Article

Effect of plant growth-promoting rhizobacteria on the growth and yield of jalapeño pepper

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Abstract

Jalapeño pepper is one of the most cultivated plants, its production has decreased due to the low fertility of the soil and the presence of diseases. Plant growth-promoting rhizobacteria are alternatives to improve the growth and yield of this crop. The objective was to evaluate the effect of rhizobacteria (*Serratia marcescens*, *S. plymuthica*, *S. liquefaciens* and *Arthrobacter* sp.) on the growth of jalapeño pepper. The experiment was established in 2020, in Gómez Palacio, Durango, Mexico. The effect of rhizobacteria was tested at the *in vitro*, seedbed and pot level, completely randomly designed with 15 and 8 repetitions. The variables evaluated were height, number of leaves, leaf area, chlorophyll content index, dry biomass in root, leaves and stems, number and size of fruits. The treatments evaluated were absolute control, fertilized control and the four rhizobacteria. The substrate used was a mixture prepared based on peat moss (Premier®), perlite (Agrolita®) and vermiculite (Agrolita®) with a ratio of 1:1:1 (w/w/w), the soil had a pH of 8.3, 2.2% of organic matter, 61.5 mg kg^{-1} of nitrogen (NO_3) and 20.2 mg kg^{-1} of phosphorus. The rhizobacterium *S. plymuthica* allowed greater height and leaves in seedlings (*in vitro* and seedbed), but under pot conditions, *S. marcescens* showed better height, number of leaves, leaf area and plant biomass, *S. plymuthica* increased the amount of chlorophyll, and finally *S. liquefaciens* increased the number and size of fruits. Rhizobacteria can improve the growth and yield of jalapeño pepper.

Keywords: *Arthrobacter* sp., *Capsicum annuum* L., *Serratia liquefaciens*, *Serratia marcescens*, *Serratia plymuthica*.

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Introduction

The genus *Capsicum* is one of the most cultivated and produced species worldwide (Antonio *et al*., 2018), belongs to the *Solanaceae* family, they are herbaceous plants of annual and perennial behavior, it is native to Mexico, archaeological evidence has made it possible to estimate that it is cultivated since 9000 BC, in the regions of Tehuacán, Puebla and Ocampo, Tamaulipas (García-Jiménez *et al*., 2018). In 2020, chili production worldwide was more than 40.2 Mt and Mexico ranked second with more than 2.8 Mt, surpassed only by China with more than 16.9 Mt (FAOSTAT, 2022).

The great diversity of uses of jalapeño pepper has highlighted its socioeconomic importance, this fruit is used fresh, dried or powdered, being one of the basic condiments in Mexican families (Campos *et al*., 2022). It is consumed in sauces, in mole, in fillings, in slices and in condiments for various foods (Pérez-Vargas *et al*., 2017; Campos *et al*., 2022). In addition, it has nutraceutical properties as it produces capsaicin, phenols, flavonoids, antioxidants, protein and other health-beneficial compounds (Espinosa-Palomeque *et al*., 2020; Medina *et al*., 2022; Campos *et al*., 2022), mainly for the treatment of cardiovascular diseases, cancer and diabetes (Chamikara *et al*., 2016; Parvez, 2017; Bonaccio *et al*., 2019).

More than 40 varieties of chili are grown in the country, and the following stand out: jalapeño, serrano, bell pepper, poblano, chilaca, Anaheim, mirasol, soledad, de árbol and piquín (SIAP-SAGARPA, 2022). The production of jalapeño pepper can be limited by various factors, with the following standing out: diseases by fungi, bacteria and viruses (Morra and Bilotto, 2015; Hernández-Huerta *et al*., 2021), low soil fertility, frost conditions, pests and little government support, causing a decrease in low production, economic losses and low interest in the crop (Galindo, 2007; Sánchez-Toledano *et al*., 2021).

Fungi that affect the chili culture are *Phytophthora capsici*, *Fusarium* sp., *Sclerotiorum* sp., *Rhizoctonia solani* and *Pythium*, causing Damping off or wilting (Morra and Bilotto, 2015; Hyder *et al*., 2021). To counteract the problems of fertility in soils and the presence of diseases, producers rely on the use of chemicals, which, by making excessive use of these, cause pollution in ecosystems and the environment, resulting in soil salinity, eutrophication of water and the accumulation of nitrites and nitrates, which can be a source of environmental pollution and a threat to human health (Castellanos *et al*., 2017).

To reduce the excessive use of chemicals and avoid soil contamination, there are environmentally friendly and sustainable alternatives; for example, using beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPR). PGPR naturally inhabit the rhizosphere of plants, they can be grown and inoculated as biofertilizers due to their ability to promote plant growth, through mechanisms such as increased mobilization and absorption of nutrients, especially N, P and K (Dahiya *et al*., 2019; Etesami and Adl, 2020), biological control of pathogens and production of plant growth regulators (phytohormones) (Yadav *et al*., 2015; Chauhan *et al*., 2015).

The strains used in this work were identified as *Serratia marcescens* (nitrogen fixer), *S. plymuthica* (it solubilizes phosphorus), *S. liquefaciens* (it produces indoleacetic acid) and *Arthrobacter* sp. (it produces indoleacetic acid), with GenBank accession numbers KX259560, KX259564, KX259559 and KX258420 (González *et al*., 2017). These rhizobacteria have been studied as growth promoters in poblano pepper (González *et al*., 2017; Quiroz-Sarmiento *et al*., 2019), but they have not been studied in seedlings and plants of jalapeño pepper, the objective of this work was to evaluate the effect of previous strains as PGPR on the growth and yield of plants in this crop.

Materials and methods

Establishment of the experiment

The study was carried out at the Faculty of Agriculture and Zootechnics, of the Juárez University of the State of Durango, located at km 32 of the Gómez Palacio-Tlahualilo Road, of the Venecia ejido, municipality of Gómez Palacio, Durango, Mexico, with geographical coordinates 25° 78’ 60” north latitude and 103° 35’ 07” west longitude, altitude of 1 110 m. An experiment was established at the greenhouse level, using four strains of plant growth-promoting rhizobacteria (PGPR) from rhizospheric soil of the poblano pepper culture, sampled in the Sierra Nevada, Puebla, Mexico, identified as *Serratia marcescens*, *S. plymuthica*, *S. liquefaciens* and *Arthrobacter* sp. (González *et al*., 2017).

The greenhouse used was of metal structure covered with white plastic and 50% shade mesh, with an average temperature of 22 °C (minimum of 9 °C and maximum of 35 °C). Before carrying out the experiment in the greenhouse, the effect of PGPR on jalapeño pepper seedlings was tested at the *in vitro* (Petri dishes) and seedbed levels, in which five treatments (SM, SP, SL, AB and control) and 15 repetitions were tested, evaluating the variables height and number of leaves; the first was measured with a rule graduated in cm and the second variable visually. In these experiments, peat moss, perlite and vermiculite (ratio 1:1:1 w/w) were used as substrate, sterilized in autoclave at 18 lb of pressure for 6 h.

The seed used was jalapeño pepper collected in the same faculty, they were disinfected before sowing with 1% chlorine for 3 min and washed three times with distilled water. Once the seed went through the disinfection process, they were sown in the Petri dishes and in the seedbed (germinating tray with 288 cavities). Eight days after germination, with heights of 2 cm (±0.5), the seedlings were inoculated with the PGPR, adding 1 mL of the nutritive broth medium, which contained the bacterial load of 10^9 CFU ml of liquid medium (quantification by serial dilution).

The experiment was established in a greenhouse, the effect of PGPR on the growth and yield of jalapeño pepper was evaluated, so the seedlings were transplanted into pots of black plastic bag of 15 x 30 cm, previously filled with approximately 5 kg of soil. The physical-chemical characteristics of the soil were analyzed according to the official Mexican standard (NOM-021-RECNAT-2000), having a moderately alkaline pH (8.4) (soil-water ratio 1:2), organic matter of 2.2% (Walkley and Black), total nitrogen of 0.09% (micro Kjeldhal), inorganic N of 61.5 mg kg^{-1}, available phosphorus of 20.2 mg kg^{-1} (Bray and Kurtz), extractable potassium and calcium of 12 404 and 375 mg kg^{-1} (saturation extract), the texture was loamy-clayey (Bouyoucos).

The experimental design was completely randomized with six treatments [absolute control (AC), fertilized control (FC), *Serratia marcescens* (SM), *S. plymuthica* (SP), *S. liquefaciens* (SL) and *Arthrobacter* sp. (AB)] and 15 repetitions each. The seedlings were transplanted after 57 days of sowing in seedbeds, duly inoculated with the rhizobacteria and processed according to this section, the heights at the time of transplantation were 6.5 (AC), 6.7 (FC), 6.7 (AB), 6.5 (SP), 7.6 (SL) and 7.4 cm (SM). Two seedlings per pot were transplanted, removing one of them later, these were watered every third day. Fertilization in this treatment was based on the dose of 120 kg N ha^{-1} in three applications.

Characteristics of plant growth-promoting rhizobacteria

Serratia marcescens (SM) is a strain with the capacity to fix atmospheric nitrogen, with accession number to GenkBank KX259560, inhibits *Phytophthora capsici* by 44% and *Fusarium* sp. by 5%. The strain *S. plymuthica* (SP) solubilizes phosphorus in an amount of 744 µg ml^{-1}, inhibits the development of *P. capsici* by 42% and *Fusarium* sp. by 37%. Its accession number to GenBank is KX259564 (González *et al*., 2017).

S. liquefaciens (SL) have the ability to produce auxins (indoleacetic acid 23.9 µg ml^{-1}), inhibits the development of *P. capsici* by 34.5% and *Fusarium* sp. by 39.4%, with accession number to GenBank KX259559. Finally, *Arthrobacter* sp. (AB) is a rhizobacterium with the capacity to produce auxins in 22.5 µg ml^{-1}, inhibits 44.6% of *P. capsici* and 12% of *Fusarium* sp., its accession number to GenBank is KX258420 (González *et al*., 2017). Before their inoculation, the strains were reactivated in solid nutritive Agar medium and sown in Petri dishes by cross streaking and incubating at 28 °C.

Variables evaluated

At 121 days after transplantation (DAT), the experiment was completed and the following variables were evaluated: height, number of leaves, leaf area, dry biomass (in root, stems and leaves), chlorophyll content index, number, length and width of fruits. The seedling height was measured with a ruler graduated in cm, from the root neck to the apex of the plant, the number of leaves was visually quantified on each of the plants. The leaf area was determined with a LI-COR area meter, model LI-3100, at the end of the experiment the leaves were separated and passed in the band of the leaf area meter, the production of dry biomass in root, stems and leaves was obtained by separating the indicated parts and they were put on brown paper, subsequently, they were subjected in a drying oven at 70 °C for 72 h, the dry weight was measured with an AdventurePro analytical balance.

To determine the chlorophyll content index (CCI) in the plants, a chlorophyll meter (Fluorimeter) of the Opti-Sciences brand (CCM-200 Plus) was used. The number of fruits was quantified visually, this variable was determined at the beginning of fruiting and as the only measurement, because the winter period began, the diameter and length of the fruits were determined using an electronic vernier graduated in mm.

Statistical analyses

The data obtained from the variables studied were subjected to a test of normality and homogeneity of variances, the variables that did not comply with these tests were transformed to a natural logarithm, then an analysis of variance and a comparison of means according to the Tukey test ($p \leq 0.05$) were performed. All data were analyzed with the help of the statistical package SAS 2002, recommended for Windows, version 9.0.

Results and discussion

Effect of PGPR on seedling growth

The results indicated that the height and number of leaves in jalapeño pepper seedlings sown at the *in vitro* level (Petri dishes) and in seedbeds were statistically different depending on the inoculated strains (Tukey, $p \leq 0.05$) (Figure 1). At the *in vitro* level, the seedlings inoculated with the *Serratia plymuthica* (SP) strain showed greater height with 8 cm, followed by treatment with *S. marcescens* (SM) with a height of 7.8 cm. The non-inoculated plants (control) showed lower height with 5.8 cm, the increases in height of the treatment with SP were 38% more compared to the height of the seedlings in the control.

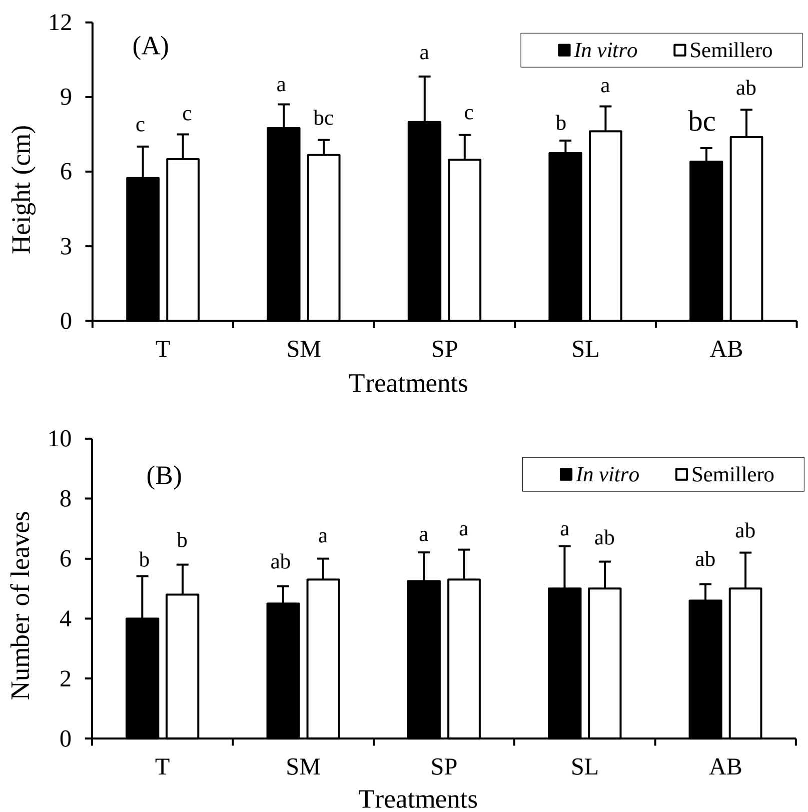

Figure 1. Height (A) and number of leaves (B) in jalapeño pepper seedlings (*in vitro* and seedbed), according to the effect of different rhizobacterial strains. SM= *Serratia marcescens*; SP= *S. plymuthica*; SL= *S. liquefaciens*; AB= *Arthrobacter* sp., T= control. Different letters on the bars indicate significant statistical differences (Tukey, $p \leq 0.05$); n= 15 ± standard error.

In seedbed, *S. liquefaciens* (SL) was better, it showed an average height of 7.6 cm per seedling, the control showed again lower height with 6.5 cm. The increases in SL with respect to the control were 17% more in height (Figure 1A). The number of leaves in seedlings was also different according to the inoculated PGPR, a greater number of leaves was found in seedlings treated with SP, both *in vitro* and in seedbeds, both with 5.3 leaves (Figure 1B). The increases were 33 and 10% more in number of leaves compared to seedlings (*in vitro* and seedbed) without microbial inoculation.

The positive effects of these strains have not been reported with jalapeño pepper, but with poblano pepper (González *et al*., 2017) reported increases of 15, 30 and 11% more in height, leaf area and production of dry biomass in seedlings when they inoculated with *S. plymuthica*, with this same strain, but in jalapeño pepper, this work reported an increase of 38% in height. Another comparable work, in which they evaluated different strains of PGPR in poblano pepper seedlings, found the best effects when inoculating *S. plymuthica* and *S. liquefaciens*, representing increases of up to 28 and 26% in height (13.3 cm) and number of leaves (7.2), in addition to increasing the leaf area and the production of dry biomass (Quiroz-Sarmiento *et al*., 2019). The positive effects found in height and number of leaves due to bacterial strains are possibly related to their ability to solubilize phosphorus (SP, 744 μg ml^{-1}), produce auxins (SL, 23.9 μg ml^{-1}) and inhibit *Fusarium* sp. and *Phytophthora capsici* (González *et al*., 2017).

Effects of PGPR on jalapeño pepper growth and yield

The inoculation of PGPR in jalapeño pepper plants, transplanted into pots, influenced the growth and yield of greenhouse-grown plants. The results indicated that the height and number of leaves were statistically different between the treatments (Tukey, $p \leq 0.05$) (Table 1). At the end of the experiment, greater height was found in plants inoculated with *Serratia marcescens* (SM), with an average of 32 cm (Tukey, $p \leq 0.05$) (Table 1); the lowest value was found in the treatments with *S. plymuthica* (SP) and *S. liquefaciens* (SL), plants with 21 cm respectively. The final height in the plants inoculated with SM represented 45% more than the height found in the absolute control (AC, 22 cm), on the other hand, compared to the fertilized control (FC, 24 cm), 33% more in height of the plants was found.

Table 1. Effect of plant growth-promoting rhizobacteria in height, number of leaves, leaf area and chlorophyll content index (CCI) in jalapeño pepper plants.

Treatments	Abbreviation	Height (cm)	Leaves	Leaf area (cm^2)	Chlorophyll (CCI)
Absolute control	AC	22 (±4) bc	24 (±7) c	574 (±65) bc	36 (±16) c
Fertilized control	FC	24 (±6) b	27 (±10) bc	486 (±103) c	39 (±12) bc
Arthrobacter sp.	AB	23 (±6) bc	38 (±14) ab	490 (±245) c	38 (±14) bc
Serratia plymuthica	SP	21 (±2) c	31 (±13) bc	609 (±203) bc	52 (±10) a
Serratia liquefaciens	SL	21 (±2) c	29 (±7) bc	688 (±143) ab	48 (±9) ab
Serratia marcescens	SM	32 (±6) a	45 (±16) a	782 (±151) a	40 (±14) bc

Different letters in the columns indicate significant statistical differences (Tukey, $p \leq 0.05$). n= 8, ± standard error.

Higher leaf production was observed in plants inoculated with PGPR, the highest value (45 leaves) was found in plants inoculated with SM, followed by plants inoculated with AB, obtaining on average 38 leaves per plant. Again, plants without microbial inoculation showed the lowest values, with 24 (AC) and 27 (FC) leaves per plant. The number of leaves found with the SM strain, at the end of the experiment, represents 88% more than the number of leaves found in plants without bacterial inoculation (AC, 24 leaves) and 67% more compared to fertilized plants (FC, 27 leaves) (Table 1).

The leaf area of the plants increased in three of the four inoculated strains, these being statistically different from the other treatments (Tukey, $p\leq 0.05$) (Table 1). The results showed greater leaf area with 782 cm^2 in plants treated with SM, the fertilized control obtained less leaf area with 486 cm^2. Inoculation with SM produced 61% more leaf area compared to the fertilized control and 36% more than the AC, which presented 574 cm^2.

The chlorophyll content index (CCI) was statistically different between the different treatments evaluated (Tukey, $p\leq 0.05$) (Table 1). The plants treated with *Serratia plymuthica* had greater chlorophyll with 52 units of CCI, the absolute control had less chlorophyll with 36 CCI units. The chlorophyll content in plants with SP represents 44 and 33% more than the CCI found in the AC (36 CCI) and FC (39 CCI) treatments.

The production of dry biomass in leaves (DBL), root (DBR) and stem (DBS) showed significant statistical differences due to the effect of the inoculation with PGPR (Tukey, $p\leq 0.05$) (Figure 2). The highest value in DBL (8519 mg), DBR (2690 mg) and DBS (5594 mg) was found in plants inoculated with the *Serratia marcescens* strain; the lowest values were found in the AC (3 553, 1 218 and 1 783 mg of DBL, DBR and DBS) and FC treatments (3 056, 1 406 and 1 702 mg of DBL, DBR and DBS), respectively. The increases in the production of total dry biomass, found in plants with SP (16 803 mg), were 156 and 172% more compared to the total biomass found in the AC (6 554 mg) and FC treatments (6 164 mg).

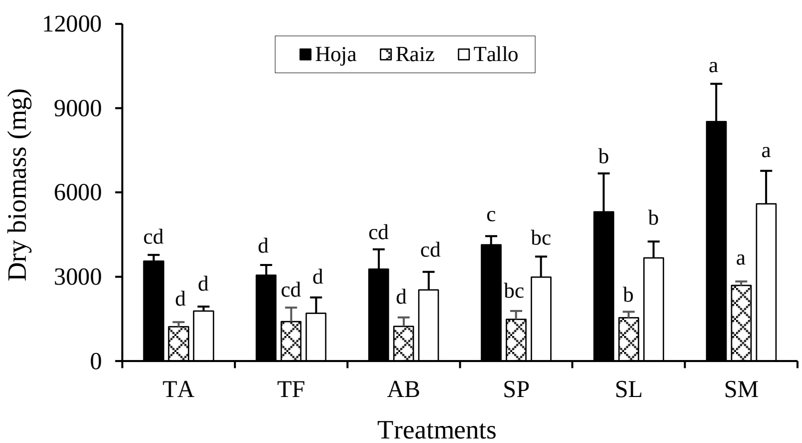

Figure 2. Production of dry biomass in leaves, stem and root, in jalapeño pepper plants according to the effect of different rhizobacterial strains. SM= *Serratia marcescens*; SP= *Serratia plymuthica*; SL= *Serratia liquefaciens*; AB= *Arthrobacter* sp., T= control. Different letters on the bars indicate significant statistical differences (Tukey, $p\leq 0.05$). n= 8 ± standard error.

Serratia marcescens managed to increase the variables height, number of leaves, leaf area and production of dry biomass in root, leaves and stems, this bacterium is a ubiquitous microorganism, capable of surviving in multiple environments (Adeolu *et al*., 2016). The effects of this rhizobacterium have been reported as plant growth promoters for its antifungal effect (Troskie *et* *al*., 2014; González *et al*., 2017) and for improving the growth and development of agricultural crops, mainly *Capsicum annuum* (Amaresan *et al*., 2012; González *et al*., 2017; Ahmed *et al*., 2022). The strains of PGPR used in this work have the ability to produce auxins and solubilize phosphates, in addition, they have the ability to inhibit the development of phytopathogens such as *Fusarium* sp. and *Phytophthora capsici* (González *et al*., 2017), so their positive effects on growth may be related to these attributes.

In a study conducted by Amaresan *et al*. (2012), by inoculating various species of PGPR, including *S. marcescens*, in *Capsicum annuum* grown in pots, they found a greater number of primary and secondary roots, greater height and better production of dry biomass; in this regard, *S. marcescens* represented 56 and 43% more height (19.9 cm) and production of aerial dry biomass (0.02 g) compared to the control treatment (12.8 cm height and 0.014 g of dry biomass), the authors conclude that this improvement in growth is due to the fact that this strain can inhibit *Sclerotium rolfsii*, *Colletotrichum capsici*, *Fusarium oxysporum* and *Pythium* sp., in addition to its ability to produce siderophores, solubilize phosphates and produce indoleacetic acid.

In another study, by inoculating *Bacillus subtilis* in bell pepper, it demonstrated the inhibition of *Phytophthora capsici*, managing to increase the height (32.02 cm) and the production of dry biomass (8.75 g), these variables increased by 5 and 13% more compared to plants without microbial inoculation, which were 30.45 cm tall and had 7.78 g of dry biomass (Irabor and Mmbaga, 2017). The inoculation of SM in oat culture, under pot conditions, improved 45 and 28% the height and fresh weight of plants (Liu *et al*., 2015), in the production of *Camellia sinensis* tea, increases of up to 54% in the production of dry biomass (between 5 and 10 g) were reported when inoculated with SM with respect to non-inoculated plants (1.3 and 6.5 g) (Chakraborty *et al*., 2013).

The number of fruits produced per plants were relatively low, because the experiment was still in process and the climate was not favorable for fruiting, this due to the cold of winter, however, the production of fruits per plant and their size (length and width) presented significant statistical differences between the treatments (Tukey, $p \leq 0.05$) (Table 2). The highest value was obtained with the inoculation of SL, producing 5.3 fruits per plant, while the absolute control (AC) and the fertilized control (FC) showed fewer fruits, with 2.7 and 2.3, respectively.

Table 2. Effect of plant growth-promoting rhizobacteria on the number of fruits, length and diameter of the fruit of jalapeño pepper.

Treatments	Abbreviation	Fruits	Fruit length (mm)	Fruit width (mm)
Absolute control	AC	2.7 (±0.6) bc	57 (±0.9) a	20 (±1) b
Fertilized control	FC	2.3 (±0.7) c	57 (±0.7) a	21 (±0.6) ab
Arthrobacter sp.	AB	3.7 (±1) b	56 (±0.6) a	23 (±0.7) ab
Serratia plymuthica	SP	3 (±0.5) bc	60 (±1.1) a	24 (±1.1) a
Serratia liquefaciens	SL	5.3 (±1.1) a	62 (±1) a	21 (±0.8) ab
Serratia marcescens	SM	3 (±0.9) bc	59 (±1) a	22 (±1) ab

Different letters between the columns indicate significant statistical differences (Tukey, $p \leq 0.05$). n= 8. ± standard error.

The size of the fruits varied between treatments, the length of the fruit was better when the PGPR SL and SP were inoculated, both with 62 and 60 mm in length, although no significant statistical differences were found, SL represented an increase of 9 and 11% in fruit length compared to the plants of AC (57 mm) and FC (57 mm). The highest value, in the width of the fruit, was found in the plants treated with the SP and AB rhizobacteria, both with 24 and 23 mm, respectively, the lowest values were found in the plants without microbial inoculation with 20 (AC) and 21 mm (FC) (Table 2). The width obtained with SP was 20 and 14% more compared to the width of fruit found in AC and FC.

Inoculating crops with PGPR substantially reduces the use of synthetic fertilizers and negative impacts on the soil, increases crop growth and yields, contributing to food security, sustainable production and the lowest risk to human health (Odoh, 2017). The results of this work showed that the inoculation of PGPR improved the growth of the chili plant, the yield and the size of the fruit, so they can be used in the production of plants, both in seedbed and in greenhouse. Similar results, but with different PGPR, were found by Gou *et al*. (2020), by inoculating *Bacillus* sp. and *Pseudomonas* sp. in *Capsicum annuum*, they reflected an increase in the previous variables; Raheem *et al*. (2018) under tests in pots with wheat (*Triticum aestivum* L.), found increases in yield of 34% when inoculated with *B. amyloliquefaciens*.

Irabor and Mmbaga (2017), by inoculating *Bacillus subtilis* in bell pepper, found an increase in the chlorophyll content and in the number of fruits; the reported chlorophyll content (8.96) did not show statistical differences; however, they were higher than in plants without microbial inoculation (7.24), representing increases of 24% more the CCI compared to the control. Regarding the number of fruits, the inoculated plants (two fruits) were better, representing an increase of 166% more than the control (0.75 fruits).

Conclusions

The rhizobacteria *Serratia plymuthica* and *S. marcescens* improved the height and number of leaves on jalapeño pepper seedlings in the first experiments (*in vitro* and seedbed), in pot conditions under greenhouse the rhizobacteria *S. marcescens* and *S. liquefaciens* stood out, the first increased the growth of the plants and the second showed greater number and size of the fruits, in addition it was observed that *S. plymuthica* induced greater size in the width of the fruit. These rhizobacteria can be alternatives to implement their use as biofertilizers in jalapeño pepper cultures.

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