Article

# Promotion of saladette tomato growth with *Bacillus cereus* and solarized manure in greenhouse

Alfonso Andrade-Sifuentes[1]
Manuel Fortis-Hernández[1]
Pablo Preciado-Rangel[1]
Jorge Sáenz-Mata[2]
Yessica Coria-Arellano[2]
César Guigón López[3§]

[1]National Technological Institute of Mexico-Technological Institute of Torreón. Torreón, Coahuila, Mexico. CP. 27170. Tel. 56 14133562. (fortismanuel@hotmail.com; ppreciador@yahoo.com.mx; ing.andrade_85@hotmail.com). [2]Faculty of Biological Sciences-Juarez University of the State of Durango. Gomez Palacio, Durango, Mexico. CP. 35010. (jsaez_mata@ujed.mx). [3]Faculty of Agricultural and Forestry Sciences-Autonomous University of Chihuahua. Delicias, Chihuahua, Mexico. CP. 33000.

[§]Corresponding author: c_guigon@hotmail.com.

## Abstract

In the Comarca Lagunera, Mexico, there are greenhouses and shade houses devoted to growing tomatoes (*Solanum lycopersicum* L.), with high productivity. The search for alternatives to improve production and meet the demand for healthy foods has recently begun. The objective of the work was to characterize a bacterium isolated from the endorhizosphere of tomato plants and evaluate its combined use with solarized manure to promote tomato growth and yield under greenhouse conditions. The bacterium was identified as *Bacillus cereus* by analyzing the 16S rRNA gene and showed the ability to solubilize phosphates (solubilization halo 5.123 ±0.702 mm), produce siderophores (halo 6.54 mm) and indoleacetic acid (5.9 μg $ml^{-1}$). In a greenhouse, seeds of tomato variety saladette TOP 2299 were inoculated with *B. cereus* at a concentration of 1 $\times 10^8$ CFU $ml^{-1}$ and 46 days after sowing, the seedlings were transplanted into soil enriched with solarized manure at the rate of 0, 40, 80 t $ha^{-1}$, or with chemical fertilization (N-P-K 366-95-635). The results show that the application of *B. cereus* + 40 t $ha^{-1}$ of solarized manure has a positive influence on tomato plants as it promoted greater height (16%), more root volume (42%) and increases in yield (20%).

**Keywords:** *Solanum lycopersicum*, biofertilization, rhizobacteria, sustainable agriculture.

Reception date: April 2022
Acceptance date: July 2022

## Introduction

Tomato (*Solanum lycopersicum*) is a widely cultivated and consumed vegetable, with a global production of 243 635 433 t in 2019. The main producer was China, with 125 739 004 t and Mexico ranked tenth, with a production of 3 441 639 t (FAOSTAT, 2021). In 2019, the state of Coahuila, Mexico, produced 121 579 t, with a value of MXN 816 million. In the Comarca Lagunera, there are 1 090 ha of greenhouses and shade houses for tomato production, generating a harvest of 145 769 t, with a yield of 135 t $ha^{-1}$ and a value production of MXN 911 369 000 (SIAP, 2021).

The high productivity of the crop depends on the use of high amounts of chemical fertilizers, which has aggravated a number of problems, among them the salinization of soils in the region. Therefore, the use of alternatives such as biofertilization and organic fertilizers should be promoted. The use of biofertilizers to increase crop production has intensified in the last decade, considering their advantages for nutrient supply, the stimulation of plant growth and protection against pathogens (Ahmed *et al*., 2021).

Beneficial microorganisms represent an option that strengthens the sustainable agriculture approach (Pretty *et al*., 2008; Bhattacharyya and Jha, 2012), because they increase yields by stimulating root growth and increasing the uptake of nutrients from the soil, thus contributing to better crop nutrition (Raffi *et al*., 2020). Inoculation with bacteria, among them those of the genus *Bacillus*, has improved the growth and development of tomato crops and they improve its nutraceutical quality (Chandrasekaran *et al*., 2019).

On the other hand, the conversion of organic waste into compost reduces environmental pollution and generates a fertilizer and soil improver (Chia *et al*., 2020). The use of solarized bovine manure can provide organic matter and nutrients to the soil, improve its physical and chemical characteristics, and favor the microbial population present, while reducing some diseases induced by phytopathogenic fungi (López *et al*., 2014).

The advantages in tomato production by using composted manure are recognized (Ravindrana *et al*., 2019) and usually increase when organic fertilizer is complemented by the activity of rhizobacteria (Khan *et al*., 2021). The present work was established with the objectives of characterizing a bacterium isolated from the endorhizosphere of tomato plants and evaluating its use alone and in combination with solarized manure to promote the growth and yield of saladette tomato under greenhouse conditions.

## Materials and methods

The isolation and characterization of the bacterium was carried out in the laboratory of Microbial Ecology of the Faculty of Biological Sciences, UJED and the study in the greenhouse was carried out in the Sapioriz ejido, municipality of Lerdo, Durango, during the spring-summer 2021 agricultural cycle.

### Isolation of bacteria

Tomato plants with remarkable characteristics in terms of yield and quality of fruits were selected, which came from a shade mesh located 21 km from the Torreón-San Pedro highway, Coahuila, Mexico, at parallel 25° 43' 30" north latitude 103° 19' 19" west longitude. Root samples of the selected plants were taken with a spatula and sterile tweezers, were placed in 15 ml Falcon tubes inside a container with ice they were transported to the laboratory of Microbial Ecology of the Faculty of Biological Sciences-UJED.

For the isolation, one gram of root was weighed, washed with 70% ethanol, and rinsed with sterile 0.5x phosphate buffer (PBS). Subsequently, it was macerated with a sterile mortar with 10 ml of 0.5x PBS solution, serial dilutions ($10^{-1}$, $10^{-2}$ and $10^{-3}$) were performed and 100 µl was inoculated in Petri dishes with nitrogen-free (NFb) culture medium with congo red and incubated at 30 °C for 1-3 days. Once the colonies were observed, those that turned scarlet red were purified. Finally, a suspension of pure bacteria in 70% glycerol was prepared and stored at -70 °C for preservation.

## Identification of rhizobacteria

### Gram stain

When the bacterium was properly purified, gram stain was performed for its observation under the compound microscope.

### Molecular analysis

The identification of the bacterium was carried out by DNA extraction using the 2x CTAB technique, according to the method of Doyle and Doyle (1990), then partial amplification of the 16S rRNA gene was performed by PCR using oligonucleotides 27F 5'AGAGTTTGATCMTGGCTCAG 3' and 1492R 5'GGTTACCTTGTTACGACTT 3'. The amplification program consisted of 5 min 94 °C, 35 cycles of 45 s 94 °C, 1 min 50 °C, 1 min 72 °C and finally 5 min 72 °C. The PCR product was sent for sequencing to McLAB in San Francisco, CA, USA. The sequences obtained were analyzed using the BioEdit 7 program and were subjected to comparison using Blast (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine their taxonomic identity.

### Characterization of rhizobacteria

The ability of the bacterium to produce indoleacetic acid (IAA) was determined in NFb medium added with L-tryptophan, using the Salkowsky technique modified and described by Bric *et al*. (1991). The concentration of IAA was quantified colorimetrically at 620 nm and compared with a standard curve (0.5, 5, 25, 50, 75 and 100 µg $ml^{-1}$). The production of siderophores was determined using the Chrome azurol S (CAS) medium, (Sigma-Aldrich) (Schwyn and Neilands, 1987). The presence of siderophores was confirmed by the formation of a clear halo around the bacterial colony.

Phosphate solubilization was determined with the National Botanical Research Institute Phosphate growth medium (NBRIP) (Nautiyal, 1999). The solubilization halos were measured with a digital vernier (Carbon Fiber Composites Digital Caliper, Industrial & Scientific) and the solubilization index (SI) and production index (PI) were calculated with the help of the formula SI or PI (as appropriate). Each of the tests was performed in triplicate. SI or PI= $\frac{\text{Colony diameter + solubilization halo}}{\text{colony diameter}}$.

## Preparation of the inoculum for the greenhouse experiment

The bacterium was reactivated in an NFb culture medium with Congo red and malic acid as the main carbon source. Incubation conditions were continuous stirring at 120 rpm and a temperature of 30 °C. Between 14 and 16 h (logarithmic phase), the bacterial concentration was determined in a spectrophotometer (Fisher Scientific 415 Spectro Master) by measuring the absorbance at 540 nm. The suspension was adjusted to an absorbance of 1, equivalent to $1\times10^6$ CFU $ml^{-1}$ and finally a concentration $1\times10^8$ CFU $ml^{-1}$ was prepared to establish the treatments shown in Table 1.

**Table 1. Established treatments with rhizobacteria and doses of solarized manure in tomato plants under shade mesh conditions.**

| Treatments | Description |
|---|---|
| T1= (Bc) | Only bacteria[z] |
| T2= control | Control without bacteria, without manure |
| T3= (Bc)+M40 | Bacteria + solarized manure (40 t $ha^{-1}$) |
| T4= M40 | Solarized manure (40 t $ha^{-1}$) |
| T5= (Bc)+M80 | Bacteria + solarized manure (80 t $ha^{-1}$) |
| T6= M80 | Solarized manure (80 t $ha^{-1}$) |
| T7= (Bc) + FQ | Bacteria + chemical fertilization |
| T8= FQ | Chemical fertilization |

[z]= the rhizobacteria was inoculated at a concentration of $10^8$ CFU $ml^{-1}$ in the corresponding treatments.

## Manure solarization

The manure was obtained from a barn, 12 days after being evacuated by Holstein dairy cattle, fed with protein-rich formula consisting of 1.8 kg of broken corn, 0.9 kg of wheat bran, 0.5 kg of soybeans and 1.3 kg of minerals (salts of $Mg^{++}$ and $K^+$). It was covered for 90 days with transparent plastic (Plastic Poly Sheeting) 30 microns thick without albedo, reaching temperatures of up to 60 ±3 °C. In the end, manure showed a pH of 7.79, EC of 6.8 dS $m^{-1}$, 5.35% of organic matter, total nitrogen of 0.86% and 0.084 mg $kg^{-1}$ of $NH_4$. Finally, the fertilizer was applied to the experimental site 30 days before sowing and incorporated to a depth of 30 cm.

## Chemical fertilization

It consisted of a formula of 366 -95 -635 ($N$-$P_2O_5$-$K_2O$). The fertilizers applied were urea [CO ($NH_2$) 2: 46% -00% -00%], monoammonium phosphate (11% -52% -00%) and NK (12% -00% -46%). The nitrogen was divided into three equal parts (five days before transplanting, flowering and fruiting) and was applied to one side of the plants. Phosphorus-based fertilizer was applied five days before transplantation.

## Inoculation of tomato seeds

Seed of the variety Top 2299 (Ahern Seeds®), saladette type, was washed with sterile distilled water to remove chemicals. Afterwards, they were immersed in 70% alcohol for 10 s, washed with sterile distilled water. They were immersed in 2% sodium hypochlorite for 2 min and then in sterile distilled water. The seeds were inoculated by immersing one gram (360 seeds $g^{-1}$ ±5) in a bacterial suspension $1\times10^8$ CFU $ml^{-1}$ for 1 min. Subsequently, the inoculated seed was placed in a 50 ml Kitazato flask and subjected individually to a vacuum at 600 mm Hg for 5 min.

The seeds were then placed in growth containers containing a previously disinfested peat moss substrate. They were covered with a black rubber for 24 h. Once the emergency began, the containers were placed in greenhouse conditions (36 °C, RH 40% and 3 000 lux) for 45 days. The seedlings were inoculated at 14 and 26 days after sowing, immersing the root system in a bacterial-based solution $1\times10^8$ CFU $ml^{-1}$.

## Establishment in greenhouse

Seedlings were selected for their good appearance and were transplanted into a soil in border strips 10 m long and one meter wide, with a line of five plants per meter in beds one meter wide and a distance between beds of 1.4 m. In each bed there were 50 cm between rows of each treatment. The soil characteristics were: sandy-loam texture, with a pH of 6.5, EC of 1.6 dS $m^{-1}$, 1.4% of organic matter, 10.25 mg $kg^{-1}$ of total nitrogen, 10.2 ppm of $NO_3$ and 15.16 mg $kg^{-1}$ of $NH_4$. The environment inside the greenhouse is shown in Table 2.

**Table 2. Environmental conditions (temperature, relative humidity, photosynthetically active radiation and average annual insolation) during the different phenological stages of saladette tomato production (variety TOP 2299) treated with bacteria and manure.**

| Phenological stage | T (°C) | RH (%) | PAR (meq $m^{-2}$ $s^{-1}$) | AAI (h $day^{-1}$) |
|---|---|---|---|---|
| Seedling/transplantation (April) | 39 ±2 | < 39 | 540.33 | 13.2 |
| Pre-flowering (May) | 39 ±3 | < 42 | 535.22 | 13.3 |
| Flowering and fruiting (June-Julio) | 37 ±3 | < 50 | 520.32 | 11.5 |
| Harvest (August) | 35 ±4 | < 46 | 478.43 | 10.4 |

T= temperature; RH= relative humidity; PAR= photosynthetically active radiation; AAI= average annual insolation. Greenhouse data.

## Crop management

The plants were pruned after 60 days and tutored to two stems. Insect pest control included *Beauveria bassiana*, extracts of garlic and oregano. The irrigation was supplied, according to the phenological stage of the crop, through a surface drip system monitored with tensiometers model SR 24", the Irrometer®. The water was characterized by pH of 5.2, EC of 2.5 dS $m^{-1}$ and 0.23 ppm of $NO_3$. The harvest began when 30 to 60% of the surface of the fruits showed a pink color, according to the color classification (Coromoto *et al*., 2018).

### Experimental design and statistical analysis

The study consisted of eight treatments that were analyzed in a randomized block experimental design with factorial arrangement. The response variables were plant height, root volume and yield. An Anova was performed followed by a Tukey test ($p$= 0.05). Analyses were performed using the SAS program.

## Results and discussion

### Identification of rhizobacteria

The rhizobacterium was characterized by its shape of short bacilli, of pink color, which indicated that it is gram positive (Figure 1). The analysis of the sequence of the 16S rRNA gene showed high homology with *Bacillus cereus* sequences reported in the NCBI GenBank (Table 3).

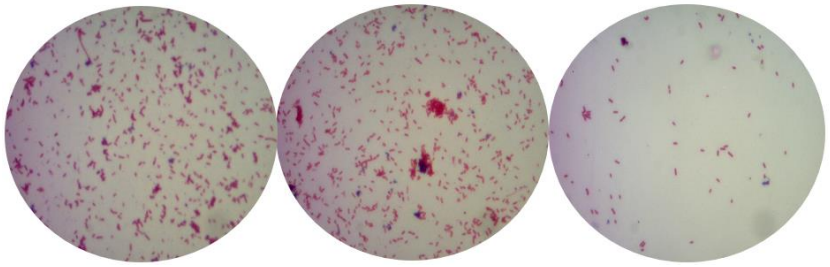

**Figure. 1. Characteristics of the bacterium observed during gram stain showing short and pink (gram positive) bacilli.**

**Table 3. Molecular identification of rhizobacteria isolated from tomato plants.**

| Genus/species | Sequence size (bp) | Identity (%) | No. Access |
|---|---|---|---|
| *Bacillus cereus* | 963 | 99.76 | EU327888.1 |

Bacteria can be important allies of plants, since some, when they are in interaction, produce various metabolites that stimulate or inhibit metabolic pathways that favor plant growth (Glick, 2012). In the particular case of tomato crop, plants benefit from their interaction with these bacteria (Noh *et al*., 2014). Coincidentally, in this work the native strain of *B. cereus* was found in tomato plants with characteristics of high yield and good fruit quality.

In the tomato-*B. cereus* interaction, the presence of the bacterium favors the growth of the crop even under stressful conditions (Khan *et al*., 2020; Mukhtar *et al*., 2020) and improves its health due to the antagonism against important fungi such as *Fusarium oxysporum* and *Alternaria solani* (Karthika *et al*., 2020) or by modifying the radical exudates of the plants to induce control of *Ralstonia solanacearum* (Wang *et al*., 2019).

## Characterization of rhizobacteria

The bacterium showed the ability to produce IAA (5.9 µg $ml^{-1}$) (Figure 2A) and siderophores, exposed by the formation of a crystalline halo around the bacterial growth, which reached 6.54 mm (Figure 2B). The solubilization of phosphates was detected by the formation of a halo of 5.12 ±0.702 mm around the bacterium (Figure 2C).

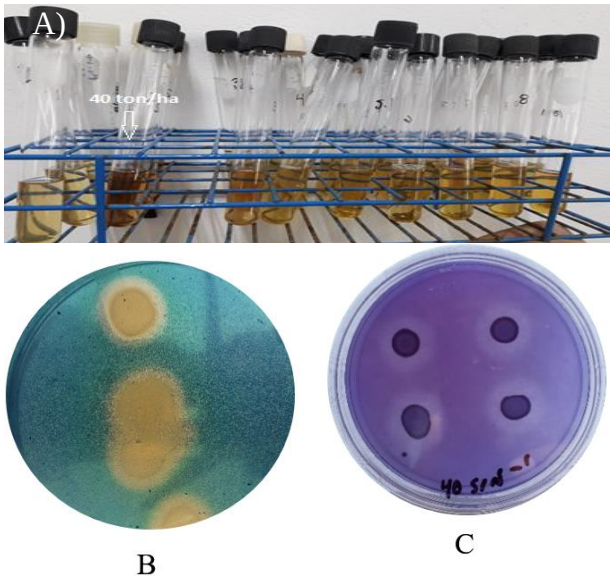


**Figure 2. Ability of *B. cereus* to produce: A) IAA in NFb medium supplemented with L-tryptophan; B) to produce siderophores in CAS medium; and C) to solubilize phosphates in NBRIP medium.**

The mechanisms for the promotion of plant growth by bacteria can be diverse and complex (Glick, 2012). In the case of the native strain of *B. cereus*, it may be that the beneficial effects are a consequence of the production of growth regulators and the absorption of macro- and micronutrients (Beltran *et al*., 2020).

The production of IAA is related to root development, proliferation of lateral roots and root hairs, which improve the absorption of water and nutrients (Spaepen *et al*., 2014). The production of IAA by rhizobacteria is well known, in addition to the fact that they stimulate plants to increase the endogenous production of this auxin (Haque *et al*., 2020). Siderophores improve the assimilation of minerals such as iron, zinc and copper and promote increases in stem and root length and biomass accumulation (Karthika *et al*., 2020; Zhao *et al*., 2020).

The production of IAA and siderophores promoted important changes in the root and stem architecture of tomato plants, which were complemented by phosphate solubilization (Haque *et al*., 2020) necessary for the regulation of various processes such as protein synthesis, enzyme activity, photosynthesis, and tolerance to abiotic stress (Tariq *et al*., 2017).

## Greenhouse growth

Significant differences were observed between the factors studied. Plant growth was better in the combination *B. cereus* +40 t ha$^{-1}$ of solarized manure, which equaled or exceeded the growth of chemically fertilized plants (Table 4). The most favored variable was root volume (42%), followed by yield (20%) and plant height (16%). For the three variables there was interaction between the factor's bacteria and solarized manure (Figure 3).

**Table 4. Effect of *B. cereus* and solarized manure and interaction on the variables plant height (PH), root volume (RV) and yield (Yd) of tomato plants.**

| Factor | Plant height (m) | Root volume (ml) | Yield (kg m$^{-2}$) |
|---|---|---|---|
| | Solarized manure | | |
| 0 | 2.35 ±0.09 b | 56.66 ±12.5 c | 9.83 ±0.77 b |
| 40 | 3.17 ±0.35 a | 97.5 ±40 a | 12.20 ±1.99 a |
| 80 | 2.68 ±0.67 b | 71.33 ±23 b | 10.56 ±1.2 b |
| Chemical fertilization | 3.16 ±0.7 a | 83.83 ±15 b | 12.45 ±1.45 a |
| | Inoculation | | |
| *B. cereus* | 3.21 ±0.5 a | 76.61 ±38.5 a | 12.31 ±1.6 a |
| Without *B. cereus* | 2.74 ±0.53 b | 54.33 ±24.5 b | 10.07 ±1.9 b |
| | Bc+ Solarized manure | | |
| | * | * | * |

Means of treatments ± standard deviation. For each variable, values for each factor with unequal letters are statistically different (Tukey $p \leq 0.05$); *= it indicates statistically significant interaction between bacteria and solarized manure (Tukey $p \leq 0.05$).

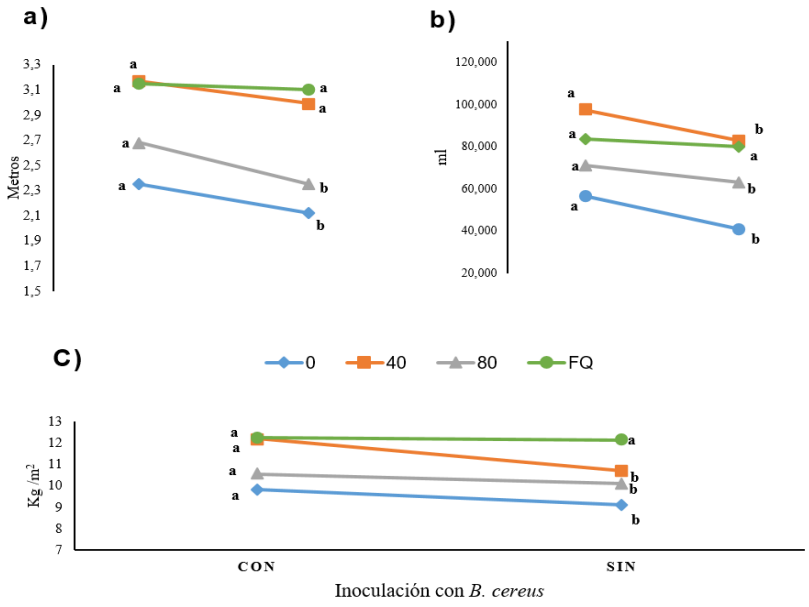


**Figure 3. Interaction of *B. cereus*-fertilization in plant height (a); root volume (b); and yield per m$^2$ (c). Values 0, 40, 80= t ha$^{-1}$ of manure. FQ= chemical fertilization. Different literals indicate a significant difference Tukey ($p \leq 0.05$).**

These results show that the growth of tomato plants was favored with the inoculation of *B. cereus* alone and in combination with solarized manure. The height of inoculated plants was higher than that of non-inoculated plants, as was the root volume, which improves their exploration capacity and enables them to assimilate more nutrients present in the soil (Khoshru *et al*., 2020). These improvements in growth led to significant increases in fruit production.

Manure helps maintain nutrient availability and plant nutritional status, improves soil physical and chemical conditions, and increases organic matter content, which also provides organic carbon that favors microbial activities (Khan *et al*., 2021). These benefits are complemented by the ability of the native strain of *B. cereus* to produce IAA, siderophores and solubilize phosphates.

Additionally, the bacteria-organic fertilizer combination causes a reduction in salinization, which is detrimental to the growth of the crop, so that reducing it generates conditions that interact positively with fruit production (Zhang *et al*., 2019).

These authors also mention that the combined use of *B. cereus* with organic fertilizer usually improves the efficiency of nitrogen use. Likewise, in the soil manure undergoes a process of mineral decomposition in which *B. cereus* and other microorganisms participate, breaking their biochemical structures into simpler nitrogenous compounds from which plants take nitrogen in the form of $NH_4$ and $NO_3$ (Andrade *et al*., 2020).

These compounds are transferred to plant tissue to be assimilated and synthesize different compounds and molecules, such as proteins that are essential for proper cellular functioning (Kour *et al*., 2020). In addition, both plants and microorganisms have the ability to produce phosphatase enzymes that typically combine to acquire P from organic forms of phosphate present in manure (Mpanga *et al*., 2018).

In La Laguna, Mexico, salinity and temperature usually reach high levels, to which the tomato is very susceptible (Khan *et al*., 2020). Consequently, the search for physiochemical and biological strategies friendly to the environment that help the crop to grow under these conditions arises. In this sense, microorganisms such as *B. cereus* exert various beneficial effects on plants, by promoting growth and mitigating adverse effects of the environment (Khan *et al*., 2020; Mukhtar *et al*., 2020). These benefits may be greater when these bacteria are combined with organic fertilizers (Pishchik *et al*., 2018; Khan *et al*., 2020).

## Conclusions

The results of this study demonstrate that the native strain of *B. cereus* develops mechanisms such as the production of indoleacetic acid and siderophores, as well as the solubilization of phosphates, which give it the ability to promote the growth of tomato plants by improving their aerial and root development that finally led it to significant increases in fruit production.

The combination of this bacterium with solarized manure equalized the growth and yields of chemically fertilized plants, so it is feasible to consider this combination to make a biofertilizer for tomato cultivation.

## Acknowledgements

Alfonso Andrade-Sifuentes thanks the financial support provided by the National Council of Science and Technology of Mexico (CONACYT, for its acronym in Spanish) for doctoral studies and the Juárez University of the State of Durango, Faculty of Biological Sciences for the unconditional support to perform the biochemical tests and PCR extraction.

## Cited literature

Ahmed, M. A.; Mahrous, Y. M. A.; Sabry, A. H.; Assem, M. A. E. G. and Mamdouh, A. E. 2021. Effect of potassium solubilizing bacteria (*Bacillus cereus*) on growth and yield of potato. J. Plant Nutr. 44(3):411-420. https://doi.org/10.1080/01904167.2020.1822399.

Andrade, S. A.; Fortis, H. M.; Preciado, R. P.; Orozco, V. J. A.; Yescas, C. P. and Rueda, P. E.O. 2020. *Azospirillum brasilense* and solarized manure on the production and phytochemical quality of tomato fruits (*Solanum lycopersicum* L.). Agronomía. 10(12):1956. Doi.org/10.3390/agronomy10121956.

Beltrán, P. E.M.; Ayala, R. J. A. y Bucio, J. L. 2020. Interrelaciones entre la disponibilidad de fosfato y el ambiente biótico del suelo en el crecimiento y desarrollo de las plantas. Cienc. Nicolaita. 78:59-74.

Bhattacharyya, P. N. and Jha, D. K. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnol. 28(4):1327-1350. Doi: 10.1007/s11274-011-0979-9.

Bric, J. M.; Bostock, R. M. and Silverstone, S. E. 1991. Rapid *in situ* assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol. 57(2):538.

Chandrasekaran, M. X.; Chun, S. C.; Oh, J. W.; Paramasivan, M.; Saini, R. K. and Sahayarayan, J. J. 2019. *Bacillus subtilis* CBR05 for tomato (*Solanum lycopersicum*) fruits in South Korea as a novel plant probiotic bacterium (PPB): implications from total phenolics, flavonoids, and carotenoids content for fruit quality. Agronomy. 9(12):838. doi.org/10.3390/ agronomy9120838.

Chia, W. Y.; Chew, K. W.; Le, C. F.; Lam, S. S.; Chee, C. S. C.; Ooi, M. S. L. and Show, P. L. 2020. Sustainable utilization of biowaste compost for renewable energy and soil amendments. Environ. Pollut. 267:115662. https://doi.org/10.1016/j.envpol.2020.115662.

Coromoto, A. Y. y Reyes, I.. 2018. Microorganismos promotores de crecimiento en el biocontrol de Alternaria alternata en tomate (*Solanum Lycopersicum* L.). Bioagro. 30:59-66.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. Focus. 12-13 pp.

FAOSTAT. 2021. Food and Agriculture Organization Corporate Statistical Database. http://www.fao.org/faostat/es/ #data/QC.

Glick, B. R. 2012. Plant growth promoting bacteria: mechanisms and applications. Scientifica. 2012:963401. https://doi.org/10.6064/2012/963401.

Haque, M. M.; Mosharaf, M. K.; Khatun, M.; Haque, M. A.; Biswas, M. S.; Islam, M. S.; Islam, M. M.; Shozib, H. B.; Miah, M. M. U.; Molla, A. H. and Siddiquee, M. A. 2020. Biofilm producing rhizobacteria with multiple plant growth-promoting traits promote growth of tomato under water-deficit stress. Front. Microbiol. 11:542053. Doi:10.3389/fmicb.2020. 542053.

Karthika, S.; Midhun, S. J. and Jisha, M. S. 2020. A potential antifungal and growth-promoting bacterium *Bacillus* sp. KTMA4 from tomato rhizosphere. Microbial Pathogenesis. 142:104049. Doi:10.1016/j.micpath.2020.104049.

Khan, M. A.; Asaf, S.; Khan, A. L.; Jan, R.; Kang, S. M.; Kim, K. M. and Lee, I. J. 2020. Ampliación de la termotolerancia a las plántulas de tomate mediante inoculación con aislado SA1 de *Bacillus cereus* y comparación con la aplicación de ácido húmico exógeno. Plos One. 15(4):e0232228. Doi:10.1371/journal.pone.0232228.

Khan, M. I.; Afzal, M. J.; Bashir, S.; Naveed, M.; Anum, S.; Cheema, S. A.; Wakeel, A.; Sanaullah, M.; Ali, M. H. and Chen, Z. 2021. Improving nutrient uptake, growth, yield and protein content in Chickpea by the Co-Addition of phosphorus fertilizers, organic Manures, and Bacillus sp. MN-54. Agronomy. 11(3):436. https://doi.org/10.3390/agronomy 11030436.

Khoshru, B.; Mitra, D.; Khoshmanzar, E.; Myo, E. M.; Uniyal, N.; Mahakur, B.; Mohapatra, P. K.; Panneerselvam, P.; Boutaj, H. and Alizadeh, M. 2020. Current scenario and future prospects of plant growth-promoting rhizobacteria: An economic valuable resource for the agriculture revival under stressful conditions. J. Plant Nutr. 43(20):3062-3092.

Kour, D.; Rana, K. L.; Yadav, A. N.; Yadav, N.; Kumar, M.; Kumar, V. and Saxena, A. K. 2020. Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatal. Agric. 23:101487. https://doi.org/10.1016/j.bcab. 2019.101487.

López, M. J. D.; Salazar, S. E.; Trejo, E. H. I.; García, H. J. L.; Navarro, M. M. y Vázquez, V. C. 2014. Producción de algodón con altas densidades de siembra usando fertilizantes orgánicos. Phyton Rev. Inter. Bot. 83:237-242.

Mpanga, I. K.; Dapaah, H. K.; Geistlinger, J.; Ludewig, U. and Neumann, G. 2018. Soil type-dependent interactions of p-solubilizing microorganisms with organic and inorganic fertilizers mediate plant growth promotion in tomato. Agronomy. 8(10):213. Doi.org/10.3390/agronomy8100213.

Mukhtar, T.; Rehman, S.; Your, S. D.; Sultan, T.; Seleiman, M. F.; Alsadon, A. A.; Ali, S.; Chaudhary, H. J; Solieman, T. H. I.; Ibrahim, A. A. and Saad, M. A. O. 2020. Mitigation of Heat Stress in *Solanum lycopersicum* L. by ACC-deaminase and Exopolysaccharide Producing *Bacillus cereus*: effects on biochemical profiling. Sustainability. 12(6):2159. Doi:10.3390/su12062159.

Nautiyal, C. S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett. 170(1):265-270.

Noh, M. J.; Yam, C. C.; Borges, G. L.; Zúñiga, A. J. J. y Godoy, H. G. 2014. Aislados bacterianos con potencial biofertilizante para plántulas de tomate. Terra Latinoam. 32(4):273-281.

Pishchik, V. N.; Vorobyev, N. I.; Ostankova, Y. V.; Semenov, A.V. and Totolian, A. A. 2018. Impact of *Bacillus subtilis* on tomato plants growth and some biochemical characteristics under combined application with humic fertilizer. Inter. J. Plant Soil Sci. 22(6):1-12.

Pretty, J. 2008. Agricultural sustainability: concepts, principles and evidence. Phil. Trans. R. Soc. B. 363(1491):447-465.

Raffi, M. M. and Charyulu, P. B. B. N. 2020. Azospirillum-biofertilizer for sustainable cereal crop production: current status. Recent Dev. Appl. Microbiol. Biochem. 2:193-209. Doi:10.1016/b978-0-12-821406-0.00018-7.

Ravindrana, B.; Lee, S. R.; Chang, S. W.; Nguyen, D. D.; Chung, W. J.; Balasubramanian, B.; Mupambwa, H. A.; Arasu, M. V.; Al-Dhabi, N. A. and Sekaran, G. 2019. Positive effects of compost and vermicompost produced from tannery waste-animal fleshing on the growth and yield of commercial crop-tomato (*Lycopersicon esculentum* L.) plant. J. Environ. Manag. 234:154-158. https://doi.org/10.1016/j.jenvman.2018.12.100.

Schwyn, B. and Neilands, J. B. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160(1):47-56.

SIAP. 2021. Servicio de Información Agroalimentaria y Pesquera. Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA). http://infosiap.siap.gob.mx/aagricola_siap_gb/ientidad/index.jsp.

Spaepen, S.; Bossuyt, S.; Engelen, K.; Marchal, K. and Vanderleyden, J. 2014. Phenotypical and molecular responses of *Arabidopsis thaliana* roots as a result of inoculation with the auxin-producing bacterium *Azospirillum brasilense*. New Phytol. 201(3):850-861.

Tariq, A.; Pan, K.; Olatunji, O. A.; Graciano, C.; Li, Z.; Sun, F. and Liu, C. 2017. Phosphorous application improves drought tolerance of Phoebe zhennan. Front. Plant Scie. 8:1561. Doi: 10.3389/fpls.2017.01561.

Wang, N.; Wang, L.; Zhu, K.; Hou, S.; Chen, L.; Mi, D. and Guo, J. H. 2019. Plant root exudates are involved in *Bacillus cereus* AR156 mediated biocontrol against *Ralstonia solanacearum*. Front. Microbiol. 10:98. Doi:10.3389/fmicb.2019.00098.

Zhang, X.; Xin, L.; Lan, Z.; Tian, Y. and Li, J. 2019. Conjunctive use of composted leguminous shrub Caragana microphylla-straw and *Bacillus cereus* reduces nitrogen input but enhances nitrogen use efficiency and cucumber yields in alkaline soils. Appl. Soil Ecol. 139:69-78. https://doi.org/10.1016/j.apsoil.2019.03.017.

Zhao, L.; Wang, Y. and Kong, S. 2020. Effects of *Trichoderma asperellum* and its siderophores on endogenous auxin in *Arabidopsis thaliana* under iron-deficiency stress. Inter. Microbiol. 23:501-509.