, and kept in darkness for 10 days. The percentage of germinated seeds was recorded based on radicle emergence, and the vigor index was calculated using the following equation (*Díaz-Vargas et al., 2001*):

$$Vigorindex(VI) = \left(M_{Radiclelength} + M_{Hypocotyllength}\right)xGP$$

Where: M is the root's length mean and hypocotyl length, and PG is the germination percentage.

Field Experiment

Experimental Design and Greenhouse Cultivation Conditions

The experimental design consisted of eight treatments (Table 1) with seven replications, where each plant served as an experimental unit and was placed in a greenhouse in a completely randomized design. As part of the treatments, the Steiner Nutrient Solution (*Steiner, 1961*) at 50% nitrogen, phosphorus, and calcium was used for the bacteria-inoculated plants, as well as for control with the same concentration of elements, but without the bacterial inoculation (T50%) (Table 2). The remaining macro and micronutrients were applied at 100% of the Steiner nutrient solution (*Hoagland and Arnon, 1938*). A control was also treated using the 100% Stainer solution (T100%).

Seeds of the commercial hybrid Saladette El Cid F1 (Harris Moran Mexicana S.A. de C.V.) were disinfected and inoculated. They were planted in polystyrene trays with 200 cavities filled with pre-conditioned peat moss substrate and placed in an area with natural light, with irrigation applied every 24 h. The plants were transplanted into 40 x 40 cm black polyethylene bags, filled with a substrate composed of tezontle and soil (1:1) 40 days after germination when the plants

had 4 to 5 true leaves. The bags were arranged in double rows with a separation of 30 cm, and Azoxystrobin (Bankit 25SC) was applied at a dose of 400 mL/ha as a preventive phytosanitary control against phytopathogenic fungi. From transplanting and throughout the crop cycle, nutrients were applied using Steiner´s nutrient solution (*Steiner, 1961*) at the concentration corresponding to each treatment, applying one L of solution per day distributed in eight irrigations/minute through a drip system. Each plant was inoculated three times during the crop cycle with 1 mL of the bacterial suspension corresponding to each treatment, adjusted to 1x10^9 CFU/mL, distributing the inoculum around the base of the stem towards the root zone. The T50% solution treatment plants were inoculated with one mL of sterile culture medium; the T100% control treatment did not receive this application. Inoculations were performed at 10, 70, and 110 days after transplantation. The staking was carried out by guiding the plant using polypropylene raffia and pruning the axillary shoots to allow only the growth of the main stem. Since the variety is of indeterminate growth, this was stopped at the fifth cluster by cutting the apical shoot. Pollination was carried out manually by gently moving the staking raffia when the first floral clusters were formed. Defoliation was performed by cutting the leaves lower than the cluster formed to facilitate illumination, ventilation, and avoid excess humidity.

Analysis of plant response variables and the sensory and physicochemical quality of fruits

The variables related to the vegetative part of each experimental unit were evaluated at 40, 70, 90, and 120 days after planting. Plant height, stem diameter, and leaf nitrogen content were measured. Leaf nitrogen content was measured indirectly in SPAD (Soil Plant Analysis Development) units using a SPAD-502 device, and its conversion to leaf nitrogen percentage was calculated using the following equation (*Rodríguez et al., 1998*):

$$\%N = \frac{SPAD + 4.26}{16.15}$$

The fruits were harvested when more than 90% of their surface was red. Firmness was evaluated by making two punctures per fruit at opposite positions located in the equatorial zone using a digital texturometer. The individual weight of each fruit was measured on an analytical balance. The fruits were mixed using an ULTRA-TURRAX homogenizer to obtain puree. Brix degrees were determined as the total soluble solids (TSS) content by placing a drop of tomato juice obtained from the previously filtered puree in a refractometer. Titratable acidity was determined by taking 10 mL of the puree, which was then adjusted to 25 mL and neutralized with 0.1 N NaOH using a potentiometer until a pH of 8.2 was reached. Acidity was calculated using the following equation, expressed as a percentage (%) of citric acid (*Nielsen, 2003*).

$$\%acidity = NaOH\frac{meq}{ml}VNaOH(mL)$$

For lycopene quantification, 0.1 g of tomato puree was weighed and placed in glass tubes covered with aluminum foil. Lycopene was then extracted by adding 8 mL of a mixture of hexane: ethanol: acetone (2:1:1) (v/v). The tubes were shaken for 10 minutes, and then one mL

of deionized water was added, followed by vortex stirring to induce phase separation. After 10 minutes of sedimentation, the upper fraction containing the lycopene extracted in hexane was collected. The absorbance of the solution was measured in a spectrophotometer at a wavelength of 503 nm, using hexane as a blank. The concentration of lycopene was estimated using the molar extinction coefficient according to the following equation (*Gawad, Jaiswal and Narayan, 2014*):

$$Lycopene\left(\frac{mg}{kg}\right) = \frac{(A_{503})(537)(10)(0.55)}{(0.1)(172)}$$

Where: A503 is the absorbance obtained from the top layer; 537 is the molecular weight of lycopene; 10 is the volume (mL) of the added HEA mixture; 0.55 is the volume of the top layer; 0.1 is the weight of the sample in grams, and 172 is the extinction coefficient of lycopene in hexane.

Finally, yield was determined by quantifying the weight of fruit obtained per plant and calculated as grams of fruit per plant at the fifth cluster.

Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA); a Tukey´s post hoc test was performed to compare means to determine significant differences between treatments, with a significance level of $\alpha = 0.05$. Calculations were performed using the statistical package SAS, 2012 (Statistical Analysis System).

Results

The *Pseudomonas* sp. isolates improve the nutrient bioavailability

The five isolates tested were positive for atmospheric nitrogen fixation (Table 3). *Azospirillum brasilense* was used as a positive control for this assay since this bacterium has been widely reported as a nitrogen fixer (*Zaidi et al., 2017*). In *A. brasilense,* a fixation of 12.7 µg/mL ammonium was quantified. The five *Pseudomonas* sp. exceeded this value isolates since the C13 strain fixed 15 µg/mL ammonium, *P. fluorescens* 20 µg/mL, and *Pseudomonas* sp. C15 and *P. putida* ACJ14 achieved a similar fixing activity, with a concentration close to 30 µg/mL. *Pseudomonas* sp. C14 exhibited the highest value, 35 µg/mL. On the other hand, the *Pseudomonas* isolates showed the ability to solubilize phosphate and calcium from an insoluble source. A concentration of solubilized phosphate in the form of orthophosphates was quantified in the range of 20 to 90 µg/mL. *Pseudomonas* sp. C14 and *P. putida* ACJ14 exhibited the lowest solubilization values, totaling 20 µg/mL, while *Pseudomonas* sp. C13, *Pseudomonas* sp. C15 and *P. fluorescens* C30 showed values close to 90 µg/mL. Regarding calcium solubilization, the quantitative analysis showed that the C13 isolate had the highest value, with 583.74 µg/mL of soluble calcium. However, atomic absorption spectrometry did not detect soluble calcium for the *Pseudomonas* sp. C14 and C15. Similarly, the *Pseudomonas* sp. isolates exhibited the ability to

produce IAA; *P. fluorescens* C30 exhibited the highest efficiency (12680 µg/L), while the rest of the isolates, a phytohormone concentration in the range of 61.33 to 840.67 µg/L was recorded.

The *Pseudomonas* sp. isolates improve the germination rate and vigor of tomato seeds

The viability of the seeds used in this study was low, with a germination rate of 70% in the control treatment (Table 4). Under these conditions, the germination percentage increased from 8.5 to 18.5% due to *Pseudomonas* sp. inoculation, but without significant differences according to the statistical analysis (Table 3). Despite this, the effect on germination positively correlated with IAA concentration since bacteria with the highest production levels of this phytohormone, such as *P. fluorescens* C30 (12.6 µg/mL), *Pseudomonas* sp. C14 (0.67 µg/mL) and *P. putida* ACJ14 (0.47 µg/mL), generated the most significant increase in germination. In contrast, *Pseudomonas* sp. C15 and *Pseudomonas* sp. C13, which produced the lowest concentrations of IAA, 0.12 µg/mL and 0.06 µg/mL, respectively, did not exert an evident effect. On the other hand, all isolates of *Pseudomonas* sp. statistically increased the plant vigor index, with the highest values recorded for *Pseudomonas* sp. C15; this isolate achieved a 50% increase compared to the control, followed by the bacterial consortium with a 45% increase.

The *Pseudomonas* sp. isolates improve vegetative parameters in tomato plants with reduced doses of N, P, and Ca

Significant differences were observed in plant height, stem diameter, and leaf nitrogen percentage at the beginning of the reproductive stage (Fig. 1 A-C). The control with 50% Steiner nutrient solution (T50%) had the lowest height, minor stem diameter, and leaf nitrogen content compared to the other treatments from the first 90 days after planting. However, the difference widened at 130 days after planting. T50% of plants had on average, a height of 146 cm, stem diameter of 6.6 mm, and a leaf nitrogen concentration of 2.7%, which corresponds to 22%, 25%, and 18% less, respectively, than the control plants fertilized with the complete nutrient solution (T100%), which showed 177 cm in height, 8.9 mm in stem diameter, and 3.4% leaf nitrogen content. The results demonstrate that a 50% reduction in nitrogen, phosphorus, and calcium consumption affected tomato plant development. The inoculation of the bacteria in plants fertilized with 50% of N, P, and Ca positively influenced plant height, stem diameter, and leaf nitrogen content, with their effect being observed from the reproductive stage, mainly in inoculations with *Pseudomonas* sp. C14, *P. fluorescens* C30, and *P. putida* ACJ14. Inoculation with the C14 isolate induced the greatest stimulation of plant height, with an average of 194 cm, corresponding to an increase of 9.7% compared to T100%. Additionally, it generated the highest leaf nitrogen content, consistent with the *in vitro* tests, since this strain fixed the highest ammonia concentrations. On the other hand, stem diameter increased significantly due to the application of *P. fluorescens* C30 and the consortium, as the plants had a diameter of approximately 10 cm, 13% larger than T100%. Additionally, they led to an increase in leaf nitrogen content, along with *P. putida* ACJ14. It is important to mention that the T50% Steiner nutrient solution control, without bacterial inoculation, showed lower levels of leaf nitrogen content for a tomato plant, typically between 3 and 5% (*Argerich and Troilo, 2010*), indicating an improvement in nitrogen absorption due to reduced chemical fertilization. However, nitrogen absorption was favored by the *Pseudomonas* sp. inoculation, as observed by a nitrogen percentage in inoculated plants that was more significant than 3% in all cases.

Analysis of the survival of *Pseudomonas* sp. in the tomato rhizosphere under greenhouse conditions.

At the end of the crop cycle, the bacterial isolations were determined from soil adhered to each plant treatment's roots to confirm the bacteria's persistence. Colonies with morphologies to the bacteria inoculated in the plants were identified, and their cellular shape was verified with the Gram staining and siderophores production observable under UV light (*Cezard, Farvacques and Sonnet, 2014*). Finally, characteristics associated with growth plant promotion were performed (Table 5). In the first instance, a higher bacterial load was observed in the treatments inoculated with the *Pseudomonas* sp. isolates compared to the no-inoculated controls. In all treatments, *Pseudomonas* presumptive colonies were isolated concerning their macroscopic morphology, even in both controls; however, the latter were discarded as they did not present fluorescence when observed under UV light, and because they did not have activities of nitrogen fixation, phosphate and calcium solubilization and IAA production.

Effect of inoculation with *Pseudomonas* sp. isolates on the root system of tomato

At the end of the crop cycle, the effect of *Pseudomonas* sp. inoculation on the root system was evaluated by quantifying the dry weight. The decrease in nutrient concentration in T50% plants did not significantly affect root weight compared to T100%, with weights of 8.2 g and 12.7 g, respectively (Fig. 2, Fig. 3). The isolates C13, C15, C30, and consortium did not generate significant differences from the controls. On the other hand, inoculation with *Pseudomonas* sp. C14 and *P. putida* ACJ14 resulted in a 63% and 60% increase in root weight, respectively, compared to T100%.

Analysis of the quality of tomato fruit fertilized with 50% N, P, and Ca after inoculation with *Pseudomonas* sp.

Tomato fruits were harvested when red on more than 90% of the surface. Regarding firmness, the results showed that the 50 % reduction of nutrients affected this parameter, with the lowest firmness observed in the T50% treatment, with an average of 4.6 N (Table 6). Likewise, fruit firmness was increased with the complete application of fertilizers and bacterial inoculation. In addition, there was an increase in total soluble solids (TSS) due to the application of the complete Steiner solution and bacterial inoculation, compared to T100% treatment, whose fruits presented 6.2° Brix. The highest SSC was obtained by inoculation with the isolate C14 (7.3° Brix). On the other hand, the fruits showed values between 0.29 and 0.41% of titratable acidity, with the lowest value being observed in the treatment with 50% fertilization without inoculation, and increased significantly due to the application of complete fertilization, as well as by inoculation in the consortium, by 41% in both cases. The rest of the treatments also showed an increase in SSC of up to 37% in the case of the *P. putida* ACJ14 isolate; however, the difference was not statistically significant. Finally, lycopene content was evaluated as a nutraceutical quality parameter. Lycopene content was 94 mg/g in the absolute control fruits (T50%) and was

positively affected by bacterial inoculation, with a significant increase by isolates C14 (37.93%), C15 (37.94%), ACJ14 (40.13%), and by the bacterial consortium (30.8%).

Greenhouse tomato crop yield with reduced doses of 50% N, P and Ca in response to inoculation with *Pseudomonas* sp.

The 50% reduction in nutrients significantly affected fruit production, as the T50% treatment yielded only 933 g of fruit per plant (Fig. 3). It also affected fruit development, resulting in the production of smaller fruit (Fig. 4). However, yield increased by 19.4% with complete fertilization, as the total weight in T100% was 1,115.97 g of fruit per plant. Moreover, nutrient deficiency was compensated by bacterial activity since inoculation with *Pseudomonas* sp., characterized as PGPR for their stimulation of morphological variables of plant growth, led to an increase in tomato yield under reduced fertilizer conditions, even surpassing the yield generated by conventional agricultural fertilization treatments. Inoculation with the isolates C13 and C15 increased yield compared to T100% treatment by 14.3% and 10.3%, respectively. Inoculation with *P. fluorescens* C30 resulted in a 32.4% increase compared to T100% and a 58.17% increase compared to T50%. The effect generated by *P. putida* ACJ14 and the bacterial consortium was similar, with yields of 1,727 and 1,711 g of tomato per plant, respectively, representing an increase of about 54% over T100% in both cases. Unlike the isolates C13, C15, and C30, the yield increase was statistically significant compared to the yield obtained when the complete nutrient concentration was applied. A significantly more significant increase was achieved by inoculation with *Pseudomonas* sp. C14, which produced 1,893 g of tomatoes per plant. This yield represents a 102% increase compared to plants fertilized with the reduced dose and a 73% increase compared to the control with the complete Steiner solution. Plants inoculated with this strain showed improved growth from transplanting, generating the most significant root volume, increasing yield, and fruit quality.

DISCUSSION

Fertilizers, mainly phosphates and nitrogen-based, are essential for the global food system and achieving high agricultural yields (*Erisman et al., 2013*). However, current greenhouses practices significantly exceed the concentration of elements to meet nutritional requirements and prevent deficiencies (*Burchi et al., 2018*). About 55% of nitrogen, 54% of calcium, and 36% of phosphorus are lost before being absorbed by plants due to various factors (*Sanjuan-Delmás et al., 2020*). In this sense, soil bacterial communities play a fundamental role by transforming nutrients into biologically assimilable forms. This research evaluated the effectiveness of *Pseudomonas* species isolated from sugarcane in biological nitrogen fixation and phosphate and calcium solubilization. Most *Pseudomonas* species lack the presence of the coding genes necessary for the biological nitrogen fixation process (*Yan et al., 2008*). However, *Fox et al., (2016)* reported that *Pseudomonas protegens* is a nitrogen fixer, while *Wang, Elmerich and Ma (2017)* reported the ability of *Pseudomonas stutzeri* A1501 to carry out the fixation process under microaerophilic and element-limiting conditions. *P. stutzeri* has been the most studied

species of the genus. In this sense, *Ke et al., (2018)* demonstrated an improvement in maize growth and nitrogen availability with the same strain and an increase in the population of diazotrophs in the rhizosphere. In addition, it had previously been determined that several Brazilian sugarcane varieties could obtain up to 70% of their nitrogen requirement through this process *Li et al., (2017)*. On the other hand, the genus *Pseudomonas* has been classified as one of the principal producers of organic acids responsible for the solubilization of tricalcium phosphate and rock phosphate (*Vyas and Gulati, 2009*). Therefore, the application of these rizobacteria can represent a safe and sustainable source of these elements, contributing to the reduction of dependence on inorganic nitrogen, as well as reducing the energy required for its production (*Mahmud et al., 2020*), as it has been estimated that up to 65% of this element supplied to cropping systems by biological nitrogen fixation (*Kuan et al., 2016*). On the other hand, *Gao et al., (2015)* reported the production of 18.47 µg/mL of IAA by *Burkholderia* sp. 7016, observing significant increases in shoot height and weight, root length and weight, and stem diameter in greenhouse tomato plants as well as an increase in tomato yield in the field. Thus, the ability of bacteria to produce IAA constitutes an ecological alternative in sustainable agriculture due to its role in the development of the root system, leading to an increase in root hair surface area and proliferation, thereby improving nutrient acquisition. From another perspective, the production of IAA by the evaluated *Pseudomonas* strains could be associated with the ability to enhance the percentage of germination and vigor of tomato seeds, particularly in the emergence of seedlings, by stimulating the cellular elongation of the embryonic axis (*Li et al., 2016*).

Balanced nutrient intake is essential at all stages of plant growth. For greenhouse tomatoes, *Bodale et al., (2021)* identified the first 90 days of growth as the period of highest nutrient consumption, mainly nitrogen, phosphorus, and potassium, due to their involvement in stem growth and leaf and flower formation. Thus, plant growth is limited if nutrients are scarce, and crop yields are reduced. Determining the activities related to the increase in nutrient availability by the isolated *Pseudomonas* allowed a reduction in the concentration of N, P, and Ca in the Steiner nutrient solution by 50%. Data obtained from measurements of height, stem diameter, and leaf nitrogen content indicated a nutritional compensation in tomato plants with reduced fertilization generated by inoculation with *Pseudomonas* sp., probably due to their participation in facilitating the availability of essential macronutrients at these stages through their ability to fix nitrogen and solubilize phosphate, or through the action of substances synthesized by the bacteria that stimulated plant elongation and nutrient acquisition, improving root development. Consistently, bacteria with greater capacity to perform the activities above showed an enhanced effect on tomato growth. These results showed a clear pattern of nutrient deficiency in uninoculated plants when the nitrogen, phosphorus, and calcium concentration in the nutrient solution was reduced by 50%. Under this condition, plants exhibited reduced stem diameter, shorter height, low leaf nitrogen percentage, and smaller root volume, indicating that nutrient reduction can jeopardize crop yield by affecting plant development. However, the improvement in the variables measured in the inoculated plants suggests that the *Pseudomonas* sp. isolated from sugarcane and agave contributed significant amounts of these elements to the crop through

nitrogen fixation and phosphate and calcium solubilization, allowing a better plant development, even more critical than when fertilized with the recommended dose of Steiner solution (*Steiner, 1961*).

Furthermore, the genus *Pseudomonas* has an additional advantage for root colonization due to its ability to form biofilms, which favors its persistence (*Zboralski and Filion, 2020*). The strains in this study demonstrated the ability to establish themselves near the tomato root and a high probability of surviving in the rhizosphere under uncontrolled conditions for at least 30 days, considering the last inoculation. Additionally, it was confirmed that they maintain their ability to fix nitrogen, solubilize phosphate and calcium, and produce indoles.

Proper nutrition is essential for plant development throughout the life cycle, so that an imbalance can affect both vegetative and reproductive growth in yield and quality (*Bodale et al., 2021*). Firmness is an important parameter related to the stage of fruit's ripeness, i.e., as ripeness increases, firmness decreases due to the hydrolysis of starches and pectins or the degradation of cell walls (*Cárdenas-Coronel et al., 2012*). An optimal calcium application also provides greater cell wall rigidity, directly influencing fruit firmness (*Ahmed et al., 2022*). This could explain the low firmness levels in the treatment with reduced fertilization T50%. However, fruit firmness also depends on other factors. For example, *Mena-Violante and Olalde-Portugal (2007)* obtained a similar value for fruit firmness in a control treatment (4.97 N) and an increase of 1.11 units with *Bacillus subtilis* BEB-13BS inoculation, which they associated with a decrease in the activity of the polygalacturonase enzyme responsible for the degradation of polyuronide, a component of the tomato cell wall. On the other hand, the role of ethylene in the ripening process is well-known (*Zhao, Nakano, and Iwasaki, 2021*). Furthermore, the expression of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase by *Pseudomonas* sp. bacteria has been widely described as a growth-promoting mechanism (*Glick and Nascimento, 2021*), which could modulate ethylene synthesis, slowing down the ripening process and thus reducing the loss of firmness. This parameter is important in fruits as it provides resistance to pathogen attack or damage caused by product handling; therefore, the results suggest that inoculation with *Pseudomonas* sp. could contribute to reducing tomato postharvest loss and prolonging its shelf life by improving fruit firmness. Total soluble solids (TSS) and titratable acidity determine tomatoes' flavor (*Gómez y Camelo, 2002*). The content of TSS measured as °Brix depends on the degree of ripeness and tomato variety (*Katsenios et al., 2021*). Since most TSS are composed of sugars, the observed increase in TSS could be related to higher photosynthetic efficiency. Authors such as *Hernández et al., (2020)* observed a significant increase in sugar concentration in fruits grown with lower nitrogen concentration because nitrogen limitation reduces vegetative growth, resulting in greater light irradiation to the fruit, thus improving its photosynthetic activity and, consequently, its sugar content. In this study, vegetative growth was not affected by nitrogen reduction in the inoculated treatments; however, removing the lower leaves at fruit ripening was performed so the conditions mentioned above could have been met. In the same vein, *Aini et al., (2019)* related the increase in nutrient concentration to sugar content, associating it to nitrogen as a basic component of chlorophyll and to the increase in the synthesis of

photoassimilates, which could explain the increase obtained by inoculation with *Pseudomonas* sp., since they generated an increase in leaf nitrogen content. On the other hand, TSS content is directly related to the degree of ripeness and, consequently, to the loss of firmness (*Kaur et al., 2006*). However, the values obtained in each of the treatments follow the same trend determined in firmness; that is, the treatments with higher firmness had higher TSS content, which translates into a high capacity for mechanical resistance, which indicates that inoculation with growth-promoting *Pseudomonas* sp. significantly improved two factors that define tomato quality.
Finally, lycopene content was evaluated as a nutraceutical quality parameter, being the most important bioactive component in tomatoes due to its antioxidant capacity (*Assar et al., 2016*). Lycopene content increased due to bacterial inoculation. Previous studies have analyzed the role of microorganisms in modulating bioactive compounds in tomatoes (*González-Rodríguez et al., 2018*) (*Singh and Pandey, 2021*), although contradictory results have been reported. For example, *Inculet et al., (2019)* obtained an increase in lycopene concentration in tomatoes inoculated with PGPR and attributed it to the improvement of the plant's nutritional status. However, *Katsenios et al., (2021)* analyzed the effect of *Pseudomonas* sp. 19Fv1T and *P. fluorescens* C7 on 70% fertilized tomatoes, where they ruled out an alteration in lycopene content in tomato fruits due to nutrient limitation. In the same context, *Hernández et al. (2020)* correlated lycopene content with nitrogen application depending on the phenological stage. On the one hand, they observed a higher lycopene accumulation in tomatoes with high nitrogen concentrations after transplanting. However, when nitrogen concentration is restricted at the flowering stage, lycopene concentration increases due to the decrease in vegetative growth, which favors irradiation to the fruit, stimulating carotenoid synthesis (*Hernández et al., 2020*). From another perspective, the increase in lycopene in tomato fruits was related to the ability of PGPR to reduce the negative effects caused by stress, leading to the production of antioxidant molecules (*Dorais, Ehret, and Papadopoulos, 2008*) (*González-Rodríguez et al., 2018*). Therefore, the increase in lycopene content observed after inoculation with *Pseudomonas* sp. could be explained by the improvement in leaf nitrogen content in the early stages of plant development, facilitated by nutrient availability favored by bacteria, together with leaf removal practices in later stages, leading to increased light irradiance to the fruit. However, lycopene accumulation depends on several biotic and abiotic factors, not on a direct response (*de la Osa et al., 2021*).
According to the *in vivo* trial results, the inoculation of *Pseudomonas* sp. increased the morphological parameters evaluated in plants fertilized with 50% nitrogen, phosphorus, and calcium from the Steiner nutrient solution, confirming its ability to promote plant growth. Additionally, the fruits maintained firmness, high sugar concentration, titratable acidity, and lycopene content, surpassing the conventional treatment.
In agricultural practice, achieving high yields is the primary goal of producers. Authors such as *Aini, Yamika, and Pahlevi, (2019)* point out a direct relationship between nutrient concentration, vegetative development, and crop yield. Tomato cultivation requires large amounts of nitrogen, phosphorus, and potassium, so a deficiency in any of these elements severely affects its growth

and yield (*Zaidi et al., 2017*), as observed in the T50% treatment, where the reduction in nutrient concentration caused a decrease in fruit yield, which was increased by inoculation with *Pseudomonas* sp. According to *Oteino et al., (2015)*, current research suggests that inoculation with phosphate-solubilizing bacteria may reduce up to 50% in phosphate application without significantly reducing yield. This study demonstrated that inoculation with *Pseudomonas* sp. exhibiting characteristics associated with plant growth promotion, including phosphate solubilization, allows for a 50% reduction in application rates of phosphorus, nitrogen, and calcium, resulting in a significant increase in tomato yield, and the size of harvested fruits. The tomatoes were classified as marketable fruits according to the Mexican standard NMX-FF-031-1997-SCFI Productos Alimenticios No Industrializados Para Consumo Humano - Hortalizas Frescas - Tomate - (*Lycopersicun esculentum* Mill.) – Especificaciones (*NMX-FF-031-1997-SCFI, 1997*), which establishes that their equatorial diameter must be greater than 38 mm. Several authors have observed similar effects following inoculation with *Pseudomonas* sp. on different solanaceous crops. For instance, *Chiquito-Contreras et al., (2017)* evaluated the impact of three strains of *P. putida* individually and in combination on the growth and productivity of habanero peppers in a greenhouse, applying complete and 25% reduced inorganic fertilization. They observed significant increases in yield, remarkably when plants were fertilized with a 75% nutrient solution, reducing production costs and environmental pollution while achieving an increase in productivity of up to 36%. However, no differences were observed in inoculation combined with complete fertilization; they argue that the efficacy of PGPR is particularly remarkable at reduced doses of fertilizer. This suggests that the action of these plant growth-promoting microorganisms is especially significant when nutritional elements are scarce in the rhizosphere. Similar results were reported by *Batool and Altaf, (2017)*, who found no positive effects on bell pepper yield with PGPR inoculation when conventional nutrition was applied. In addition, based on the yields obtained, it was determined that inoculation allows for a reduction of between 75 and 80% in fertilizer concentration, achieving yields statistically equivalent to those of plants with traditional inorganic fertilization. However, doses below 75% significantly affect productivity. Thus, despite the improved efficiency in plants due to the inoculation of beneficial microorganisms, their use does not replace inorganic fertilizers but complements them. The involvement of bacteria in yield enhancement has been associated with various activities. *Narendra et al., (2015)* proposed that the beneficial effects of PGPR are attributed to enhanced nutrient uptake, which is facilitated by their ability to solubilize phosphorus and fix nitrogen. Specifically, improved phosphorus uptake has been related to its participation in the flowering process. It could provide a more significant number of fruits per plant or potentially through its role in root development (*Coutinho-Edson et al., 2014*). *Pérez-Rodriguez et al., (2020)* attributed the increase in tomato growth and yield to the improvement in the root system resulting from inoculating with *Pseudomonas fluorescens*, which increased the exploratory potential for water and nutrient *uptake*. Meanwhile, *Espinosa-Palomeque et al., (2019)* attributed the observed increase in tomato yield to an improvement in chlorophyll content, which led to higher photosynthetic efficiency and, subsequently, an increase in tomato weight. In this case,

the growth promotion and yield improvement observed in plants inoculated with *Pseudomonas* sp. and fertilized under a reduced nutrient regime could be attributed to increased nutrient uptake and improved nutritional status due to their demonstrated ability to fix nitrogen, solubilize phosphate, solubilize calcium, and produce IAA, as observed in leaf nitrogen content. Additionally, improved root system development may have contributed to these observed effects.

Conclusions

Inoculation with *Pseudomonas* sp. isolated from sugarcane and agave from the state of Michoacán resulted in a 50% reduction in the application of nitrogen, phosphorus, and calcium fertilizers in tomatoes grown in greenhouses using hydroponic irrigation with the Steiner Universal Solution. This increases tomato fruit yield by up to 73% while maintaining or improving the quality parameters of the plants fertilized with the conventionally recommended dose. When applying the isolates in a consortium, it was not observed that they stood out compared to the use individually. The finding demonstrates the potential for utilizing this PGPR approach as a viable alternative for optimizing chemical fertilizers, resulting in a reduction of approximately 32% in the total cost of the nutrient solution and a notable decrease in the use of chemical fertilizers that are detrimental to the environment.

Acknowledgments

The authors thank at Centro de Bachillerato Tecnológico y Agropecuario No. 7 (CBTA 7, Morelia, Michoácan, México) for allowing field research to be conducted in their greenhouse.

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Table 1(on next page)

Greenhouse trial treatments with *Pseudomonas* sp. isolates

Table 1 Greenhouse trial treatments.

Number of treatments	Description
1	T50%
2	T100%
3	Strain C13 + SNS 50%
4	Strain C14 + SNS 50%
5	Strain C15 + SNS 50%
6	Strain C30 + SNS 50%
7	Strain ACJ14 + SNS 50%
8	Consortium of the five strains SNS 50%

SNS: Steiner´s Nutrient Solution.

Table 2(on next page)

Concentration of macro and micronutrients for the treatments with Steiner nutrient solution.

Table 2 Concentration of macro and micronutrients for the treatments.

	Nutrients (meq/L)											
	N	**P**	**Ca**	**K**	**S**	**Mg**	**Fe**	**Mn**	**Cu**	**Zn**	**B**	**Mo**
SNS 50%	8.6	0.4	6.5	7.1	14	8.3	0.08	0.04	0.04	0.008	0.04	0.002
SNS 100%	16.4	0.8	13									

SNS: Steiner´s Nutrient Solution.

Table 3(on next page)

Isolates activity in attributes associated with nutrient availability.

Table 3. **Strain activity in attributes associated with nutrient availability.**

Strain	Ammonia (mg/mL)	Production IAA (µg/L)	Phosphate solubilization (µg/mL)	Calcium solubilization (µg/mL)
Azospirillum brasilense sp. 245	12.68 ± 0.60 d	487.33±70.7 b	NA	NA
Pseudomonas sp. C13	15.50 ± 0.9 cd	61.33±17.2 b	91.73±1.72 cd	583.74±4.8
Pseudomonas sp. C14	35.07 ±0.83 a	840.67±5.8 b	24.25±0.94 e	-
Pseudomonas sp. C15	30.04 ± 1.6 b	122.53±5.8 b	87.75±1.05 d	-
P. fluorescens C30	20.45 ±0.89 c	12680±22.9 a	95.62±2.02 c	59.42±0.4
P. putida ACJ14	29.53 ± 1.08 b	470.67±13.7 b	21.93±0.17 e	257.27±2.1

Table 4(on next page)

Effect of *Pseudomonas* sp. isolates on germination rate and vigor index of tomato seeds.

Table 4 Effect of *Pseudomonas* sp. strains on germination rate and vigor index of tomato seeds.

Treatments	Germination (%)	Vigor index
Control	70.0 ± 5.7	346.79
Pseudomonas sp. C13	70.0 ± 5.0	409.72
Pseudomonas sp. C14	83.3 ± 4.4	409.74
Pseudomonas sp. C15	76.7 ± 1.6	522.56
P. fluorescens C30	81.7 ± 4.4	455.39
P. putida ACJ14	80.0 ± 5.0	475.72
Consortium	80.0 ± 2.88	504.99

Table 5(on next page)

Qualitative tests to evaluate the growth-promoting characteristics of bacteria isolated from previously inoculated soils.

Table 5 Qualitative tests to evaluate the growth-promoting characteristics of bacteria isolated from previously inoculated soils.

Treatment	Nitrogen fixation	Phosphate solubilization	Calcium solubilization	IAA production	Fluorescence under UV light
T50%	-	-	-	-	-
T100%	-	-	-	-	-
Pseudomonas sp. C13	+	+	+	+	+
Pseudomonas sp. C14	+	+	+	+	+
Pseudomonas sp. C15	+	+	+	+	+
P. fluorescens C30	+	+	+	+	+
P. putida ACJ14	+	+	+	+	+
Consortium	+	+	+	+	+

Table 6(on next page)

Effect of *Pseudomonas* sp. isolates on tomato quality parameters.

Table 6 Effect of *Pseudomonas* sp. on tomato quality parameters.

Treatments	Firmness (Newton)	TSS (°Brix)	Titratable acidity (% Citric acid)	Lycopene (mg/g)
T50%	4.26 ± 0.15 b	6.2 ± 0.17 b	0.29 ± 0.004 b	94.40 b
T100%	5.42 ± 0.27 ab	6.9 ± 0.24 ab	0.41 ± 0.028 b	112.85 ab
Pseudomonas sp. C13	5.15 ± 0.21 ab	6.6 ± 0.29 ab	0.39 ± 0.022 ab	116.74 ab
Pseudomonas sp. C14	5.56 ± 0.25 a	7.3 ± 0.19 ab	0.39 ± 0.016 ab	130.22 a
Pseudomonas sp. C15	4.95 ± 0.16 ab	6.8 ± 0.21 ab	0.38 ± 0.017 ab	130.66 a
P. fluorescens C30	5.30 ± 0.45 ab	6.9 ± 0.3 ab	0.39 ± 0.047 ab	116.87 ab
P. putida ACJ14	5.20 ± 0.34 ab	7.0 ± 0.22 ab	0.40 ± 0.027 ab	132.29 a
Consortium	5.46 ± 0.17 ab	7.1 ± 0.29 ab	0.41 ± 0.008 a	123.53 a

TSS: Total soluble solids.

Figure 1

Effect of *Pseudomonas* sp. inoculation on tomato vegetative variables. A) Plant height; B) Stem diameter; and C) Leaf nitrogen. Values correspond to the mean of seven replicates.

Bars represent the mean ± standard error. Letters correspond to means with significant differences ($p < 0.05$).

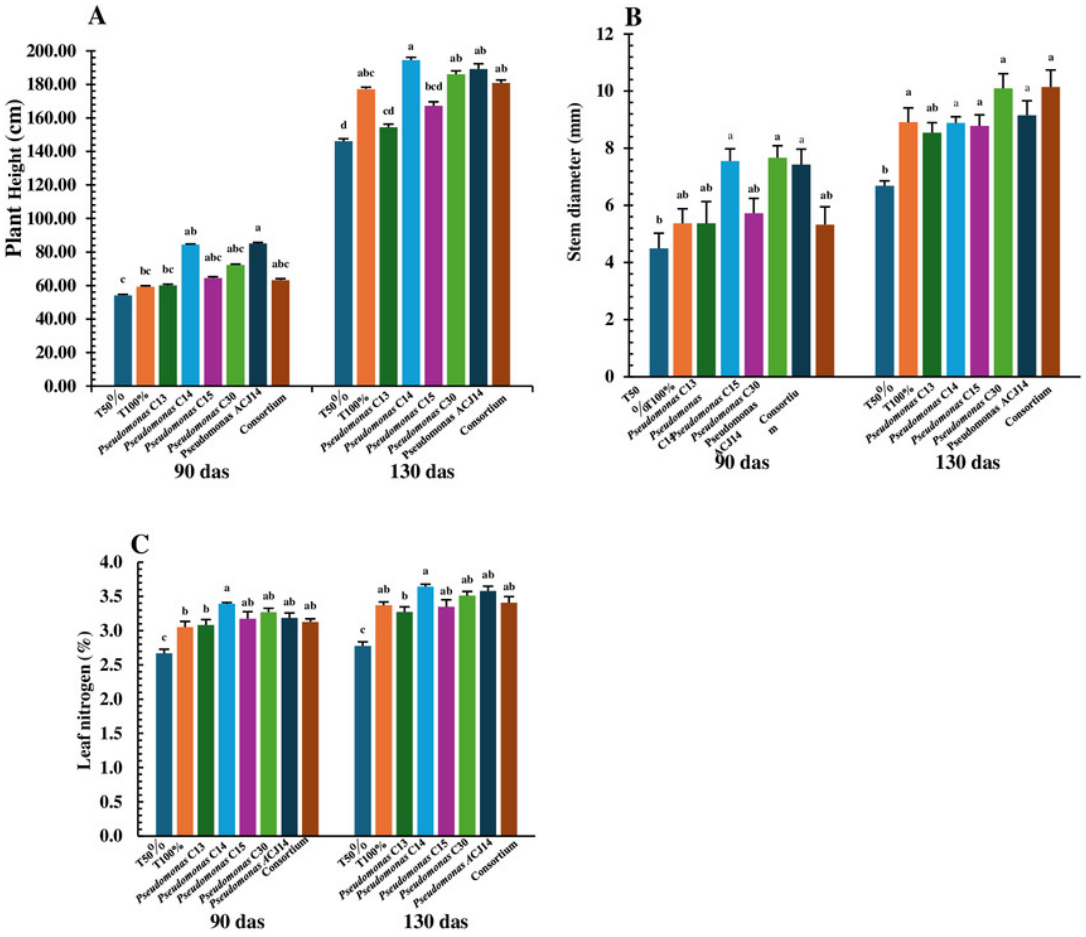
A
Plant Height (cm)
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
c bc bc ab abc abc a abc
d abc cd a bcd ab ab ab
T50% T100% Pseudomonas C13 Pseudomonas C14 Pseudomonas C15 Pseudomonas C30 Pseudomonas ACJ14 Consortium
90 das
130 das
B
Stem diameter (mm)
12
10
8
6
4
2
0
b ab ab a ab a a ab
b a ab a a a a a
T50% T100% Pseudomonas C13 Pseudomonas C14 Pseudomonas C15 Pseudomonas C30 Pseudomonas ACJ14 Consortium
90 das
130 das
C
Leaf nitrogen (%)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
c b b a ab ab ab ab
c ab b a ab ab ab ab
T50% T100% Pseudomonas C13 Pseudomonas C14 Pseudomonas C15 Pseudomonas C30 Pseudomonas ACJ14 Consortium
90 das
130 das

Figure 2(on next page)

Effect of inoculation of *Pseudomonas* sp. on the weight of the tomato root at the end of the growing cycle.

The mean of seven replicates is represented. Bars represent the mean standard error.

Letters correspond to means with significant differences ($p<0.05$).

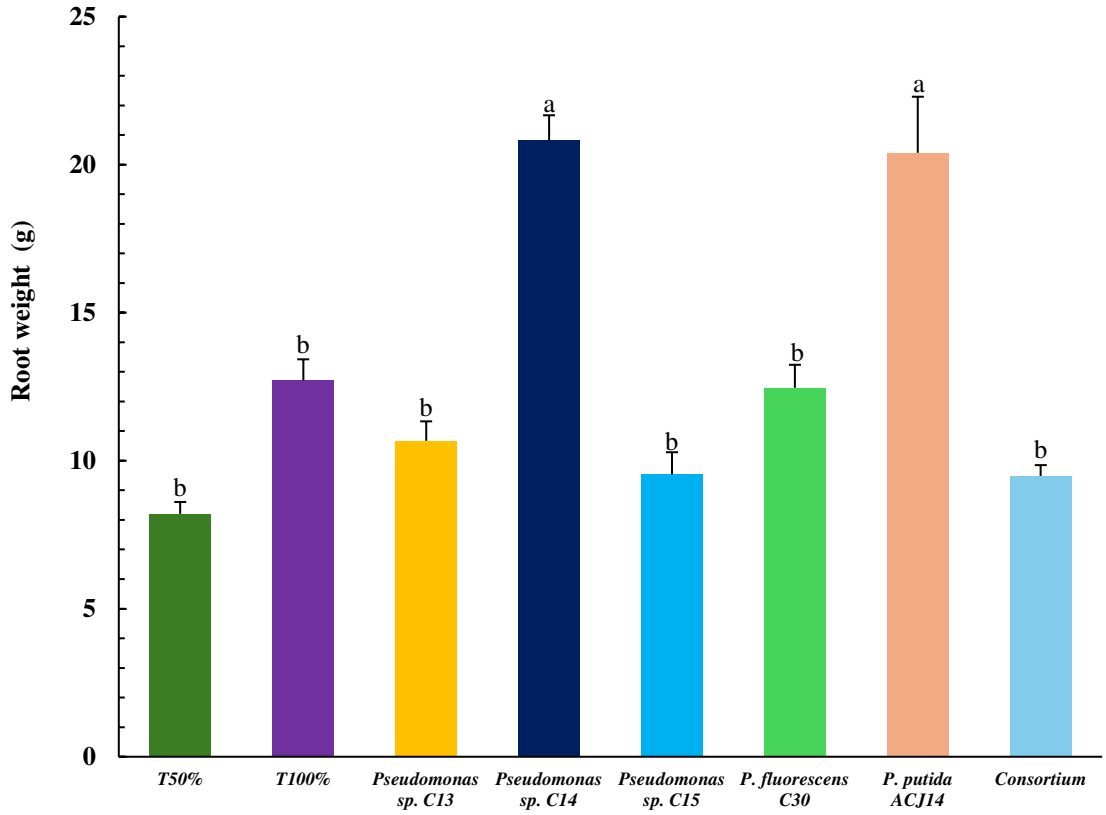
Root weight (g)
25
20
15
10
5
0
a
b
b
b
b
b
a
b
b
T50%
T100%
Pseudomonas sp. C13
Pseudomonas sp. C14
Pseudomonas sp. C15
P. fluorescens C30
P. putida ACJ14
Consortium

Figure 3

Root system of plants inoculated and not inoculated with *Pseudomonas* sp. in greenhouse tomato cultivation

Representative root of each of the treatments with *Pseudomonas* sp. inoculation.

T50%

Peudomonas sp. C15

T100%

P. fluorescens C30

Pseudomonas sp. C13

P. putida ACJ14

Peudomonas sp. C14

Consortium

Figure 4

Effect of *Pseudomonas* sp. inoculation on the yield of tomato fertilized with 50% of N, P, and Ca. The mean of seven replicates is represented

Bars depict the standard error mean. Letters correspond to means with significant differences ($p<0.05$).

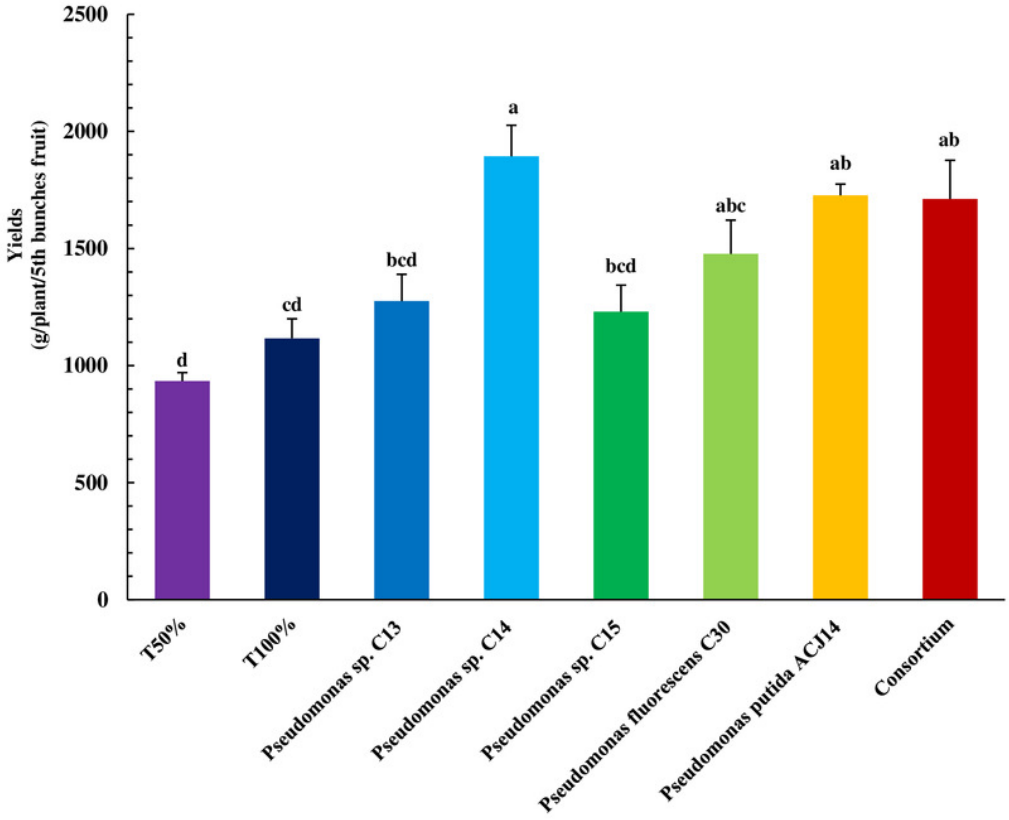
Yields
(g/plant/5th bunches fruit)
2500
2000
1500
1000
500
0
d
cd
bcd
a
bcd
abc
ab
ab
T50%
T100%
Pseudomonas sp. C13
Pseudomonas sp. C14
Pseudomonas sp. C15
Pseudomonas fluorescens C30
Pseudomonas putida ACJ14
Consortium

Figure 5

Tomato fruits harvested from plants with and without inoculation of *Pseudomonas* sp.

Representative fruits of each of the treatments with*Pseudomonas* sp. inoculation.