

## Influence of rhizobacteria in production and nutraceutical quality of tomato fruits under greenhouse conditions

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### Abstract

An alternative in organic agriculture is the use of biofertilizers base rhizobacteria promoting plant growth and organic fertilizers “plant growth promoting rhizobacteria (PGPR) by its acronym in English”. The objective of the present work was to evaluate the effect of the inoculation of PGPR (*Bacillus* sp., *Aeromonas* sp. and *Pseudomonas lini*), using two substrates: S1= compost + river sand + perlite, and S2= river sand and as witnesses both substrates without PGPR (total of eight treatments), on the yield and quality of tomato fruits produced in the greenhouse. The experimental design used was completely randomized with three repetitions in a factorial arrangement (2 × 4), where factors A and B were: a) substrates and b) PGPR. The results indicate that the substrate S1 increased the contents of SST, lycopene, total sugars, ascorbic acid and the percentage of citric acid in tomato fruits. The inoculation of the strain *Bacillus* sp., Produced the highest contents of SST, lycopene and ascorbic acid in tomato fruits. Based on the set of responses in tomato fruits developed with different substrates and PGPR, the best treatment was T<sub>1</sub> (*Bacillus* sp. + S1) which increased by 17.54, 8.77, 17.34, 31.31 and 11.52%, yield, contents of SST, lycopene, reducing sugars and ascorbic acid, respectively, in relation to the rest of the treatments. Therefore, the strain *Bacillus* sp. and the substrate base compost could be an alternative, because they improve the nutraceutical quality of fruits, without diminishing the yield of tomato in the greenhouse.

**Keywords:** *Solanum lycopersicum* L., biofertilizers, compost, lycopene, PGPR.

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## Introduction

The tomato (*Solanum lycopersicum* L.) is one of the main crops worldwide, because the fruit of this vegetable is an important component in the daily diet of the population of many countries since it is a source of antioxidants, such as vitamins A, C and E, carotenoids, flavonoids, lycopene and phenolic compounds (Dorais *et al.*, 2001; George *et al.*, 2004). These molecules are able to counteract free radicals and inhibit DNA oxidation, thus avoiding some types of cancer, preventing blockages in the arteries, as well as the degradation of the nervous system and aging (Waliszewski and Blasco, 2010). Currently, the tendency of consumers is to prefer foods free of the use of pesticides and inorganic fertilizers, innocuous and with high nutritional value (Marquez-Hernández *et al.*, 2013).

Derived from the above, there is evidence that the use of biofertilizers base rhizobacteria promoting plant growth, plant growth promoting rhizobacteria (PGPR) by its acronym in English (Kloepper and Schroth, 1978)] (Ashrafuzzaman *et al.*, 2009) and substrates organic compost base, can partially or totally replace the supply of inorganic pesticides and fertilizers both in open field production systems and protected conditions (López *et al.*, 2001; Marquez-Hernández *et al.*, 2013). In addition, these alternatives strengthen the focus of organic agriculture (Pretty, 2008).

The PGPR are able to colonize the root system of plants and perform various mechanisms involved in promoting the growth and yield of plant species; these mechanisms are classified as direct and indirect. The direct mechanisms are those where these microorganisms stimulate the development of plants, through the production of growth regulators (auxins, cytokinins, gibberellins, abscisic acid), biological nitrogen fixation, solubilization and mineralization of phosphates (Ahemad and Kibret, 2013; Pii *et al.*, 2015).

While the indirect mechanisms are carried out when the PGPR are able to inhibit the growth of one or more phytopathogenic microorganisms, due to the synthesis of antibiotics or siderophores (Vessey, 2003, Ortiz-Castro *et al.*, 2014), together these mechanisms have the potential to improve the quality of the fruits and the efficiency of the supply of synthetic fertilizers and pesticides (Kloepper *et al.*, 2004). Certain bacterial genera are the most commonly used in agriculture such as: *Acinetobacter* spp., *Aeromonas* spp., *Azospirillum* spp., *Bacillus* spp., *Erwinia* spp., *Flavobacterium* spp., *Burkholderia* spp., *Pseudomonas* spp., *Rhizobium* spp., *Serratia* spp., among others (Beneduzi *et al.*, 2008; Esitken *et al.*, 2010).

On the other hand, compost as an organic substrate provides considerable amounts of nutrients that could satisfy the demand of the crops, its application entails an improvement in the physical and chemical properties of the substrates, which is reflected in a better growth, development and higher yields of vegetable crops (Marquez-Hernández *et al.*, 2006). According to Marquez *et al.* (2008) mixing the compost with inert media improves its physical and chemical characteristics of growth substrates avoiding hypoxia, in this sense, it is allowed to assume that the application of compost, in addition to satisfying the nutritional demand of crops, favors the antioxidant quality and activity of the fruits. Additionally, tomato production under greenhouse conditions is an option to increase production, compared to open field (Marquez-Hernández *et*

*al.*, 2013). In protected production systems, a higher yield and an improvement in the quality of the products are obtained, as well as an efficient use of fertilizers and water (Moreno *et al.*, 2011). The objective of the present work was to evaluate the effect of the inoculation of *Bacillus* sp., *Aeromonas* sp. and *Pseudomonas lini* using two substrates based on compost or river sand, on the performance and nutraceutical quality of greenhouse tomato fruits.

## Materials and methods

The experiment was carried out, in the Spring-Summer cycle, 2015, under greenhouse conditions, at the Antonio Narro Autonomous Agrarian University in Torreon, Coahuila, Mexico (25° 05' and 26° 54' north latitude, 101° 40' and 104° 45' west longitude, at an altitude of 1139 m) (Schmidt, 1989). The greenhouse has an area of 200 m<sup>2</sup>, is semicircular in shape, with reinforced acrylic cover, gravel floor and automatic cooling system with wet wall and extractors, the minimum and maximum temperature inside the greenhouse fluctuated between 17.4 and 32.6 °C respectively, while the minimum and maximum relative humidity oscillated between 30 and 70%.

The three PGPR used as inoculants were; *Bacillus* sp., *Aeromonas* sp. and *Pseudomonas lini* (Palacio-Rodríguez *et al.*, 2017), which were obtained from the microbial collection of the Microbial Ecology Laboratory of the Faculty of Biological Sciences of the Juárez University of the State of Durango, Gomez Palacio, Durango, Mexico. For the preparation of the bacterial inocula, the three strains were individually inoculated in Luria Bertani<sup>®</sup> liquid medium and then placed in a shaking incubator of 200 rpm (Precisión Scientific 815<sup>®</sup>) for 24 h at 30 °C, the bacterial concentrations were adjusted to 1 × 10<sup>8</sup> UFC mL<sup>-1</sup> with phosphate buffered saline (PBS) at 0.5x.

The vegetal material that was used was tomato cv. Aphrodite, an indeterminate type of saladette, which was planted in 200-well polystyrene trays using Peat moss (Premier<sup>®</sup>) as a substrate. These were placed in black polyethylene bags for 72 h, applying a spray every 24 h to drain. The inoculation of the bacterial strains was carried out 12 days after the emergence of the seedlings, by means of the immersion method, during a period of 5 min, in a bacterial suspension of 4 L, with a concentration of 1 × 10<sup>8</sup> UFC mL<sup>-1</sup>, whereas the control treatments were only supplied with distilled water.

The substrates evaluated consisted of different percentages of compost, river sand and perlite: substrate 1 (S1)= 50% compost + 40% river sand + 10% perlite and substrate 2 (S2)= 100% river sand. The chemical composition of the substrates is presented in Table 1. From the interaction of the substrates × PGPR the following treatments were formed: T<sub>1</sub>: *Bacillus* sp. + S1; T<sub>2</sub>: *Aeromonas* sp. + S1; T<sub>3</sub>: *P. lini* + S1; T<sub>4</sub>: without PGPR + S1 (control 1); T<sub>5</sub>: *Bacillus* sp. + S2; T<sub>6</sub>: *Aeromonas* sp. + S2; T<sub>7</sub>: *P. lini* + S2 and T<sub>8</sub>: without PGPR + S2 (control 2). The transplant was carried out 46 days after sowing, when the plants presented an average height of 15 cm, establishing a plant by pots that consisted of a black polyethylene bag with a capacity of 18 L, which were filled with the substrates corresponding.

**Table 1. Chemical analysis of compost and river sand used as growth medium of tomato cv. Aphrodite in the greenhouse.**

Substratum	N	P	K	Ca	Mg	Na	Fe	Zn	Mn	pH	CE <sup>†</sup>
	(mg kg <sup>-1</sup> )										(dS m <sup>-1</sup> )
Compost	120.1	42	610.6	90	85	3	7.5	5.1	4.1	8.56	6.7
River sand	1.15	11.2	100.2	45	4.3	0.17	5.75	0.7	4.43	7.5	0.65

†= electric conductivity.

The pots were placed in a double row with a separation of 1.6 m between the row, with a staggered arrangement, at a separation of 0.3 m, the density of sowing was four plants per square meter. The river sand used in all treatments was washed and sterilized with a 5% solution of sodium hypochlorite, then washed and dried in the environment for three days. The development of cultivation was to a single stem, with weekly pruning and the phytosanitary control was made in a preventive way, applying Cinna-Mix<sup>®</sup>, approved input for organic products (IFOAM, 2003). The pollination was carried out daily between 11:00 and 14:00 h at the beginning of the flowering and until the mooring of the fifth cluster, mechanically with an electric vibrator.

The volume of irrigation water was supplied to the pots according to the phenological stages of the crop, from four days after the transplant (DDT) 0.5 L of water pot<sup>-1</sup> day<sup>-1</sup> was applied, later it was increased to 0.8 and 1.9 L pot<sup>-1</sup> day<sup>-1</sup>, at 30 and 71 ddt, respectively. The nutritive solution used for the treatments without inoculation was the one recommended by Castellanos and Ojodeagua (2009). The nutritional demand of the crop for the treatments inoculated with the PGPR was covered using Maxifrut and Maxiquel, both products of the company BioCampo<sup>®</sup>, to apply macro and micro elements, respectively.

These products have been approved by the IFOAM (2003) certified organic production standards. Of both products, mother liquors were prepared at a rate of 10 and 50 g in 20 L of irrigation water, and for the fertilization of the plants by pots dilutions of 1 and 0.5 L in 1 000 L of water were made, respectively. The dilution of the Maxifrut was applied daily and that of the Maxiquel every week.

The tomato fruits were harvested in a state of maturity between 30 and 60% to perform determinations of: total soluble solids (SST), titratable acidity (expressed as percentage of citric acid), lycopene content, vitamin C, sugar content totals and reducing sugars. The yield was obtained per plant when harvesting, from the first to the fifth bunch, the fruits of the plants of each treatment and corresponding replica. For the determination of the SST of the fruits was performed with a manual refractometer ATAGO PR-100 with a scale of 0-32%, while for titratable acidity the methodology of the AOAC (1990) was used. The content of vitamin C, expressed in milligrams of ascorbic acid 100 g<sup>-1</sup> fresh fruit (FF), was determined according to the method of the AOAC (1984). The content of total sugars was made by alcoholic extraction and quantified by the Antrona method (Witham *et al.*, 1971), obtaining for the calculations a standard curve, expressing the results in milligrams of glucose 100 g<sup>-1</sup> of FF.

The concentration of reducing sugars was quantified by the method of Nelson (1944) and Somogyi (1952) the results were expressed in milligrams of glucose 100 g<sup>-1</sup> of FF. The extraction of lycopene was carried out using the methodology proposed by Fish *et al.* (2002) using hexane, acetone, ethanol (2:1:1 v:v:v) and for the calculation of lycopene the equation proposed by Javanmardi and Kubota (2006) was used.

The experimental design used was completely randomized with three replications, with a factorial arrangement (2 × 4), where factor A corresponded to substrates, while factor B corresponded to PGPR. The data were analyzed statistically by analysis of variance and mean comparisons using the Tukey test ( $p \leq 0.05$ ) (SAS, 2004).

## Results and discussion

### Total soluble solids, percentage of citric acid, yield and number of fruits

The results indicate that the substrates used in the present work caused the tomato fruits to show significant differences in the TSS content and the percentage of citric acid ( $p < 0.05$ ), but not for the yield and number of fruits. According to the PGPR factor, no significant difference was observed in the yield, number of fruits and titratable acidity; however, it presented a highly significant difference in the SST content ( $p < 0.01$ ). In relation to the interaction substrates × PGPR, statistical significance was found in the TSS content and the percentage of citric acid ( $p < 0.05$ ), in the same way, there were highly significant differences in the yield and number of fruits ( $p < 0.01$ ). (Table 2).

**Table 2. Yield, number of fruits, total soluble solids and titratable acidity in tomato fruits by effect of different substrates and PGPR.**

Factor	Performance (kg m <sup>-2</sup> )	Number of fruits (num.)	SST (°Brix)	Titratable acidity (% of citric acid)
Substratum				
‘S1’	8.86 a	30.41 a	4.84 a	0.67 a
‘S2’	8.6 a	28.58 a	4.26 b	0.56 b
PGPR				
<i>Bacillus</i> sp.	9.84 a	29.83 a	4.9 a	0.63 a
<i>Aeromonas</i> sp.	8.72 a	30 a	4.5 b	0.64 a
<i>Pseudomonas lini</i>	7.93 a	27.33 a	4.6 b	0.61 a
Without inoculating	8.46 a	30.83 a	4.3 b	0.57 a
Substrates×PGPR				
Significance	**	**	*	*
CV (%)	16.47	14.31	4.04	8.58

Means with equal letters in a column for each factor are not statistically different (Tukey,  $p \leq 0.05$ ); SST= total soluble solids; S1= 50% compost + 40% river sand + 10% perlite; S2= 100% river sand; PGPR= plant growth promoting rhizobacteria; CV= coefficient of variation; \* = significant  $p < 0.05$ ; \*\* = highly significant  $p < 0.01$ .

The content of SST and the percentage of citric acid in the tomato fruits, were increased when using the S1 substrate, in 11.98 and 16.42% in relation to the S2 substrate, respectively, these increases in the SST could be related to the presence and availability of salts in the radical medium (Dorais *et al.*, 2001). This behavior agrees with what was pointed out by Cuartero and Fernández-Muñoz (1999) who indicate that the content of salts, present in organic fertilizers, increases the SST content in fruits. Similar results were reported by Gutiérrez-Miceli *et al.* (2007) who found a higher SST content in tomato fruits, when using compost as a source of fertilization.

In this sense, the content of SST registered in fruits of plants developed in the S1 substrate, was higher 7.6 and 12.6% at the values reported by Rodríguez *et al.* (2009) who evaluated tomato fruits developed in compost base substrate: river sand (50:50 v: v) plus the application of compost tea and Salas-Pérez *et al.* (2016) when evaluating the nutraceutical quality of tomato fruits in compost-based substrates: river sand in greenhouse, respectively. In the case of the titratable acidity variable, the highest value was found in tomato fruits from plants grown on the S1 substrate, being higher than the average of 0.027 percent of citric acid reported by Vázquez *et al.* (2015), who evaluated tomato quality and yield in the greenhouse with different proportions of compost and compost tea. Regarding the effect of the PGPR factor, the SST content was increased when the *Bacillus* sp. Strain was inoculated, registering an increase of 24.17% in relation to the treatment without inoculation. The results of SST were superior to those reported by Dursun *et al.* (2010) who found a value of 3.63 °Brix, when evaluating the application of the co-inoculant based on *Pantoea agglomerans*, *Acinetobacter baumannii* and *Bacillus megaterium* in the tomato crop.

In the Table 3 shows the interaction substrates×PGPR, where it is indicated that the highest SST content was found in the T<sub>1</sub> treatment (*Bacillus* sp. + S1) with an average of 5.36 °Brix, being higher in 17.35 and 23.51% treatments T<sub>4</sub> and T<sub>8</sub> (controls), respectively. This behavior coincides with other researchers who report that organic substrates plus the inoculation of PGPR generate fruits with higher SST content (Orhan *et al.*, 2006), this may be due to the increase in salinity in the root medium (Dorais *et al.*, 2001), has also shown an increase in the absorption of nutrients by plants when inoculated with PGPR, this increase has been attributed to the production of phytohormones in the growth medium, which stimulates the development of the roots and therefore a better absorption of water and nutrients (Ordookhani *et al.*, 2013).

The SST of the tomato fruits developed in the treatments under study are considered adequate since they exceeded the optimum value (4 °Brix) of reference for fresh consumption (Santiago *et al.*, 1998). On the other hand, the highest percentage of citric acid was reported in the treatments T<sub>2</sub> (*Aeromonas* sp. + S1), T<sub>5</sub> (*Bacillus* sp. + S2) and T<sub>7</sub> (*Pseudomonas lini* + S2). Likewise, the results of the present study coincide with those obtained by del Amor *et al.* (2008) who indicate a higher concentration of citric acid in pepper fruits (*Capsicum annuum* L.) developed in plants inoculated with *Azospirillum brasilense* and *Pantoea dispersa*, in comparison with the fruits of plants without inoculation. In general terms, the results confirm the importance of the application of biofertilizers based PGPR and the use of organic fertilizers such as compost on the quality of the tomato fruit.

**Table 3. Effect of substrate interaction×PGPR on the production and quality of tomato fruits developed under greenhouse conditions.**

Treatment	Number of fruits (num.)	Performance (kg m <sup>-2</sup> )	SST (°Brix)	Titrateable acidity (% citric acid)
T <sub>1</sub> - <i>Bacillus</i> sp. + S1	35 a	11.86 a	5.36 a	0.7 ab
T <sub>2</sub> - <i>Aeromonas</i> sp. + S1	32.33 abc	9.78 ab	4.67 bc	0.72 a
T <sub>3</sub> - <i>Pseudomonas lini</i> + S1	34.66 ab	9.77 ab	4.89 ab	0.69 ab
T <sub>4</sub> - Without PGPR + S1	30 abc	8.11 ab	4.43 bcd	0.58 bc
T <sub>5</sub> - <i>Bacillus</i> sp. + S2	24.66 c	7.8 ab	4.36 cd	0.56 bc
T <sub>6</sub> - <i>Aeromonas</i> sp. + S2	27.66 abc	7.66 b	4.36 cd	0.56 bc
T <sub>7</sub> - <i>Pseudomonas lini</i> + S2	24.66 c	7.75 b	4.23 cd	0.53 c
T <sub>8</sub> - Without PGPR + S2	27 bc	7.15 b	4.1 d	0.58 abc
Means	29.5	8.7	4.55	0.62
DMSH	7.738	4.0705	0.5206	0.1499

Values with equal letters in each column are equal according to the Tukey test ( $p \leq 0.05$ ); SST = total soluble solids; S1 = 50% compost + 40% river sand + 10% perlite; S2 = 100% river sand; PGPR = plant growth promoting rhizobacteria. DMSH = honest significant minimum difference.

Regarding the interaction substrates×PGPR, the yield showed the highest value with 11.86 kg m<sup>-2</sup> in the T<sub>1</sub> treatment (*Bacillus* sp. + S1) which was higher in 31.61 and 39.71% compared to the treatments T<sub>4</sub> and T<sub>8</sub>, respectively (Table 3), this behavior could be due to the fact that PGPR stimulate the yield of vegetable crops, by various mechanisms such as the production of plant growth stimulating substances (phytohormones) such as indole-3-acetic acid (AIA), gibberellic acid, ethylene and abscisic acid (Arcos and Zuñiga, 2015).

While the treatment T<sub>1</sub> (*Bacillus* sp. + S1) obtained an increase in the number of fruits, obtaining 35 fruits per plant; however, they were statistically similar to the treatments T<sub>2</sub> (*Aeromonas* sp. + S1) and T<sub>3</sub> (*P. lini* + S1), this result indicates that the three bacterial strains and the compost, are considered an option to increase the number of fruits per plant, hence the yield of tomato cultivation in the greenhouse.

Which coincides with Karakurt *et al.* (2011), who mention that the PGPR have a potential to increase the number of fruits per plant and the quality of the fruits, because these bacteria are able to synthesize phytohormones such as cytokinins and AIA, they are also nitrogen fixers and solubilizers of phosphate and as well as inhibit the development of phytopathogenic microorganisms. However, in the treatments where the substrate S<sup>2</sup> (100% river sand) was used, a reduction in the number of fruits per plant was observed, both in the treatments inoculated with PGPR and in the T<sub>8</sub> treatment (control 2). On the other hand, Karlidag *et al.* (2010) indicate that PGPR may have potential to be used to increase plant growth, fruit yield and plant nutrition under salinity conditions.

## Nutraceutical quality

The statistical analysis indicates that in the substrates factor; there was a significant difference in the lycopene variable ( $p < 0.05$ ), likewise, a highly significant difference was registered in total sugars and vitamin C ( $p < 0.01$ ); however, in reducing sugars no significance was found. Regarding the PGPR factor, the contents of lycopene, total sugars, ascorbic acid and reducing sugars showed highly significant differences ( $p < 0.01$ ). The interaction substrates×PGPR was significant for lycopene ( $p < 0.05$ ), and highly significant in the variables total and reducing sugars, as well as in the content of ascorbic acid ( $p < 0.01$ ) (Table 4).

In Table 4 it is shown that the substrate S1 registered an increase of 9.18, 22.05 and 12.68% in the content of lycopene, ascorbic acid and total sugars, respectively, with respect to the substrate S2. This behavior can be attributed to the content of salts present in organic fertilizers, which can favor an increase in salinity of the radical medium (Cuartero and Fernández-Muñoz, 1999), this feature decreases the absorption of water and nutrients; which implies an ionic and osmotic stress that affects the metabolism of the plant, but the nutraceutical quality of the fruits is improved (Ruiz-López *et al.*, 2010; Díaz-Franco *et al.*, 2016).

**Table 4. Contents of lycopene, total sugars, reducing sugars and vitamin C in tomato fruits due to the effect of different substrates and PGPR.**

Factor	Lycopene (mg 100 g <sup>-1</sup> FF)	Total sugars Reducing sugars		Vitamin C (mg of ascorbic acid 100 g <sup>-1</sup> FF)
		(mg of glucose 100 g <sup>-1</sup> FF)		
Substratum				
‘S1’	4.38 a	3.55 a	1.89 a	9.48 a
‘S2’	3.95 b	3.1 b	1.87 a	7.39 b
PGPR				
<i>Bacillus</i> sp.	5.02 a	3.52 a	1.94 a	9.45 a
<i>Aeromonas</i> sp.	4.46 ab	3.62 a	2.03 a	8.72 a
<i>Pseudomonas lini</i>	4.29 b	3.44 a	1.98 a	8.42 ab
Without inoculating	2.88 c	2.71 b	1.57 b	7.18 b
Substratum × PGPR				
Significance	*	*	**	**
CV (%)	9.56	8.37	3.48	9.31

Means with equal letters in a column for each factor are not statistically different (Tukey,  $p \leq 0.05$ ); S1= 50% compost + 40% river sand + 10% perlite; S2= 100% river sand; PGPR= plant growth promoting rhizobacteria; CV= coefficient of variation; \* = significant  $p < 0.05$ , \*\* = highly significant  $p < 0.01$ .

The greater accumulation of total sugars in the fruits could be due to the decrease in the accumulation of water by the fruits, in response to this, the fruits accumulate some sugars (glucose, fructose and sucrose), thus maintaining the osmotic potential in balance and increasing Water absorption (Plaut *et al.*, 2004). Regarding the PGPR factor, the highest lycopene content was presented when *Bacillus* sp. was inoculated, increasing 42.63% compared to the treatment without

inoculation. According to Ordoorkhani *et al.* (2013) the lycopene content in fruits increases because the PGPR have the capacity to reduce the negative effects caused by a biotic and abiotic stress in the plants.

In the variable reducing sugars, the greatest increase was reported when the *Aeromonas* sp. strain was inoculated, with a value of 2.03 mg 100 g<sup>-1</sup> FF, exceeding in 22.66% the treatment without inoculation (Table 4). This behavior can be attributed to the fact that the PGPR tend to increase the photosynthetic efficiency, and consequently the chlorophyll content due to the high levels of CO<sub>2</sub> uptake and therefore there is greater accumulation of sugars in the fruits (Makino and Mae, 1999; Kai and Piechulla, 2009; Karlidag *et al.*, 2010). The content of ascorbic acid was increased when inoculating *Bacillus* sp., Although it was not different from the statistical point of view when using *Aeromonas* sp., for its part, the total sugars the inoculation of the three bacterial strains registered a statistically equal behavior, for what is presumed that the three PGPR are suitable for tomato cultivation. In the present study, the strain *Bacillus* sp. was the one that most influenced the contents of SST, lycopene and total sugars in tomato fruits produced in greenhouse conditions, which could be related to the capacity of each microorganism to synthesize phytohormones (Adriano *et al.*, 2011).

Regarding the interaction substrates×PGPR, the T<sub>1</sub> treatment (*Bacillus* sp. + S1) presented a greater increase in the lycopene content with an average of 5.65 mg 100 g<sup>-1</sup> FF, exceeding in 42.83 and 55.04% the control treatments T<sub>4</sub> and T<sub>8</sub> (Table 5). Similar results were reported by Kumar and Sharma (2014), who evaluated the strain *Azotobacter* + vermicompost + NPK 300 kg ha<sup>-1</sup> in two cycles of the tomato crop, reported values of 5.26 and 5.28 mg 100 g<sup>-1</sup> FF, respectively.

**Table 5. Effect of substrate interaction × PGPR on the nutraceutical quality of tomato fruits developed under greenhouse conditions.**

Treatment	Lycopene (mg 100 g <sup>-1</sup> FF)	Total sugars		Vitamin C (mg of ascorbic acid 100 g <sup>-1</sup> FF)
		Reducing sugars (mg of glucose 100 g <sup>-1</sup> FF)		
T <sub>1</sub> - <i>Bacillus</i> sp. + S1	5.65 a	3.4 ab	2.07 a	11.28 a
T <sub>2</sub> - <i>Aeromonas</i> sp. + S1	4.25 bc	3.95 a	2.04 a	9.98 a
T <sub>3</sub> - <i>Pseudomonas lini</i> + S1	4.41 b	3.81 ab	1.98 ab	9.49 ab
T <sub>4</sub> - Without PGPR + S1	3.23 cd	3.04 bc	1.47 d	7.18 c
T <sub>5</sub> - <i>Bacillus</i> sp. + S2	4.41 b	3.65 ab	1.81 bc	7.61 bc
T <sub>6</sub> - <i>Aeromonas</i> sp. + S2	4.67 ab	3.3 ab	2.02 a	7.45 bc
T <sub>7</sub> - <i>Pseudomonas lini</i> + S2	4.19 bc	3.08 bc	1.99 ab	7.33 bc
T <sub>8</sub> - Without PGPR + S2	2.54 d	2.38 c	1.67 c	7.18 c
Means	4.17	3.33	1.88	8.44
DMSH	1.1259	0.7873	0.1858	2.2204

Values with equal letters in each column are equal according to the Tukey test ( $p \leq 0.05$ ); S1= 50% compost + 40% river sand + 10% perlite; S2 = 100% river sand; PGPR= plant growth promoting rhizobacteria. DMSH= honest significant minimum difference.

The content of ascorbic acid was also increased with the treatment T<sub>1</sub> (*Bacillus* sp. + S1) with an average of 11.28 mg 100 g<sup>-1</sup> FF exceeding in 36.34 the treatments T<sub>4</sub> and T<sub>8</sub>, a behavior that coincides with that established by Molla *et al.* (2012), who report that the content of ascorbic acid in tomato fruits increases due to the use of biofertilizers enriched with *Trichoderma harzianum* and the application of compost. Organically produced fruits have high concentrations of absorbed acid, lycopene and low concentrations of nitrates compared to conventionally produced fruits (Worthington, 2001).

In relation to total sugars, the highest content was reported in the *Aeromonas* sp. + S1 (T<sub>2</sub>); however, it did not differ statistically from the T<sub>1</sub> treatments (*Bacillus* sp. + S1), T<sub>3</sub> (*P. lini* + S1), T<sub>5</sub> (*Bacillus* sp. + S2) and T<sub>6</sub> (*Aeromonas* sp. + S2) (Table 5). According to Kumar *et al.* (2015), the total sugars are increased in strawberry fruits (*Fragaria × ananassa* cv Chandler) when inoculating PGPR plus the application of vermicompost in comparison to control plants. For reducing sugars the greatest increase was obtained in the T<sub>1</sub> treatment (*Bacillus* sp. + S1), although it was statistically equal to the treatments T<sub>2</sub> (*Aeromonas* sp. + S1), T<sub>3</sub> (*P. lini* + S1), T<sub>6</sub> (*Aeromonas* sp. + S2) and T<sub>7</sub> (*P. lini* + S2).

This behavior coincides with that established by Pırlak and Köse (2009), who indicate that when applying the PGPR and organic fertilizers in strawberry plants, they have the potential to increase the content of reducing sugars in the fruits due to the production of stimulant substances of the increase. This allows us to suppose that the use of compost and the inoculation of PGPR are an option to increase the contents of lycopene, total sugars and ascorbic acid in tomato fruits cv. Aphrodite, which is desirable in recent years has received great interest for its antioxidant properties in relation to free radicals, suggesting that these prevent the risks of acquiring chronic diseases such as cancer and cardiovascular diseases (Waliszewski and Blasco, 2010 ).

## Conclusions

According to the results obtained, it is concluded that the use of the substrate S1, had positive effects on the contents of SST, lycopene, total and reducing sugars, ascorbic acid and the percentage of citric acid of tomato fruits cv. Aphrodite. The inoculation of plant growth promoting rhizobacteria (PGPR) increased the contents of total soluble solids, lycopene, reducing sugars and ascorbic acid in tomato fruits produced in the greenhouse. The use of the substrate based on 50% compost + 40% river sand + 10% perlite and the inoculation specifically of the strain *Bacillus* sp. they increased the yield and the nutraceutical quality of the tomato fruits. Therefore, biofertilizers based on PGPR and compost could be a viable alternative to improve the nutraceutical quality of fruits, without reducing tomato yield under greenhouse conditions.

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