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Full Length Article

Effect of Two Strains of *Stenotrophomonas rhizophila* Isolated from Compost on the Growth of Tomato, Lettuce, and Jalapeno Pepper

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Abstract

Currently, use of plant growth-promoting bacteria are considered a strategy to improve the productivity of agroecosystems. *Stenotrophomonas rhizophila* promotes plant growth, protects roots against stress, and has antagonistic activity against some pathogens. In this study, we isolated two strains of *S. rhizophila* from compost, and evaluated their potential as plant-growth promoters. Both the bacterial strains showed phosphate solubilizing activity. *S. rhizophila* MN067216 promoted the germination of tomato, lettuce, and jalapeño pepper seeds. In five-week-old seedlings, this strain increased plant height by 129.74% and chlorophyll content by 61.34% in jalapeño peppers. In tomato, the thickness of the stem was increased by 8.4%, and the number of leaves by 42.86% as compared to the control without inoculation. *S. rhizophila* MN067218 increased, in, the height of lettuce plant by 13.66%, the thickness of the stem by 13.66%, and the number of leaves by 20.53%; as well as the thickness of the jalapeño pepper stem by 116.87%, compared to the control without inoculation. The consortium between both bacteria increased 21.2% the chlorophyll content of the tomato. The results suggest that the effect of the bacterial inoculation, alone or in combination, depends on the inoculated plant. However, both the bacterial strains showed plant growth-promoting properties on the selected seedlings. Therefore, the isolated strains of *S. rhizophila* are considered promising candidates to promote the growth of tomato, lettuce, and jalapeño seedlings. © 2022 Friends Science Publishers

Keywords: Compost; PGPB; Phosphate solubilizers; Seedlings

Introduction

Currently, agriculture faces the challenge of producing a higher quantity of products to meet the population's demand. A growing world population, scarcity of farmland, water scarcity, and climate change threatens agricultural ecosystems. To ensure food and agroecosystem security it is necessary to propose strategies for the development of sustainable agriculture, which maximizes agricultural production and minimizes adverse effects on the environment (Tian *et al.* 2021). Plant growth-promoting bacteria (PGPB) are considered an alternative to solve the main agro-environmental problems related to the yield and quality of crops. PGPB can replace conventional agricultural practices of chemical fertilization and pest control (Hernández-Soberano *et al.* 2020; Sharf *et al.* 2021). These bacteria promote plant growth by releasing phytohormones, siderophores, nitrogen fixation, and phosphorus solubilization. Several bacterial species associated with the plant rhizosphere have been isolated and tested with beneficial results for plant growth under different environmental and nutritional conditions. PGPB bacteria include species from distinct genera such as *Azospirillum* (Huang *et al.* 2017), *Bacillus* (Gadhave *et al.* 2018), *Pseudomonas* (Sharma *et al.* 2018), *Rhizobium* (Afzal and Bano 2008; Javaid 2009), *Streptomyces* (Dias *et al.* 2017; Hu *et al.* 2020), and *Stenotrophomonas* (Schmidt *et al.* 2012; Nigam *et al.* 2022).

The interaction of *Stenotrophomonas* with plants occurs through three different mechanisms: colonization based on recognition, promotion of plant growth, and antagonism against pathogens. In addition, this bacterium provides osmotolerance and versatility of nutrients in plants. In the genus *Stenotrophomonas*, only two species show a

To cite this paper: Martínez-Cano B, M Toledano-Ayala, CAO Olvera, R Nava-Mendoza, GM Soto-Zarazúa (2022). Effect of two strains of Stenotrophomonas rhizophila isolated from compost on the growth of tomato, lettuce, and jalapeno pepper. Intl J Agric Biol 27:423–430 strong association with plants: Stenotrophomonas maltophilia and S. rhizophila (Wolf et al. 2002). From these species, S. rhizophila has a high potential for applications in biotechnology and biological control due to its capacity to promote plant growth and protect roots against biotic and abiotic stresses. Some mechanisms used by this strain (syn. strain e-p10) are the formation of biofilms and alginate biosynthesis. the synthesis and excretion of glucosylglycerol, and the production of spermidine; the last one is a significant compound in the protection of plant roots against osmotic stress in the environment (Alavi et al. 2013). The complete genome of S. rhizophila was analyzed, and a remarkable number of genes are presumably involved in the mechanisms responsible for the colonization of the rhizosphere: plant association, competition for nutrients, and production of important plant growth regulator compounds were found (indole acetic acid and spermidine), as well as substances that protect against stress (trehalose, glucosylglycerol, proline and glycine betaine) (Dif et al. 2022). In a study conducted by Pérez-Pérez et al. (2020). S. rhizophila was evaluated in vitro to determine its properties as a plant growth promoter, and they found that it presents nitrogenase activity, produces indole-acetic acid. ammonium, and some siderophore compound. There are few in vivo studies to evaluate the potential as a plant growth promoter of S. rhizophila. Schmidt et al. (2012) found that, under non-sterile conditions and with environmental humidity, the bacteria grows endophytically and colonizes the root hairs of the tomato when it is inoculated at 10⁵ CFU mL⁻¹ in seeds; thus, promoting the growth of 87-day-old tomato seedlings because of an indirect effect, which consists of the elimination of harmful organisms in the rhizosphere. Moreover, Egamberdieva et al. (2016) found that S. rhizophila at a concentration of 10^8 CFU mL⁻¹ in combination with Bradyrhizobium japonicum has a beneficial effect on 42-day-old soybean seedlings. The test was done in a greenhouse in hydroponics under controlled conditions; an increase in root size, root dry weight, shoot length, and shoot weight was observed in saline conditions compared to uninoculated control. Nigam et al. (2022) found that a strain of Stenotrophomonas spp. acts as an effective protector to reduce yield loss induced by salinity due to the strong profile of low molecular weight proteins, its strength of ionic homeostasis, and higher activity of ascorbate peroxidase.

Although some studies evaluate the effect of *S. rhizophila*, most of them have been done in vitro; so it is necessary to study the potential of the bacterial strain under the desired environmental conditions and cultures of interest to consider it as alternative fertilization. In this context, the objective of this study was to isolate and characterize plant growth-promoting bacteria from mature compost to verify its activity as a phosphate solubilizer. In addition, the bacteria were inoculated, individually and in a consortium, to improve germination and morphological variables of tomato, lettuce, and jalapeño pepper seedlings.

Materials and Methods

Isolation and identification of PGPB

Isolation bacteria: The samples were from a 75-day maturation compost. Compost was prepared from manure from stables located on the Amazcala, Campus of the Autonomous University of Querétaro, El Marqués, Querétaro, Mexico. The sampling was carried out by separating the compost into three parts: in depths of 0–25 cm, 50–75 cm, and 100–125 cm, in addition to left, center, and right. The samples were placed in sterile sealed plastic bags, homogenized, and, subsequently, the bacterial strains were isolated.

Phosphate solubilizing bacteria were chosen using selective media Pikovskaya (Pikovskaya 1948). Approximately 3 g of the compost was transferred to a laboratory flask with 99 mL of sterile distilled water and stirred vigorously. A 10-fold dilution series was prepared up to 10^{-8} . 0.1 mL aliquots were spread on the selective medium and incubated at $28 \pm 2^{\circ}$ C in an incubator for 72 h.

Two bacterial strains with the ability to solubilize phosphate in the Pikovskaya medium were selected. Gram staining was performed to classify the bacterial strains (Islam et al. 2016). In addition, the bacteria were inoculated in Luria Bertani culture broth for molecular characterization. Molecular characterization: Bacterial genomic DNA of both strains was extracted using Kirby's technique (1967), which consists of lysing cells and DNA solubilizing. After extraction, DNA was amplified with the 16S rRNA gene. Sequencing was carried out at the National Laboratory of Agricultural, Medical, and Environmental Biotechnology of the IPICyT using the dideoxynucleotide method marked with the Genetic Analyzer 3130 sequencer. The DNA sequencing of the 16S ribosomal gene obtained from the amplicon was compared with others in the GenBank database using the NCBI BLAST nucleotide sequence program website on the https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on January 12, 2022).

The complete sequences were deposited in NCBI GenBank. **Phosphate solubilization activity:** The phosphate solubilizing activity was tested by inoculating the bacteria *S. rhizophila* MN067216, and *S. rhizophila* MN067218 grown in PDB on the surface of the Pikovskaya medium and incubated at $28 \pm 2^{\circ}$ C for five days (Pikovskaya 1948). The plates were observed for the formation of light areas around the colonies. In this test, to have a qualitative analysis of the phosphate solubilizing activity of the bacteria, we calculated the phosphate solubilization index using the formula (Kumar and Narula 1999):

$$PSI = \frac{Halo \text{ zone diameter}}{Colony \text{ diameter}}$$

And the relative efficiency of solubilization (Vera *et al.* 2002):

$$RSE = \frac{Solubilization mean diameter}{Colony mean diameter} \times 100.$$

Assessment of plant growth

Preparation of inoculum and seed selection: The *S. rhizophila* MN067216 and *S. rhizophila* MN067218 strains were inoculated, alone and in combination, in 100 mL of LB broth and incubated at $28 \pm 2^{\circ}$ C with constant rotational shaking of 150 rev min⁻¹ for 48 h until the exponential growth phase (Yu *et al.* 2012). The standardization of the inoculums was carried out through dilutions with 0.9% sterile saline solution until obtaining a bacterial cell density of 1×10^{9} CFU mL⁻¹.

For the germination test, commercial seeds of saladette-type tomato (*Solanum lycopersicum* L.), lettuce (*Lactuca sativa* L. var. *Longifolia*), and jalapeño pepper (*Capsicum annuum* L.), Hortaflor brand from the producer Rancho Los Molinos in Mexico, were used. The seeds were disinfected with 70% ethanol (volume/volume) for one minute, then 2.5% sodium hypochlorite for 20 min, and washed three times with sterile distilled water. We discarded the floating seeds.

Assessment of seed germination: Squared and sterilized filter paper was placed inside Petri dishes. A tomato, lettuce, or jalapeño pepper seed was placed in each space until there were 45 seeds per Petri dish. The seeds were moistened with five ml of sterile distilled water and 0.1 mL of *S. rhizophila* MN067216, *S. rhizophila* MN067218, or consortium per seed. The plates were sealed and kept at room temperature for 20 days. Control was 45 uninoculated seeds in a Petri dish. The number of germinated seeds was recorded daily, and at 20 days, germination percentage was calculated with the equation (Islam *et al.* 2016):

germination % =
$$\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

The test had a completely randomized one-factor design with three replications.

Assessment of seedlings: Tomato, lettuce, and jalapeno seeds previously disinfected were sown in seedbeds with sterile peat. Under aseptic conditions, one seed was placed per cavity. Daily irrigation with sterile distilled water was maintained until the seedlings emerged. The inoculation of three treatments, *S. rhizophila* MN067216, *S. rhizophila* MN067218, and a consortium of both strains, began seven days after sowing; it consisted of adding one mL of standardized bacterial inoculum to each well, close to roots of the seedling every seven days for five weeks (Zhang *et al.* 2010). Non-inoculated seedlings were used as a control.

The experiment was established in a greenhouse at an average temperature of 21.5°C, a humidity of 81.9%, and a photoperiod of 10 hours, approximately. The seedlings were watered every three days with sterile distilled water at field capacity. The height and stem thickness of the seedlings were measured every seven days, while at the end of the

experiment (five weeks after sowing), height, stem thickness, chlorophyll content, and the number of leaves were measured. For the measurements, a digital caliper was used, range 0–6"/0–150 mm, resolution 0.0005"/0.01 mm, Surtek brand, model 122200, producer Urrea Herramientas. Chlorophyll was measured with a SPAD-502 Plus Chlorophyll Meter.

The experiment had a completely randomized univariate design with three replications, with experimental units of ten seedlings.

Statistical analysis

The experiments were performed in a completely randomized factorial design. The experimental data statistically were analyzed by an analysis of variance (ANOVA) and a Tukey test with a 95% confidence level with the Statgraphic software. It was considered the null hypothesis that the effect of the bacterial inoculation is equal to the effect of the control without inoculation.

Results

Isolation of bacterial strain

On PDA agar first bacteria presented a medium, spindleshaped, convex colony; with a complete edge, smooth, creamy, and yellow. The second bacteria had a small, pointed, convex, whole-edged, flat, creamy, whitish colony. When applying Gram stain and observing them under the microscope, both bacteria were Gram-negative bacilli.

Both bacterial isolates were selected, among many, for their ability to solubilize phosphate on Pikovskaya agar, an assay used to identify the strains that would be potential PGPB.

Molecular characterization of isolated bacteria and sequence analysis

The phylogenetic tree constructed from 16S rRNA sequences showed that the selected isolates are members of the genus *Stenotrophomonas*. Furthermore, both bacteria are 99% similar to *S. rhizophila*. The partial sequences were deposited in NCBI GenBank with the following access numbers:

S. rhizophila: SUB5832921 Seq1 MN067216

S. rhizophila 2: SUB5832921 Seq3 MN067218

The phylogenetic position of *S. rhizophila* MN067216 (Fig. 1) and *S. rhizophila* MN067218 (Fig. 2) were constructed by retrieving 16S rRNA gene sequences from closely related bacterial species using BLAST pairwise alignments at https://www.ncbi.nlm.nih.gov/blast/treeview/treeView.cgi (accessed on January 12, 2022).

Phosphorus solubilizing activity

In the Pikovskaya medium, both S. rhizophila strains



Fig. 1: Phylogenetic neighbor-binding tree reconstructed based on the 16S rRNA gene sequence showing the phylogenetic relationship between the isolated *S. rhizophila* strain (MN067216) and similar strains



Fig. 2: Phylogenetic neighbor-binding tree reconstructed based on the 16S rRNA gene sequence showing the phylogenetic relationship between the isolated *S. rhizophila* strain (MN067218) and similar strains

showed a translucent halo from the second day after inoculation. The diameter of the bacterial colony and the diameter of the solubilization halo, measured after 24 h, are contained in Table 1, just like the solubilization index (SI) and the relative solubility efficiency (RSE) calculated.

S. rhizophila improves plant growth

Assessment of seed germination: In this work, 45 seeds of each variety of tomato, lettuce, and jalapeno pepper were used, which report a germination percentage of approximately 92, 88 and 92%, respectively.

In tomato seeds, *S. rhizophila* MN067216 increased the germination percentage by 2.2%, while *S. rhizophila* MN067218 and the consortium decreased germination compared to the control.

In lettuce seeds, the germination percentage with *S. rhizophila* MN067216 increased by 16.9%. In addition, in jalapeño pepper seeds, the germination of the control was $80.9 \pm 0.99\%$, while *S. rhizophila* MN067216 presented a germination percentage of $94.2 \pm 1.6\%$. The complete results of the germination percentages are observed in Fig. 3.

Assessment of seedlings: The effect of *S. rhizophila* MN067216, *S. rhizophila* MN067218, and a consortium on

the growth of tomato, lettuce, and jalapeno pepper seedlings was evaluated. Height, stem thickness, leaf number, and chlorophyll content were measured and compared with an uninoculated control. Inoculation with *S. rhizophila* MN067216 significantly increased the height of the jalapeño pepper seedlings, while the bacterial consortium increased it in the lettuce seedlings compared to the control. Otherwise, both strains of *S. rhizophila* do not have a beneficial effect on the height of tomato seedlings (Fig. 4a).

The stem thickness of lettuce and jalapeño pepper seedlings increased significantly when inoculated with *S. rhizophila* MN067218. Moreover, *S. rhizophila* MN067216 increased the stem thickness of tomato seedlings (Fig. 4b).

The inoculation of *S. rhizophila* MN067218 significantly increased the leaf number of jalapeño pepper and lettuce seedlings; this is a distinctive characteristic of the bacteria since the leaves and part of the stem constitute the consumption organ of the lettuce. In tomato seedlings, the leaf number increased when inoculating the *S. rhizophila* MN067216 (Fig. 5a).

The amount of chlorophyll in jalapeño pepper seedlings increased significantly in the presence of *S. rhizophila* MN067216, while in lettuce is enhanced with the consortium (Fig. 5b).

Table 1: Phosphate solubilization index and relative solubilization efficiency of *S. rhizophila* MN067216, *S. rhizophila* MN067218. SI = solubilization index. RES = relative efficiency of solubilization. * Values are the average of three replicates ($\bar{x} \pm SD$)

Bacteria	Colony diameter* (mm)	Solubilization halo diameter* (mm)	SI (mm)	RES (%)
S. rhizophila MN067216	3.5 ± 0.5	21.2 ± 1.3	7.1	604.8
S. rhizophila MN067218	4.9 ± 0.2	22.6 ± 0.5	5.6	455.0

SI = solubilization index. RES = relative efficiency of solubilization. * Values are the average of three replicates ($\bar{x} \pm SD$)



Fig. 3: Germination percentage of tomato, lettuce, and jalapeño pepper seeds inoculated with *S. rhizophila* MN067216, *S. rhizophila* MN067218, and a consortium between both bacteria compared to a sterile distilled water control. Error bars represent the standard deviation. The different letters indicate statistically significant differences between the Tukey treatments for $P \le 0.05$ (\Box) *S. rhizophila* MN067216, (\blacksquare) *S. rhizophila* MN067218, (\blacksquare) *S. rhizophila* MN067216, (\blacksquare) *S. rhizophila* MN067218, (\blacksquare) *S. rhizophila* MN067216, (\blacksquare) *S. rhizophila* MN067216, (\blacksquare) *S. rhizophila* MN067218, (\blacksquare) *S. rhizophila* MN067216, (\blacksquare) *S. rhizophila* MN067216, (\blacksquare) *S. rhizophila* MN067218, (\blacksquare) *S. rhizophila* MN067216, (\blacksquare) *S. rhizophila* MN067216, (\blacksquare) *S. rhizophila* MN067218, (\blacksquare) *S. rhizophila Hizophila Hiz*



Fig. 4: a) Stem height of seedlings with inoculation of *S. rhizophila* MN067216, *S. rhizophila* MN067218, and a consortium between both bacteria compared with a control of sterile distilled water. b) Stem thickness of seedlings with inoculation of *S. rhizophila* MN067216, *S. rhizophila* MN067218, and a consortium compared with a control of sterile distilled water. The error bars represent the standard deviation. The different letters indicate statistically significant differences between the Tukey treatments for $P \le 0.05$ (\Box) S. *rhizophila* MN067216, (\blacksquare) S. *rhizophila* MN067218, (\blacksquare) S. *rhizophila* MN067216, (\blacksquare) S. *rhizophila* MN067218, (\blacksquare) S. *rhizophila* MN067216, (\blacksquare) S. *rhizophila* MN067218, (\blacksquare)



Fig. 5: a) Leaf number of seedlings with inoculation of *S. rhizophila* MN067216, *S. rhizophila* MN067218, and a consortium between both bacteria compared with a control of sterile distilled water. b) Chlorophyll content of seedlings with inoculation of *S. rhizophila* MN067216, *S. rhizophila* MN067218, and a consortium compared with a control of sterile distilled water. The error bars represent the standard deviation. The different letters indicate statistically significant differences between the Tukey treatments for $P \le 0.05$. (\Box) S. *rhizophila* MN067216, (\blacksquare) S. *rhizophila* MN067218, (\blacksquare) S. *rhizophila* MN067218,

Discussion

In the composting process, many bacteria participate in the degradation of organic compounds, and some of them have properties that promote plant growth. In this work, two bacterial strains were isolated from mature compost that improve plant growth, which shows that compost can be used as a source of PGPB (Lin *et al.* 2014).

The two isolated bacteria were *S. rhizophila*, with similar sequences; however, according to the results, they were considered two phenotypically different strains. Both bacteria can solubilize phosphate; however, they do so in different proportions. In addition, the promotion of plant growth is distinct for each bacterium in the vegetables evaluated. The variability of *Stenotrophomonas* strains has been previously evaluated. Wolf *et al.* (2002) demonstrated that a group of three plant-associated isolates was phenotypically different from other *Stenotrophomonas* strains due to isolation sources, heterogeneity in their physiological parameters, and differences in their genome.

Stenotrophomonas species are dominant members of the plant-associated bacterial community (Zhu *et al.* 2012). They can fix nitrogen, promote plant growth and protect them from pathogens. Furthermore, they can induce tolerance to saline stress in crops (Berg *et al.* 2013).

For the phosphorus solubilizing activity, according to Sanclemente et al. (2017), a solubilization halo higher than 10 mm indicates a high potential for solubilizing capacity. In this study, S. rhizophila MN067216 and S. rhizophila MN067218 presented solubilization halos of 21.2 mm and 22.6 mm, respectively, which indicates that both bacteria have a high potential as phosphate solubilizers. The importance of phosphate solubilization is that, after nitrogen, phosphorus is an essential element for plant development. However, soluble phosphorus is a nutrient that is limited in natural ecosystems (Siddique et al. 2021). When farmland is fertilized with inorganic phosphorus, more than 90% of this element is stored in the soil as insoluble phosphorus, since it is not used by plants (Wang et al. 2021). Phosphate solubilizing bacteria take up insoluble phosphorus and release it as dibasic and monobasic phosphates, and they can be assimilated by plants (Lacava et al. 2021).

In soil, various microorganisms solubilize phosphate, and they are widely used to increase plant growth. Some of them are *Azotobacter chroococcum* with a PSI of 2.1% (Kumar and Narula 1999), *Bacillus subtilis* has a PSI of 4%, and an RSE of 303.3% (Mumtaz *et al.* 2017), *Streptomyces* spp. have a PSI between 1.09–1.89 mm (Wahyudi *et al.* 2019); thus, the bacteria isolated from *S. rhizophila* have a higher phosphate solubilization capacity than those previously reported and used.

Several methods have been used to increase the germination rate in seeds: partial hydration pre-germination treatments in osmotic solutions or chemo-conditioning treatments with bioactive additives; these treatments aim to reinvigorate aged seeds, accelerate and standardize germination to increase the resistance of plants to environmental stresses (Pérez *et al.* 2016).

S. rhizophila has been studied mainly for its effect on the control of phytopathogens. However, it has also been found to promote plant growth. Particularly in germination, this strain, when inoculated in seeds, has presented high colonization in the roots, stems, and leaves of tomato and promotes growth and germination (Schmidt *et al.* 2012). The significant increase in germination with the *S. rhizophila* strain for tomato, lettuce, and jalapeño pepper could be because the bacteria produce some plant growth regulator such indole acetic acid, a hormone that stimulates cell division to promote embryo growth (Berg *et al.* 2010; Dif *et al.* 2022). However, it is necessary to carry out laboratory tests to check the release of hormones in these bacteria.

S. rhizophila is capable of producing phytohormones that partially modify the metabolism of plant cells (Bano and Yasmeen 2010). In addition, the auxins produced by the bacteria increase the surface of the root, which facilitates the absorption of essential nutrients and water, and it will increase the growth of plants (Berg *et al.* 2010; Egamberdieva *et al.* 2016). Furthermore, a study by Schmidt *et al.* (2012) points out that *S. rhizophila* promotes the growth of tomato plants in non-sterile soil by indirect effect for the suppression of pathogens and harmful microbes.

PGPB can enhance growth and influence the metabolism of many plant species in many families; thus, it is not yet clear whether the affinity of genus/species for specific plants is evident and whether there is a host specificity (Pereg *et al.* 2016). However, it is clear that the soil-plant-microorganism-environment interaction has a direct impact on the growth and development of plants, so the beneficial effects of PGPB will depend on the above factors (Pedraza *et al.* 2010). The above may explain that the inoculation of *S. rhizophila* MN067216, *S. rhizophila* MN067218, and the consortium have a different effect on each type of plant; however, the beneficial result obtained is observable.

The results observed in the present study show that inoculation with two strains of *S. rhizophila* significantly improves the growth of the morphological variables of different types of plants.

The effect of beneficial bacteria on the improvement of plant growth is known; however, it is still necessary to explore the physiological and molecular mechanisms, as well as the bacterium-plant interaction, and thus, obtain better use of PGPB according to its specificity with plants. That can help bridge the gap for commercial application of the bacteria in the field.

The *S. rhizophila* strains used in this study have not yet been evaluated in unsterilized soils and the field. Furthermore, the effect of bacteria cannot be verified in other vegetables besides tomatoes, jalapeño peppers, and lettuce; so, it is necessary to study and investigate how *S. rhizophila* exert their beneficial effects on the desired plants.

Conclusion

Two bacterial strains with plant growth-promoting properties were isolated and characterized from mature compost. The results of the investigation suggest that inoculation with isolated strains of *S. rhizophila* significantly improve germination and growth of some physiological variables of seedlings of lettuce, tomato, and jalapeño pepper, so they are considered promising candidates to be used as biofertilizer.

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Author Contributions

BMC planned and performed the experiments and wrote the original draft. MTA did a formal analysis of the manuscript, revised and edited. CAOO reviewed and edited the manuscript. GMSZ planned the experiments, data analysis, supervision, and writing.

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