Article

Effect of plant growth-promoting rhizobacteria inoculation on tomato under commercial shade-house conditions

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Abstract

In the present study, the effect of the inoculation of Plant Growth Promoting Rhizobacteria (root-dwelling bacteria that promote plant growth through various mechanisms, commonly known by the acronym PGPR); LBEndo1 (*Bacillus paralicheniformis*), NFbEndo2M2 (*Acinetobacter guillouiae*), KBEndo3 (*Aeromonas caviae*), and KBEcto4 (*Pseudomonas lini*) were evaluated in tomato plants (*Solanum lycopersicum* L. *cv* 'Top1182') into two soil preparations and the use of compost under commercial shade house conditions. Root weight of tomato plant were increased significantly by inoculation with LBEndo1 and KBEcto4 strains, 119.3 and 81.9%, respectively, on composted flatted soil conditions compared to tomato plants control uninoculated. The PGPR treatments also increased fruit number per plant on both soil condition preparations. KBEcto4 was the treatment with the highest number of fruits with 23 tomatoes plant^{-1}, compared with 18.6 fruits plant^{-1} control uninoculated on composted flatted soil conditions. The yield and marketable yields were also enhanced by the inoculation of LBEndo1 and KBEcto4 strains in both soil preparations. The plant growth promoting rhizobacteria and the use of organic fertilizer have the potential to be useful under shade house production and is a viable alternative to improving the yield of tomato.

Keywords: compost, PGPR, protected cultivation, tomato, yield.

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Introduction

Tomato fruits (*Solanum lycopersicum* L.) production is one of the most important consumed vegetal crops worldwide. Tomato plants are characterized by rapid growth and high yields. Nutritionally, tomato fruits are rich in antioxidants such as vitamin C, lycopene, and carotenes as well as minerals, sugars, and fibers that play an important role in human health (Dumas *et al*., 2003; Naika *et al*., 2005; Nzanza *et al*., 2012). Greenhouse, shade house or protected cultivations are alternatives for using soil, water, and other resources more efficiently; moreover, is the best alternative for production of tomato in quantity and quality because the process is clean and free from insect pests and diseases (Gruda 2005; Mahajan and Singh, 2006; Cervantes-Vázquez *et al.*, 2021).

Currently millions of tons of chemical fertilizers are applied to crops to improve the yield; however, the effectiveness is diminished by excesses in fertilizers uses. It is also being important to highlight the negative effects of fertilizers on environment due to pollution of the soil and groundwater (Ayala and Prakasa-Rao, 2002; Son *et al*., 2006; Pastor *et al*., 2014). Another limiting factor on productivity of crops is water deficit, in special in arid and semiarid regions (Armada *et al*., 2014).

Therefore, it is necessary to search alternatives to increase yield and quality of crops without affecting the environment and to reduce the water use in protected cultivations Kumari *et al*. (2019). The use of soil and rhizosphere beneficial microorganisms for increasing the nutrients and water uptake capacity and efficient use by cultivated plants is a great potential possibility. The biofertilizers like plant growth-promoting rhizobacteria (PGPR) are a feasible manage for crop yield increases (Mena-Violante and Olalde-Portugal, 2007; Armada *et al*., 2014; Ruzzi and Aroca, 2015). PGPR can exert a beneficial effect on plants by numerous mechanisms involved on enhance the growth, these mechanisms are protection against phytopatogens (fungi, bacteria, nematodes, etc.), enhancing the availability of nutrients to the host plant, lowering the ethylene production or by enhancing stimulatory compounds, as such as phytohormones (Gravel *et al*., 2007; Copetta *et al*., 2011).

The assays for determine the capacity of increase in growth is easily controlled in vitro sterile conditions, but under nonsterile soil uncontrolled conditions (pot, protected agriculture, and open agriculture), the inoculated PGPR lost the growth promotion effect by compete with soil microbiota. Despite this, there are several examples of growth and yield increase in vegetables, fruit crops, and flower plants. The PGPR positive effect in horticulture was review recently (Ruzzi and Aroca, 2015).

The tomato production in North of Mexico is almost exclusively in protected cultivation since arid and semiarid conditions are predominant. Tomato production to enabling winter production in México and the potential to be a year-round supplier of North America (Cook and Calvin, 2005). Arid and semiarid soils are characterized by lack of structure and organic matter. Not only does the applications of organic amendments (humus, compost, nutrients, etc.) improve the soil structure, but also increase the microbial activities (Trejo *et al*., 2012; López *et al*., 2013; Armada *et al*., 2014).

The aim of this work was to evaluate the effect of the inoculation of four strains of PGPR into plants and fruit yield of tomato in shade house conditions; moreover, to compare the effect of soil preparations and the use of compost in addition, we analyzed the growth of plants and fruit yield of tomato.

Materials and methods

PGPR strains and culture media

The PGPR strains were isolated from rhizosphere of the grass halophyte *Distichlis spicata* L. The bacterial strains used were: LBEndo1, NFbEndo2M2, KBEndo3, and KBEcto4. The LBEndo1, KBEndo3, and KBEcto4 strains were characterized previously as PGPR, these strains improved growth under standard and saline conditions, which correlated with IAA and siderophore production, as well as phosphate solubilization (Palacio-Rodríguez, 2015; Espinosa-Palomeque *et al*., 2017; Palacio-Rodríguez *et al*., 2017; González-Rodríguez *et al*., 2018; Espinosa-Palomeque *et al*., 2019). Stock of PGPR strains were stored -70 ºC in 30% glycerol and before being used were grown at 30 ºC and 180 rpm overnight in Luria Broth medium. Bacterial suspensions were adjusted to $1x10^8$ CFU ml^{-1} before making the inoculations to tomato plants.

Shade house experiments

The experiments were conducted in the commercial shade houses of the company 'Agrícola Vigo', Ejido El Pilar, Matamoros, Coahuila. The site is located at 25º 72' 38.33" north latitude, 103º 32' 73.08" west longitude. Tomato seeds (*Solanum lycopersicum* L. *cv* 'Top1182') were germinated in transplant tray (200 inverted pyramid cells of 2.5 X 6.5 cm, side length X depth) filled with peat moss (Lambert peat moss, Inc, Quebec, Canada). 43 days after germination the plantlets were inoculated by immersion in a bacterial suspension of $1x10^8$ CFU ml^{-1} one by one. After three days of inoculation the tomato plants were transplanted to shade house conditions.

The experimental design in shade house was divided into two conditions according to soil preparation (characterized by being a clayey soil): the first one, soil in flat was enriched with coconut fiber (15 kg m^{-2}) and composted cattle manure (12.5 kg m^{-2}), there were four repetitions of 200 tomato plants by each PGPR inoculated, plus a control row with 200 tomato plants uninoculated. Beds soils (≈30 cm high) were used for the second condition, four repetitions of 200 tomato plants by each PGPR inoculated, plus a control row with 200 tomato plants uninoculated (Table 1).

Table 1. Treatments divided into two groups (ten treatments): flatted soil and Beds soil, each group had the treatments: control, LBEndo1, NFbEndo2M2, KBEndo3, and KBEcto4. All treatments had four repetitions.

	Flatted soil	Beds soil
uninoculated	Control	Control
Inoculated	LBEndo1	LBEndo1
Inoculated	NFbEndo2M2	NFbEndo2M2
Inoculated	KBEndo3	KBEndo3
Inoculated	KBEcto4	KBEcto4

All the tomato plants were planted in rows with 18 cm between tomato plants and 1.8 m between rows. The tomato plants were drip-irrigated with a fertilizer solution containing (mg L^{-1}): N 2 311, Ca 626, Mg 1 158, K 723, and micronutrients 15. The plants were re-inoculated two months after the first inoculation at beginning of bloom stage (63 days after transplantation on shade-house conditions), the second inoculation was performed using a manual 20 L knapsack sprayer with 40 ml of $1x10^8$ CFU ml^{-1} of each bacterial suspension by tomato plants.

Biometric parameters and yields tomato

Throughout the experiment, two samples were taken, the effect of PGPR in the plant growth was determined in the first sampling; three plants by treatment were harvest and shoot length, root fresh weight, stem diameter, fruit weight, and number of fruits per plants were determined after 59 days of transplanted to shade house (62 days after first inoculation).

Yield and quality evaluation was determined in the second sampling, 87 days after transplanted to shade house (90 days after first inoculation). Twenty red tomato fruits were randomly sampled of harvest total by treatment. Fruit size was determined by measuring fresh weight and equatorial diameter of 20 red fruits harvested. Tomato fruits equatorial diameter was measured with a caliper (Scala, Inox 222B, México) and divided into four categories of marketable classification: extra-large (>67 mm), large (54-67 mm), medium (47-54 mm) and small (<47 mm) according to the Jones (1999) scale. Two of twenty tomato fruits were cut in half by equatorial diameter to visually examine the size and internal appearance (Table 2).

Table 2. Time in which the biometric parameters of shoot length, root fresh weight, stem diameter, fruit weight, number of fruits per plant, yield, and fruit quality were taken.

shoot length	Stem diameter	Root fresh weight	Fruit weight	Number of fruits per plant	Yield	Fruit Quality
62 days after first inoculation	62 days after first inoculation	62 days after first inoculation	62 days after first inoculation	62 days after first inoculation	90 days after first inoculation	90 days after first inoculation

Statistical analysis

Data were obtained under completely randomized design with four replications number. The data of measured parameters were analyzed using analysis of variance Anova with the help of software Microsoft Excel complement XLSTAT. Means were tested by minimum significant difference (MSD) at $p \leq 0.05$.

Molecular identification of PGPR by 16S rRNA

The four rhizobacteria were identified by molecular analysis, the LBEndo1, and KBEcto4 rhizobacteria were reported in Palacio-Rodríguez *et al*. (2017). NFbEndo2M2, and KBEndo3 rhizobacteria were subjected to 16S rRNA gene sequence analysis. The DNA was extracted by CTAB technique according to the method of Doyle and Doyle (1990). Partial amplification of 16S rRNA gene was made by PCR technique using the 27F oligonucleotides 5'AGAGTTTGATCMT GGCTCAG 3' and 1492R 5'GGTTACCTTGTTACGACTT 3', the PCR product was purified

using the kit AxyPrep DNA gel Extraction kit (Axygen) and then sent to be sequenced to McLAB in San Francisco, CA, USA. The sequences obtained from sequencing underwent compared using Blast (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the taxonomy of the bacterial strains (Weisburg *et al*., 1991; Altschul *et al*., 1997).

Results and discussion

Effects on growth promotion of tomato plants

In this study, the effect of four PGPR strains on the growth of tomato plants was investigated under shade house conditions using flatted soil added of coconut fiber and compost and raised beds soil without compost. Significant increases in root fresh weight were observed with the inoculations of PGPR in special with LBEndo1 and KBEcto4 strains. The inoculation of tomato plants with PGPR promoted the vegetative growth, increase of growth was observed on shoot length, root weight, and stem diameter.

Even though no marked differences were observed in the increase in shoot length and root weight, increase in root weight by the strains LBEndo1 and KBEcto4 on composted flatted soil conditions, by 119.3 and 81.9%, respectively, compared to tomato plants control uninoculated (Table 3 and Figure 1). The root of tomato plants inoculated with PGPR showed that they modified the architecture and increased growth of plants roots (Figure 1). Root fresh weight was increased significantly in tomato plants inoculated with LBEndo1, NFbEndo2M2, and KBEcto4 compared to controls uninoculated (Figure 1).

Table 3. Biometric parameters of tomato plants after 61 days of PGPR inoculations.

Treatment	Shoot length (cm)	Stem diameter (cm)	Root fresh weight (g)	No. fruits plant^{-1}
		Flatted soil		
Control	136 ±16.3 a	1.19 ±0.011 c	21.7 ±2.9 c	18.6 ±1.3 bc
LBEndo1	152.7 ±5.3 a	1.46 ±0.06 a	47.6 ±1.9 a	21.6 ±0.6 ab
NFbEndo2M2	148.7 ±3.3 a	1.29 ±0.047 bc	36.4 ±3.3 b	20 ±1.1 abc
KBEndo3	137.7 ±12 a	1.38 ±0.033 ab	25.4 ±2.5 c	16.6 ±1.7 c
KBEcto4	150 ±5.7 a	1.46 ±0.024 a	39.4 ±1.6 b	23 ±2.2 a
		Raised beds soil		
Control	137 ±2.2 a	1.32 ±0.028 c	13.3 ±2.4 b	17.3 ±0.6 c
LBEndo1	129.7 ±11.3 a	1.39 ±0.017 bc	27.7 ±2.7 a	22 ±1.1 ab
NFbEndo2M2	140.3 ±4.5 a	1.65 ±0.056 a	36.6 ±9 a	20 ±1.1 bc
KBEndo3	138.7 ±8.4 a	1.34 ±0.045 c	34.0 ±4.1 a	24.3 ±1.7 a
KBEcto4	146.3 ±19.6 a	1.47 ±0.068 b	26.7 ±3.2 a	21 ±1.1 b

Mean values ± SEM are shown; different letters indicate statistically significant differences ($p< 0.05$) among treatments.

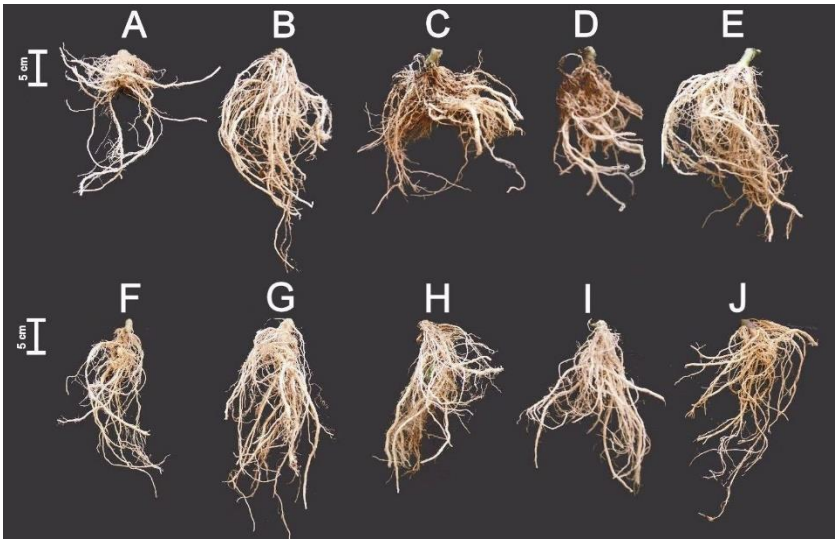

Figure. 1. Root systems of tomato plants: flatted soil preparation (A-E) and raised beds soil preparation (F-J); uninoculated control (A and F); LBEndo1 inoculated (B and G); NFbEndo2M2 (C and H); KBEndo3 (D and I); and KBEcto4 (E and J).

Our results agree with those reported by Gamalero *et al*. (2002, 2004), who worked with *Pseudomonas fluorescens* 92rk and *P. fluorescens* A6RI increased the root fresh weight and affected root architecture when they are inoculated in tomato plants. It Also agrees with what was reported with Sharma *et al*. (2015) reported a significant increase in plant growth parameters (length and weight of shoot and root) in tomato seedlings treated with *Bacillus subtillis* strain S25, which is a strain with antagonistic activity to *Phytophthora capsici*.

Stem diameter of tomato plants was increased on composted flatted soil conditions by PGPR strains LBEndo1 and KBEcto4 inoculation by 22.17 and 22.42%, respectively, for raised beds soil conditions the PGPR strains NFbEndo2M2 and KBEcto4 inoculation increased significantly by 24.71 and 11.11%, respectively, in relation to control plants uninoculated (Table 3). The increased of stem diameter in tomato plants due to inoculation of PGPR agrees with reported by Zulueta-Rodríguez *et al*. (2020), who reported an evident increase of up to 15% in stem diameter in tomato seedlings inoculated with Bacillus subtilis in a chapel-like greenhouse under semi-hydroponic conditions.

The PGPR treatments also increased fruit number per plant on both soil condition preparations. KBEcto4 was the treatment with the highest number of fruits with 23 tomatoes per plant, compared with 18.6 fruits per plant control uninoculated on composted flatted soil conditions. The increase in the growth of tomato plants by PGPR inoculations was already reported (Gamalero *et al*., 2002, 2004; Kokalis-Burelle *et al*., 2002; Gravel *et al*., 2007; Mena-Violante and Olalde-Portugal, 2007; Felici *et al*., 2008; Pastor *et al*., 2014; Sharma *et al*., 2015). There are few reports about evaluation of crops in shade house conditions, Yu *et al*. (2011) reported that *Pseudomonas chlororaphis* and *Pseudomonas fluorescens* increased the phosphorous and nitrogen uptake, and height, shoot and root dry weight of walnut seedlings under shade house conditions. However, the present study reports for the first time the effect of rhizobacteria LBEndo1, NFbEndo2M2, KBEndo3, and

KBEcto4 on the growth of tomato plants under commercial shade house conditions. Moreover, the soil preparation and organic fertilizer added were also shown to have a stimulating effect on the growth in tomato plants.

Yield and marketable tomato fruit

After 12 weeks of shade house experiments, the size and the weight of the tomato fruit was obtained of 20 randomly red fruits of each treatment and the respective control uninoculated of both soil preparations variants (flatted and raised beds soil). Yield of the tomato plants was increased significantly in two of four PGPR strains used. As shown in Table 4, strains LBEndo1 and KBEcto4 had the ability to increase the fruit weight and equatorial diameter on both soil preparations; raised beds and flatted soil supplemented with organic fertilizer where the increase in size is more evident in tomato fruits.

The average weight of tomato fruits per plant treated with LBEndo1 (236.27 and 225.8 g corresponding to 40.8% and 48.4% respectively more than control) and KBEcto4 (240.22 and 211.41 g corresponding to 43.1% and 38.9%, respectively, more than control) strains in both soil preparations were higher than those of others including the uninoculated control (Table 4). The results in the present study exceed those reported by Katsenios *et al*. (2021), where the increased of mean tomato fruit weight per plant by 30.7, 28.81, 27.52, and 26.78% by the inoculation with *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Priestia megaterium*, and *Bacillus licheniformis*, respectively compared to control on the cultivation of industrial tomato.

Table 4. Yield and marketable size classification of tomato fruits after 90 days of PGPR inoculations. According to the Jones (1999) scale the marketable classification was divided in; extra-large (>67 mm), large (54-67 mm), medium (47-54 mm) and small (<47 mm).

Treatments	Fruit weight (g)	Equatorial diameter (cm)	Marketable classification			
			Extra-large (%)	Large (%)	Medium (%)	Small (%)
		Flatted soil				
Control	167.8 ±24.7 c	6.5 ±0.37 b	25	75	0	0
LBEndo1	236.2 ±23.4 ab	7.6 ±0.32 a	95	5	0	0
NFbEndo2M2	162.3 ±20.2 c	6.4 ±0.39 b	40	50	10	0
KBEndo3	199.5 ± 0.6 abc	7 ±0.49 ab	70	20	10	0
KBEcto4	240.2 ±34 a	7.6 ±0.5 a	65	35	0	0
		Raised beds soil				
Control	152.2 ±17.6 b	6.4 ±0.33 c	40	55	5	0
LBEndo1	225.8 ±19.5 a	7.4 ±0.29 a	85	15	0	0
NFbEndo2M2	160.4 ±17.1 b	6.3 ±0.3 c	40	55	0	5
KBEndo3	164.6 ±21.3 b	6.5 ±0.44 bc	25	65	10	0
KBEcto4	211.4 ±26.3 a	7.1 ±0.36 ab	70	30	0	0

For fruit parameters the mean values ± SEM are shown; different letters indicate statistically significant differences ($p< 0.05$) among treatments.

The equatorial diameter of tomato fruit in LBEndo1 inoculated treatment was increased 16.4% (7.623 cm) and 15.2% (7.423 cm) compared to control uninoculated (6.545 and 6.445 cm) for flatted and raised beds soil, respectively (Table 4; Figure 2). The KBEcto4 treatments increased the equatorial diameter by 16.5% (7.625 cm) and 11.1% (7.166 cm) compared to controls of composted flatted soil conditions (6.545 cm) and raised beds soil (6.445 cm), respectively (Table 4 and Figure 2).

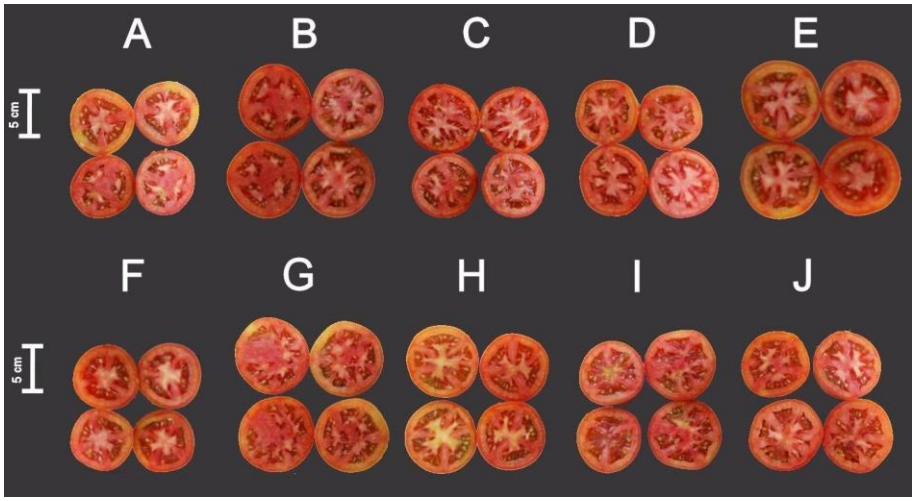

Figure. 2. Tomato fruits cut in half: flatted soil preparation (A-E) and raised beds soil preparation (F-J); uninoculated control (A and F); LBEndo1 inoculated (B and G); NFbEndo2M2 (C and H); KBEndo3 (D and I); and KBEcto4 (E and J).

This effect is also observed in the study carried out by Espinosa-Palomeque *et al*. (2017) with the same strains that we report here under greenhouse conditions, where increases in the equatorial diameter of tomato fruits are reported in the treatment with the LBEndo1 strain corresponding to *Bacillus paranichelliformis*. Gül *et al*. (2008) reported the effect of two commercial strains of *Bacillus amyloliquefaciens* (FZB24 and FZB42) on tomato yield, the application of either strain increased the yield by 8-9%. In a similar way, enhance of yield has been reported in tomato plants inoculated with PGPR *Bacillus subtilis* BEB-13bs, increasing yield and marketable grade yield (21%) compared to control (Mena-Violante and Olalde-Portugal, 2007).

Marketable tomato fruits yields were increased significantly in all the treatments of composted flatted soil conditions compared to control tomato fruits and for raised beds soil the LBEndo1 and KBEcto4 were higher than control uninoculated. In the same way as in the previous parameters the LBEndo1 was higher with 95% (composted flatted soil conditions) and 85% (raised beds soil conditions) of extra-large marketable grade yield (Table 2 and Figure 2). The Figure 2 showed tomato fruit cut in half by the equatorial diameter, in which the size of the fruit is observed between treatments, the largest fruits are observed for the LBEndo1 and KBEcto4. Although, not enough observations of tomatoes fruits cut in a half, the locules number is higher in PGPR treatments in comparison with tomatoes fruits uninoculated; this is important in the texture and firmness of fruits. Espinosa-Palomeque *et al*. (2017) determined the firmness of tomato fruits by penetrometer device, finding that tomatoes from inoculated plants have greater firmness compared to controls without inoculation.

Identification of selected strain by 16S rRNA sequencing

The selected rhizobacteria were identified by PCR amplification and sequencing of 16S rRNA gene. The LBEndo1, and KBEcto4 rhizobacteria were reported in Palacio-Rodríguez *et al*. (2017). NFbEndo2M2, and KBEndo3 rhizobacteria were subjected to 16S rRNA gene sequence analysis. The 16S rRNA gene partial sequence obtained of the sequencing were subject to homology analysis in BLAST (NCBI Database), which retrieve taxonomic positions of rhizobacteria strains, LBEndo1 is like *Bacillus paralicheniformis* with a homology 96%, NfbEndo2M2 was 98% like *Acinetobacter guillouiae*, KBEndo3 had a similarity of 99% to *Aeromonas caviae* and KBEcto4 exhibited 99% homology with *Pseudomonas lini* (Table 5).

Table 5. Molecular Identification of rhizobacteria LBEndo1, NFbEndo2M2, KBEndo3 and KBEcto4 by 16S rRNA sequences.

ID	Taxon	pb	Identity (%)	# access
*LBEndo1	*Bacillus parallicheniformis*	563	96	NR_137421.1
NFbEndo2M2	*Acinetobacter guillouiae*	1 416	98	KJ_147068.1
KBEndo3	*Aeromonas caviae*	918	99	NR_104824.1
*KBEcto4	*Pseudomonas lini*	1 387	99	NR_099042.2

*= Reported in Palacio-Rodríguez *et al*. (2017).

Conclusions

In this study the enhancements of growth, mainly observed in the root development and the increased yield of the fruit in tomato plants by application of LBEndo1 and KBEcto4 PGPR in roots and were shown in a commercial shade house test. Moreover, the use of compost and coconut fiber on flatted soil preparation improved growth and yield of tomato plants. In conclusion, PGPR and the use of organic fertilizer have the potential to be useful in shade house production and it is a viable alternative to improving the yield of tomato. Therefore, the isolation, selection, and evaluation of effective and efficient PGPR strains as bio-fertilizer should be a scalable process of laboratory, greenhouse, and field assays.

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