Article

In vivo translocation of *Klebsiella variicola* PB02 and *Klebsiella quasipneumoniae* HPA43 in fruits of *Solanum lycopersicum* cultivar DT-22

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Abstract

Currently, biofertilizers or organic compounds have been implemented in agriculture as fertilizer to increase the yield of crops of commercial interest and restore soil fertility. The use of plant growth-promoting bacteria has been one of the best alternatives that increase production yield and do not cause damage to the environment. Their use is not fully accepted by producers because of the risk they may have to the health of the consumer. Therefore, this work evaluated the effectiveness of two strains *K. variicola* PB02, *K. quasipneumoniae* HPA43 in consortium with *Trichoderma* in the production yield of *Solanum lycopersicum* cultivar DT22, a variety highly marketed in the national market, the significant increase (p= 0.003) was observed with respect to other commercial biofertilizers and the control group. The microbiological safety of the fruits, the translocation of these bacteria by the vascular system of the plant until reaching the fruit, was evaluated through the clonal profile of each of the isolates, without finding similarities between the inoculated strains and the strains isolated from endophytic tissues of the fruit, however it is necessary to carry out more genetically specific studies that verify that these bacteria have not actually developed mechanisms that allow them to translocate to aerial anatomical sites of plants and fruit, thus guaranteeing quality fruits without representing a risk to the health of the consumer and a high yield in the production of agricultural crops of commercial interest.

Keywords: biofertilizer, clonal profile, safety, translocation.

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Introduction

Bacteria associated with the rhizosphere, also called plant growth promoters (PGPB), fulfill specific functions in biogeochemical processes, interact with plants, establish protection against phytopathogens, increase nutrient availability and soil fertility level (Martínez-Romero *et al*., 2018). Among these species, the genus *Klebsiella* stands out, mainly the species of *K. variicola* and *K. quasipneumoniae.*

They fulfill mechanisms such as the modulation of the root system, promote positive phototropism, fulfill specific functions such as the production of indole-3-acetic acid, fix atmospheric nitrogen, solubilize phosphorus (Wei *et al*., 2013; Vega Celedón *et al*., 2016), which are essential for the formation of amino acids, nitrogenous compounds for metabolism and structural part of the plant, membrane phospholipid synthesis pathways, root elongation, flowering, part of the metabolism to obtain energy, repair and synthesis of fatty acids (Beltrán, 2014).

Another function of PGPB is to stimulate the development of the root system of the plant and therefore increase the absorption of nutrients, the main absorption pathway is through the root hairs present in the primary and secondary roots, through which a large number of organic compounds and molecules enter for the metabolism of the plant, it has been shown that there are microorganisms present in the soil that have developed the ability to internalize, translocate in the vascular system of the plant and persist in the fruit as is the case of *E. coli* O157: H7 (Heaton *et al*., 2008; Ocaña *et al*., 2018).

The interaction and metabolism with other microorganisms allow the association of microbial communities that establish symbiosis between bacteria with antagonist fungi that provide protection to the plants against phytopathogens present in the soil. Among the antagonist fungi present are *Trichoderma*, commonly isolated from the soil, being widely studied and marketed as biopesticides, biofertilizers and soil amendments, which allow the balance of the ecological niche (Benítez *et al*., 2004; Do Nascimento *et al*., 2017; Hirpara *et al*., 2017). Several strategies have been used to identify the molecular factors involved in this complex symbiosis interaction, including genomics, proteomics, and more recently, metabolomics, in order to improve our understanding (Vinale *et al*., 2008).

Biofertilizers based on microbial solutions have been evaluated in vegetables such as *Solanum lycopersicum*, commonly known as tomatoes (SIAP, 2019). However, it is of vital importance to monitor the internalization, persistence and translocation of these bacteria in the internal tissues of fruits in order to guarantee the health of the product. Therefore, the *in vivo* translocation of *K. variicola* and *K. quasipneumoniae* from the root system to the fruit of *S. lycopersicum* DT-22 in greenhouse was analyzed.

Materials and methods

Isolation of bacterial strains from various environments

Samples of banana, corn and tomato roots, and various water samples (river, lagoon, wastewater treatment plant and irrigation) were selected from the municipalities of Mochitlán, Acapulco, Chilpancingo and Tixtla, respectively. A sterile water wash of the root was carried out and the

nodules were located. The rhizosphere samples were taken directly with a swab and transported to the laboratory in sterile 0.85% saline solution and in vials in tubes with MacConkey agar. Dilutions were made from the water samples up to $1x10^{-3}$, following the methodology of Puerta *et al*. (2010). After 24 h at room temperature, characteristic colonies of the genus *Klebsiella*, lactose-positive, irregular-edged and highly mucoid, dome-shaped, smooth and convex colonies were selected. Oxidase tests were performed, and the conventional biochemical profile was evaluated: in Kligler, MIO, citrate, urea, lysine to separate the genus.

Molecular differentiation of *K. variicola, K. pneumoniae* and *K. quasipneumoniae* by multiplex PCR

The biochemically identified strains of the genus *Klebsiella*, the extraction of DNA was performed by thermal shock, solubilizing three colonies in 50 µl of sterile water and subjecting to a temperature of 96 °C for 10 min and quickly in ice for 5 min and then centrifuged and 10 µl of the supernatant is taken and resuspended with 90 µl of sterile distilled water and kept at -20 °C. The reaction mixture for the multiplex PCR was carried out at a final volume of 25 µl, with a concentration of 1X of buffer, 2 mM of dNTP's, 25 mM of MgCl, final of 1X of buffer, 2 mM of dNTP's, 25 mM of MgCl, 1 µl of the oligonucleotides of *K. variicola* 2006 at a concentration of 10 pmol DNA, 2 µl of *K. quasipneumoniae* 10441 at a concentration of 5 pmol and 2 µl of the oligonucleotides of *K. pneumoniae* 13883 at a final concentration of 5 pmol, 0.5 µl of Taq DNA polymerase and 3 µl of the 1% DNA solution.

The reaction mixture was subject to an initial denaturation step of 2 min, at 92 °C, followed by 30 cycles of 30 s, at 92 °C, 30 s, at 60 °C, 40 s, at 72 °C and a final extension of 3 min, at 72 °C. The PCR products were subjected to electrophoresis in a 1.5% agarose gel at 120 V for 1 h. A fragment of 275 bp of *K. variicola* 2006, one of 372 bp *K. quasipneumoniae* 10 441 and one of 650 bp of *K. pneumoniae* 13 883 were used as controls (Martínez *et al*., 2018).

Detection of plant growth promotion mechanisms: nitrogen fixation

The strains were inoculated with a sterile wooden stick in modified Rennie medium free of N_2 (Rennie, 1986) and incubated at 30 °C for 24-48 h. Growth is an indicator of nitrogen fixation. *K. variicola* PB was used as positive control and *E. coli* DH5-α was used as negative control.

Phosphate solubilization

The ability to solubilize inorganic phosphorus was evaluated in the National Botanical Research Institute's Phosphate (NBRIP) medium with tricalcium phosphate following the methodology of Nautiyal (1999), the diameter of the halo and the colony (mm) was measured for the calculation of the solubilization index (SI), which relates the sum of the halo and colony diameters, and the diameter of the colony. *K. variicola* PB was used as positive control and *E. coli* DH5-α as was used negative control.

Quantification of indole acetic acid

The production of indole acetic acid (IAA) was evaluated in trypticasein soy broth supplemented with tryptophan following the methodology and interpretation of Lara *et al*. (2011). To quantify the production of IAA, in supernatants of the strains evaluated, a standard curve was made with

different concentrations of commercial IAA (4 to 140 μg ml^{-1}) and the Salkowski reagent in a 2:1 ratio. Absorbance was measured at 542 nm in a Stat Fax-2100 reader, using *K. variicola* PB as positive control and *E. coli* DH5-α as negative control.

Siderophore production

The production of siderophores was evaluated by growing the strains in F broth, added with glycerol 10 ml L^{-1}, from a pure culture of 18-24 h, it was incubated for 24-72 h at 37 °C and the growth was examined under a faint ultraviolet light of 260 nm in a mini BioRad transilluminator. It was interpreted according to Villa *et al*. (2017), using *K. variicola* PB as positive control and F broth without inoculation as negative control.

Plant material and treatment design

The seeds of *S. lycopersicum* variety Saladette cultivar DT-22 were germinated in seedbeds of 54 cavities in December 2018, at an age of 39 days post-germination, they were transplanted in greenhouse without any fertilizer. For their study, a design of randomized complete blocks with seven plants each was carried out in accordance with NOM-032-SAG/FITO-2014, this for each treatment and distributed as shown in Figure 1, with a total of 252 plants in a greenhouse with an area of 500 m^2, under controlled conditions of temperature, moisture and irrigation (the temperature was measured with a digital thermometer inside the greenhouse, when it reached 40 °C, corrective measures were taken, such as opening the wings of the greenhouse, the same for moisture, and irrigation was measured by two tensiometers that measure the effort made by the roots to extract moisture from the soil, one was 60 cm and another 30 cm from the soil and these turned on the drip irrigation when that of 30 cm exceeded 5 kPa (kilopascals) and the irrigation was turned off when that of 60 cm reached 0 kPa, all this was to avoid generating water stress in the plants). In addition, a strain of *Trichoderma* sp., X2, provided by Dr. Sergio Ramírez of INIFAP, Zacatepec, Morelos, was added in order to evaluate together with the strains of *Klebsiella*. It should be noted that T1 is the control group, T6 a commercial biofertilizer as positive control.

Barrier furrow

		I	II	III	IV	V	
Barrier furrow with worm	Furrow without worm	Furrow + 33% more worm	T2	T4	T3	T1	T5	Barrier furrow with worm
			T1	T2	T4	T5	T6	
			T6	T3	T5	T4	T2	
			T4	T6	T1	T6	T3	
			T1	T2	T5	T1	T4	
			T3	T6	T2	T3	T5	

Barrier furrow

T1	Control
T2	*Trichoderma*
T3	*K. variicola+K. quasipneumoniae*
T4	*Trichoderma+K. variicola+ K. quasipneumoniae*
T5	*Trichoderma+K. quasipneumoniae*
T6	Commercial biofertilizer

Figure 1. Randomized complete blocks design of plants exposed to microbial treatments.

The tests of biological effectiveness and translocation to fruit were conducted during the agricultural cycle from January to June 2019. Environmental conditions were monitored throughout the cycle until the end of the production stage.

Preparation of microbial treatments

The selection of the strains PB02 and HPA43 used in this work was in accordance with their previous characterization as nitrogen-fixing, phosphate-solubilizing, indole-3-acetic acid-producing bacteria, molecularly identified by sequencing the *rpoB* gene as *K. variicola* and *K. quasipneumoniae*, respectively, and evaluated as root growth promoters *in vitro* in *Solanum lycopersicum* (Gutiérrez *et al*., 2017). As well as the results obtained when they were evaluated *in vivo* in other crops of commercial interest by this work team. A pre-inoculum of the strains was made individually in nutrient broth, adjusting an optical density of $1.5x10^8$ microorganisms and the inoculum in saline solution at a final volume of 1.5 L per each treatment, at the same concentration of microorganisms.

Inoculation of microbial treatments in the root of *S. lycopersicum* cultivar DT-22

After transplantation, 50 ml of the inoculum was added with sterile syringe at the root level, this was to each of the plants by block, a total of 3 inoculations were performed for periods of every 30 days.

Production yield of *S. lycopersicum* fruits exposed to microbial treatments

The number of fruits produced was quantified and classified by class based on weight (g): those of first class weighed 100 g or more, second class from 50 to 99 g and third class with weight less than 49 g, the absolute weight (kg) of the production by block was also obtained with respect to each treatment; it is worth mentioning that this classification is carried out based on the experience of the producer; Eng. Rubén Ocampo Espín, who has produced and placed his product for more than 15 years in the national market. Absolute weight of production, weight by class (I, II and III) were analyzed as response variables. The statistical analysis was performed in the statistical program SAS version 9.4. A bifactorial Anova test, comparison of means by the Tukey test.

Microbiological safety of the fruits of *S. lycopersicum* cultivar DT-22 exposed to the various microbial treatments

Three fruits per treatment were randomly collected in three different events, with a total of 54 fruits per greenhouse. The collection and preparation of the samples was conducted executing the specifications of NOM-109-SSA1-1994. Once the fruits were transported to the laboratory, the surface of the fruits was disinfected with iodine at a concentration of 0.5% in sterile water for 5 minutes, after this, the peel of the fruit was removed.

For the processing of the samples, 10 g of the internal tissue of the fruit was taken and diluted in 90 ml of peptone H_2O with sterile NaCl at 0.85%, the processing was carried out based on NOM-113-SSA1-1994 and NOM-210-SSA1-2014, from the resulting suspension, 100 µl were taken and inoculated in Petri dishes (culturable area 100 mm) in Mac Conkey agar, using the glass bead dispersion technique under sterility conditions for the count of CFU g^{-1} as indicated in NOM-110-SSA1-1994. For the interpretation of the result, the reference values established in NOM-113-

SSA1-1994 were taken, which indicates that the maximum permissible limit (MPL) for anaerobic mesophiles is 150 000 CFU g^{-1} and for fecal coliforms is 100 000 CFU g^{-1} in food samples, together with ISO 21528-2:2017. The isolates were biochemically identified by the Api20E® Biomerix system following the manufacturer's instructions.

Determination of the colonization of the root of *S. lycopersicum* DT-22 plants

Once the production stage finished, the *S. lycopersicum* plants were sacrificed to obtain the root, they were transported to the laboratory for their microbiological analysis, the root surface was disinfected with a 20% Tween solution for 10 min (modified from Bueno *et al*., 2007). One cubic centimeter was taken from the internal tissue of the apical meristem of the primary root. It was macerated with a sterile glass rod and diluted in 9 ml of peptone water with sterile 0.85% NaCl and vortexed at 3 500 rpm for 1 min. Subsequently, dilutions were made up to 10^{-8}, in a time not exceeding 20 min, 100 µl of each dilution were plated in Mac Conkey agar plates in duplicate, incubating at 25-30 °C for 18 h. After the incubation time, the CFU g^{-1} count was performed. The isolates were identified by characteristic colonial morphology and biochemically.

Evaluation of the translocation by genomic fingerprint of each *Klebsiella* isolate

Genomic DNA was extracted from the total strains identified as *Klebsiella* by thermal shock and they were identified based on genetic patterns by PCR of enterobacterial repetitive intergenic consensus (ERIC) sequences to evidence their clonal origin. The oligonucleotides used were: ERIC-1 (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC-2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'), under the following conditions: initial denaturation at 95 °C for 3 min, followed by 30 cycles of 93 °C for 45 s, 50 °C for 1 min, 65 °C for 8 min and a final extension at 65 °C for 16 min (Versalovic *et al*., 1991). ERIC-PCR products were analyzed in 1% agarose gels at 75 V for 2 h and stained with ethidium bromide (3 µl 100 ml^{-1} of agarose) and visualized under UV light (365/302 nm). The genomic pattern of the strains isolated from the fruit and root were compared with the type strains of *K. variicola* PB02 and *K. quasipneumoniae* HPA43. Finally, the interpretation of the patterns obtained was carried out according to Tenover's criteria (Tenover *et al*., 1995).

Results and discussion

A total of 13 strains isolated from the rhizosphere of various agricultural crops (banana, corn and tomato) and water were collected. The species of *K. variicola*, *K. pneumoniae* and *K. quasipneumoniae* were molecularly differentiated. Obtaining 37.5% (3/13) confirmed as *K. pneumoniae*, strains RS10, GN7 and KA2, 6.5% (1/13) *K. variicola* PB02, and 12.5% (2/13) identified as *K. quasipneumoniae* HPA4-3 and PB06. *K. quasipneumoniae* and *K. variicola* have had a great impact, however, a bad approach, lack of resources, ignorance of the diversity among species, lack of updating of the databases of the equipment used for bacterial identification in hospitals hinder their differentiation and diagnosis.

Therefore, it is important to use molecular methods to confirm the identification and differentiation of species within the microbiological routine, given that studies by Garza-Ramos *et al*. (2015) mention that it is not possible to differentiate *K. quasipneumoniae* and *K. variicola* from *K. pneumoniae* by biochemical and phenotypic tests, but by molecular methods (Figure 2).

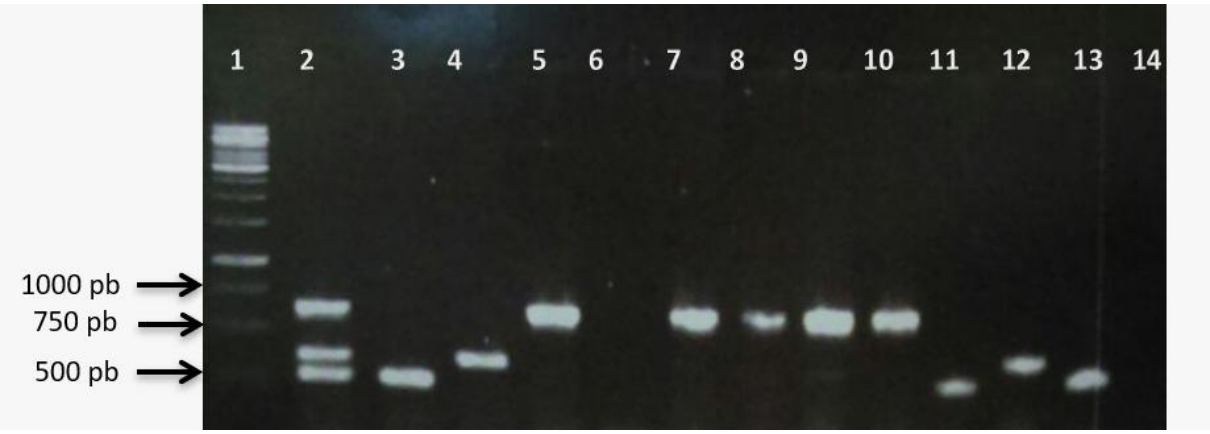

Figure 2. Differentiation of the strains of *Klesiella* sp. Lane 1= molecular weight marker 1kb DNA; lane 2= positive control; DNA *K. variicola*, *K. quasipneumoniae*, *K. pneumoniae*, lane 3= DNA *K. variicola* 2006 (control); lane 4= DNA *K. quasipneumoniae* 10442 (control); lane 5= DNA *K. pneumoniae* 13883 (control); lane 6= negative control (distilled water); lane 7= DNA strain KC1; lane 8= DNA strain KC2; lane 9= DNA strain KC3; lane 10= DNA strain RS10; lane 11= DNA strain PB02 (*K. variicola*); lane 12= DNA strain HPA4-3 (*K. quasipneumoniae*); lane 13= DNA strain PB06 (*K. variicola*).

Bernabé (2016) reports *K. quasipneumoniae* from the rhizosphere of *Opuntia ficus* identified by metagenomics directed at the 16S rRNA gene. This being the first report of *K. quasipneumoniae* in an environmental area, the HPA4-3 strain isolated from blue river water and the PB06 strain located in the corn rhizosphere. It is worth mentioning that 43.75% (7/13) of the isolates did not amplify for any species, but for the genus.

Biological nitrogen fixation

The strains identified as *K. variicola* PB02 and *K. quasipneumoniae* HPA4-3 are able to fix atmospheric nitrogen in 100%, since they present a nitrogenase complex activity at 24 h, evaluated *in vitro*. The latter is confirmed by the study of Fouts *et al*. (2008) that reports that *K. pneumoniae* has in its genome the regulon (KPK_1696-KPK_1715) of nitrogen fixation, indicating that the nitrogenase enzyme is present in the strains, which catalyzes the conversion of N_2 to NH_4. Carcaño *et al*. (2006) report *Klebsiella* sp. with a nitrogenase activity in a range of 42-122.46 nmol of ethylene per ml, where when compared with the genus *Azospirullum* sp. (8.62-70.08 nmol), it shows a significant difference in the nitrogenase activity of ethylene per ml, this indicates that the genus *Klebsiella* sp. has great potential as a nitrogen-fixing bacterium.

Phosphate solubilization

One hundred percent of the strains are solubilizers. The one with the highest index was *K. quasipneumoniae* PB06 with indices of 10.7 mm, followed by *K. pneumoniae* KA-2 with 9.66 mm and *K. quasipneumoniae* HPA-4-3 with 7.97 mm, Tereja *et al*. (2013) evaluated strains of *Bacillus* sp., where 29.31% are phosphate solubilizers, this places *Klebsiella* as a genus with great potential to perform this phenomenon. The release of soluble phosphorus is essential for plant development; it plays a fundamental role in DNA synthesis. Lara *et al*. (2011) reports a solubilization index with

a maximum range between 3 and 4.2 mm in diameter of a total of 20 strains, among which are *P. putida, P. luteola, E. sakasaki* and *E. cloacae,* evaluated under the same conditions of this working document. The difference in these results is related to the ability to fix atmospheric nitrogen. High rates of solubilization have been recorded in the presence of ammonium as a nitrogen source, which is related to the percentage of fixation in the strains evaluated in our work (Beltrán, 2014).

Production of indole acetic acid

In the production of IAA, it was observed that 100% of the strains are producers of IAA, among which the following stand out: *K. pneumoniae* RS10 and *K. quasipneumoniae* PB06, with a high concentration of 3.241 and 2.738 μg ml^{-1}, respectively, which is statistically significant with respect to the positive control *K. variicola* PB with 2.169 μg ml^{-1}. The concentration of IAA in the plant is responsible for increasing the root system and foliage of the plant (Taiz-Zeiger, 2013). In addition, this phytohormone is related to the induction of stem elongation and fruit development.

The results obtained in this study, compared to those of Bautista-Gallardo (2008), are lower. They worked with *A. vinelandii* and obtained a high IAA production. Carcaño *et al*. (2006) report strains of *Azospirillum* and *Klebsiella* with a production of up to 26.55 μg ml^{-1}, which confirms that species of the genus *Klebsiella* are producers of this auxin, however, in this study it can be compared that the strains of *Klebsiella* sp. that do not have nitrogenase activity have a higher production of indoles and vice versa, this confirms the results obtained given that, in the nitrogen-fixing strains, a production of IAA is obtained in low concentrations. Vega *et al*. (2016) mention that IAA-producing strains have a greater growth promoting effect, increasing the elongation of the primary root and the number of secondary roots. However, those that do not produce IAA delay root elongation.

Siderophore production

The production of siderophores was carried out on the molecularly identified strains: 100% of the strains evaluated are producers of siderophores. This agrees with what was mentioned by Arena *et al*. (2015), where they show that *K. pneumoniae* produces siderophores: enterobactin and aerobactin. This gives it competitive advantages in the colonization of the root, providing protection through the biocontrol that it establishes, due to the uptake of iron by siderophores, being attracted to the bacterial membrane, thus limiting the availability of iron for microorganisms that lack the assimilation system. This is consistent with what Molina *et al*. (2015) mentioned in relation to the antagonism between *B. altitudinis* and *Thanatephorus cucumeris*.

K. quasipneumoniae HPA4-3 and PB06 and *K. variicola* PB02 were selected as candidates together with *Trichoderma* sp. to be inoculated in *S. lycopersicum* Saladette cultivar DT-22, although the strain of *K. pneumoniae* RS10 shows several characteristics of plant promotion, it was ruled out because of the various published studies that demonstrate the alterations in human health.

Production yield in Saladette cultivar DT-22

A total of 11 harvests (one per week) were made, five harvests were taken at random in different events to analyze yield. The total estimated production harvest was: 1 392 kg for all treatments. The results obtained show that the treatments evaluated do not have the same effect on the

production of first class tomatoes, the Anova comparative analysis gives us a value of $p= 0.0008^{*}$, complete data are observed in Figure 3, where it is classified according to the Tukey grouping, treatments with the same letter do not have significant differences in their effect, T4 has the highest average value ($p= 0.0007^{*}$) in production (39.618 kg) per harvest, with respect to the control group (T1). Unlike the treatments where they were evaluated individually, T2 does not show a significant difference with respect to the control ($p= 0.464$), T3 has a lower production ($p= 0.999^{*}$) compared to the other treatments and the control (T1), on the other hand, T5 does not show a significant difference ($p= 0.999$) with respect to the control group (T1), the latter consortium was carried out because the HPA43 strain was able to promote *in vitro* root growth of *S. lycopersicum* L. (Gutiérrez *et al*., 2017).

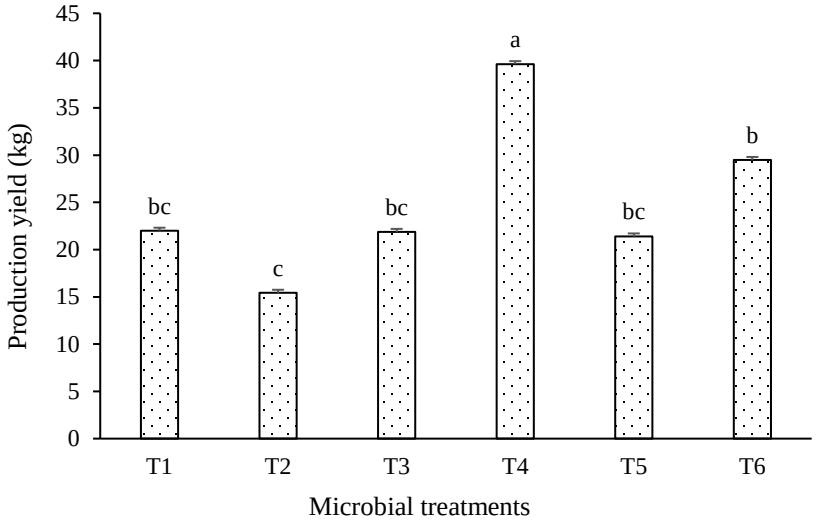

Figure 3. Graph of comparison of means of the production yield (kg) of Saladette tomato cultivar DT-22. The graphs show the standard error bar and the classification according to the Tukey grouping, which indicates that the treatments with the same letters do not present significant differences.

In addition to the microbial consortia, a commercial biofertilizer T6 was evaluated, already standardized and it was found that it does increase the production yield of the crops with respect to T1; however, it is not significant compared to our T4. These microorganisms are able to establish a mutualistic association (bacteria-fungus-plant) and potentiate the production yield, stimulate the development of the root system of the plant, influencing cell differentiation at the level of the meristem, internalizing themselves in the zone of differentiation, and they influence the differentiation of cells according to the needs of the plant and environmental conditions, therefore there is a greater number of root hairs which allow the entry of nutrients and minerals into the vascular system of the plant (Scharf *et al*., 2016; Calzavara *et al*., 2018).

Microbiological analysis of fruits exposed to microbial treatments

Table 1 shows the microbiological count of the fruits by treatment, the means and standard deviations of the Log of CFU g^{-1} of three repetitions in three different events, the number of microorganisms g^{-1} present in the sample is reported, as ISO 21528-2:2017, the values obtained from the count are within the maximum permissible limits.

Table 1. Microbiological count in fruits of *S. lycopersicum* DT-22 in each treatment.

Treatment	Microbiological count Log of CFU g^{-1} (X ±SD)	N (microorganisms g^{-1})	Pr(>F)	Reference
T3	2.6 ±2.5	3262 a	*p*= 0.469	NOM-113-SSA1-1994, ISO 4833-1:2013, ISO 21528-2:2017
T4	2.2 ±2.1	1595 a		
T5	2.5 ±2.5	2828 a		
T1	2.2 ±2	1545 a		

X= mean; SD= standard deviation; N= number; T3= *K. variicola* and *K. quasipneumoniae*; T4= *K. variicola*, *K. quasipneumoniae*, *Trichoderma* sp.; T5= *K. quasipneumoniae*, *Trichoderma* sp.; T1= control. The subscript a in each value belongs to the Tukey grouping, equal letters indicate that there is no statistical difference between the treatments.

From the CFU g^{-1} count found, it is inferred that it is the endemic microflora that is associated with the anatomical sites of the fruit, which have affinity for specific sites of the plant according to the nutritional composition of each anatomical site (Ottesen *et al*., 2013). It is of vital importance the microbiological surveillance and the study of the microbial ecology of these crops of commercial interest, such as tomato DT-22, which is marketed directly to the market and is highly consumed fresh, because the disinfectants used are of superficial use in the fruit, they do not exert an effect on the internal tissues (Barak *et al*., 2012). We must watch that the fruits do not pose a risk to the health of the consumer.

Molecular analysis of genomic fingerprint of *K. variicola* PB02 and *K. quasipneumoniae* HPA43

A total of 114 strains (12 of fruit and 102 of root) were obtained, identified phenotypically and biochemically as *Klebsiella* sp. The profiles obtained based on ERIC-PCR allowed classifying clones (38) and subclones (16) of the strains of *K. variicola* PB02 and *K. quasipneumoniae* HPA43, all from root, of the strains isolated from fruits, no isolation was determined with the same pattern of bands similar to that of the control strains, as observed in Figure 4.

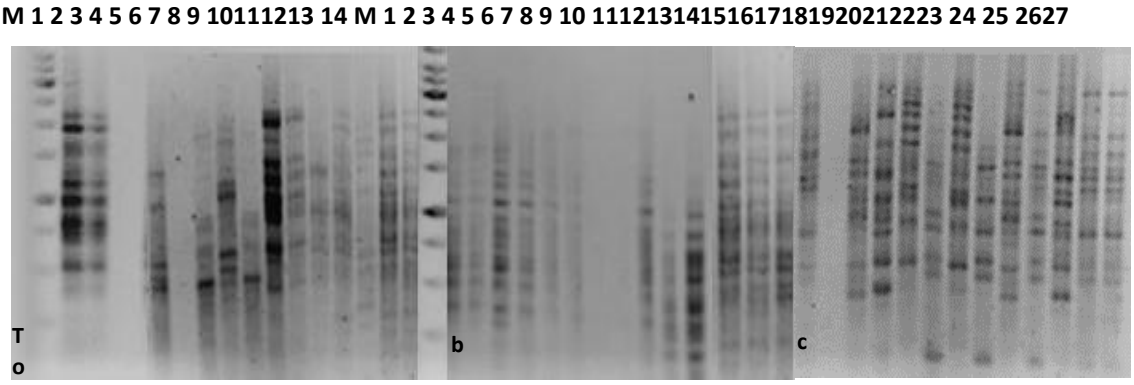

Figure 4. The clonality patterns of enterobacterial repetitive intergenic sequences are observed. M= molecular weight marker of 1Kb; 1= strain *K. variicola* PB02; 2= *K. quasipneumoniae* HPA43; 3= negative control. Fruit strains; 4= T5R3, 5= S2, 6= R4, 7= V4, 8= P3, 9= Z3, 10= T3R2, 11= R3, 12= N3. Root strains; 13= T5R5, 14= T5R1, 15= T3R4, b) M= molecular weight marker of 1Kb; 1= 3R1, 2= 3R2, 3= 3R4, 4= 3R4, 5= 3R5, 6= 5R1, 7= 5R2, 8= 5R3, 9= 5R4, 10= 5R5, 11= T5R3, 12= T5R4, 13= T3R2, 14= V5, 15= W5, 16= T5, 17= T3R1, 18= T3R4, 19= T3R5, 20= T3R, 21= 5R1, 22= A1, 23= A6, 24= B6, 25= C6, 26= D6, 27= E6.

This method has been used mainly to determine hospital outbreaks with the aim of verifying whether phenotypically similar isolates have a genetic relationship, given the high rate of genetic variability in prokaryotes caused by environmental factors, intergenic sequences conserved in enterobacteria that allow to identify by means of their fingerprint that they are derived from a common ancestor, in the same place and in a defined period, are amplified (Tenover *et al*., 1995). These profiles are useful for analyzing the spread and distribution of bacteria in the environment, how they move from one place to another and the conditions under which they persist.

In the results obtained, genetic similarity was only found between the *Klebsiella* strains isolated from the root and the strains *K. variicola* PB02, *K. quasipneumoniae* HPA43 inoculated; however, more specific molecular studies are needed to confirm that these bacteria or other PGPB do not actually translocate to the fruits, that they do not have integrated adaptation mechanisms by horizontal transfer that allow them translocation to the fruit, as is the case of *E. coli*, in response to environmental or genetic factors (Ottesen *et al*., 2013). To better understand the interaction of the microbial ecology present in the fruit and determine if the colonization of these bacteria is a determining factor in developing a health problem to consumers.

Conclusions

The use of plant growth-promoting bacteria in consortia with antagonist fungi potentiates the production yield of agricultural crops of commercial interest, helping to meet consumption demands and reducing production costs in a shorter time compared to conventional agriculture. There is a need for further studies on the effects of the amount of inoculum used on fruit yield and to use finer molecular tools of the strains isolated from the fruits to corroborate the clonal pattern and propose them with greater certainty for the study of translocation of PGPB from the rhizosphere to the fruits.

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