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Articles

Biostimulant effect of beneficial rhizobacteria on the yield and bioactive compounds of cucumber

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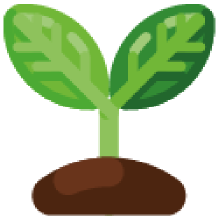
Abstract

The use of beneficial microorganisms in agriculture, such as bacteria, is a practice that allows improving the production of agricultural crops since they can act as stimulants, biofertilizers, and as antagonistic organisms against pathogens. The study aimed to assess seed priming and drench in cucumber with beneficial bacteria on the yield and synthesis of bioactive compounds of the fruits. The bacteria used were *Pseudomonas paralactis*, *Sinorhizobium meliloti*, and *Acinetobacter radioresistens*; in addition to this, a control with distilled water was used. The results indicate that the strains used improved the growth and accumulation of plant biomass compared to the control, which was reflected in a greater length, diameter, and yield of the cucumber fruit, with *A. radioresistens* increasing the yield to a greater extent compared to the control (37.78%). As for the bioactive compounds (total phenols, flavonoids, and ascorbic acid), antioxidant capacity, and proteins, the bacterial strains improved them substantially compared to the control, which showed the lowest values. These results confirm that the application of bacteria is a promising alternative to improve the production and quality of agricultural crops in a sustainable way.

Palabras clave:

Cucumis sativus L., biopriming, biostimulation.



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Introduction

The different biotic and abiotic conditions that cause stress in plants are factors that lead to the search for alternatives to improve the production of agricultural crops. These factors cause imbalances in the plant at all omic levels, which causes large losses in terms of yield and quality of the harvested products (Kőmíves and Király, 2019; Kopecká *et al*., 2023).

One of the most promising alternatives is the use of biostimulants. A biostimulant is any substance or microorganism that is applied to plants with the aim of improving nutritional efficiency, stress tolerance, and quality characteristics regardless of their nutrient content (du Jardin, 2015).

Today, biostimulants are commonly applied as seed primers, foliar products, and in drench; however, seed priming is gaining a lot of relevance due to the different responses they induce compared to the rest of the applications. Seed priming is a pre-sowing treatment in which the seeds are soaked for a certain time in a solution or dispersion of some substance or microorganisms at a specific concentration (González-García *et al*., 2022a; Corbineau *et al*., 2023).

This practice induces what is known as priming memory, which causes an adaptive modification of metabolic processes, so that plants make adjustments that lead to a more efficient use of resources (Juárez-Maldonado *et al*., 2021). These adjustments, by triggering pre-germinative metabolic processes, induce de novo synthesis of nucleic acids, proteins, bioactive compounds, ATP production, accumulation of sterols, phospholipids, and DNA repair mechanisms (Cardarelli *et al*., 2022).

Among the biostimulants, microorganisms such as plant growth-promoting rhizobacteria (PGPR) associated with plants stand out, which are part of the soil and play a fundamental role in providing the plant with a wide range of services and benefits, the plant in turn gives back by supplying reduced carbon and bioactive compounds (De Pascale *et al*., 2017; Shahraja bian *et al*., 2023). PGPRs play key roles in the solubilization and assimilation of nutrients, such as nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), and iron (Fe), and the synthesis of extracellular molecules, such as hormones, bioactive compounds, and antibiotics, all of which result in improved plant growth (Ding *et al*., 2021; Khan *et al*., 2023).

In addition, these biostimulants can improve crop tolerance to biotic and abiotic stresses, conditions that, in the existing climate change environment, are becoming more and more frequent (Pereira *et al*., 2019). These benefits help to reduce the excessive use of fertilizers and pesticides employed in production (Gómez-Godínez *et al*., 2023).

Among the most important horticultural crops is cucumber (*Cucumis sativus* L.), which, together with its economic importance, stands out for its functional importance due to the quality of its fruits, which are usually consumed fresh (Kaur and Sharma, 2021). These fruits are a source of minerals and bioactive compounds (phenols, ascorbic acid, glutathione, etc.) that improve human health by preventing cardiovascular and neurodegenerative diseases (Trejo-Valencia *et al*., 2018; González-García *et al*., 2022b).

In this regard, the cucumber is susceptible to different biotic and abiotic tensions, which can negatively affect the yields and quality of the fruits, so it is important and advisable to be able to biostimulate the crops, so that it can mitigate tensions and improve the yields and quality of the crops. In accordance with the above, the study aimed to evaluate the priming of seeds and drench of cucumber with PGPR on the yield and synthesis of bioactive compounds of the fruits.

Materials and methods

Setting up the experiment

The experiment was conducted at the Antonio Narro Autonomous Agrarian University, Laguna Unit, with geographical location -103° 21’ west longitude and 25° 33’ north latitude, using a semicircular greenhouse, controlling the temperature at 25 °C and average relative humidity of 60%. Thesubstrates used for sowing consisted of a mixture of sand, perlite, and vermicompost (Table 1) in a ratio of 50:25:25, respectively.

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Table 1. Characterization of the vermicompost used.

Parameter	Concentration
Electrical conductivity	2.4 dS m^{-1}
pH	7.9
Organic matter (MO)	24.74%
Total nitrogen (Nt)	0.95%
Phosphorus (P)	0.22%
Potassium (K)	0.06%
Calcium (Ca)	48.6 mg kg^{-1}
Magnesium (Mg)	5.6 mg kg^{-1}
Copper (Cu)	1.8 mg kg^{-1}
Iron (Fe)	26 mg kg^{-1}
Zinc (Zn)	12 mg kg^{-1}
Manganese (Mn)	21.2 mg kg^{-1}

The substrate used was solarized for seven days. Subsequently, 10 L black polyethylene bags were filled with the substrate. Poinset 76 cucumber seeds were used, which were washed and disinfected with 5% sodium hypochlorite for 1 h. Afterward, the seeds were inoculated by immersion for 24 h in beakers with 50 ml of each strain corresponding to *Pseudomonas paralactis*, *Sinorhizobium meliloti* and *Acinetobacter radioresistens*, donated by the Microbial Ecology Laboratory of the Faculty of Biological Sciences of the Juárez University of the State of Durango, Mexico.

The concentration of the inocula was adjusted to $1x10^8$ CFU ml^{-1} (Zapata-Sifuentes *et al*., 2024). The seeds were then sown directly into the pots previously prepared with the substrate. Additionally, the bacterial strains were reinoculated at 55 days after sowing (DAS) with 15 ml at a concentration of $1x10^8$ CFU ml^{-1} applied to the base of the stem (drench).

To meet the nutritional needs of the crop, Steiner's (1961) nutrient solution was used, considering the nutritional contribution of vermicompost and irrigation water. The nutrient solution was applied for 150 days, which was the length of the experiment; to do this, it was applied at 25% during the phenological phase of vegetative development, at 50% during flowering, at 75% during fruit setting, and at 100% during fruit filling. Training, pruning, and pest and disease control practices were carried out to guarantee the good condition of the plants.

The experimental design was in randomized complete blocks with four treatments and four replications, which consisted of inoculating seeds and plants with the strains of *P. paralactis*, *S. meliloti*, and *A. radioresistens* and a control with distilled water.

Variables evaluated

Agronomic variables, such as plant height, stem diameter, root length, dry stem biomass (aerial part), and fruit yield per plant and its components (length, diameter, and kg $plant^{-1}$) were assessed. For the dry stem biomass, the material was dried for 72 h at 70 °C and weighed on a digital balance (VE-CB2000, Velab).

The protein analysis was carried out following Bradford's (1976) methodology, using Coomassie blue dye as a reaction agent. Total phenols were determined following Singleton *et al*. (1999) methodology, using the Folin-Ciocalteu reagent. Flavonoids were quantified using the methodology described by Zhishen *et al*. (1999), using $AlCl_3$ as a reaction agent. Ascorbic acid was determined by titration following the methodology described in Hernández-Hernández *et al*. (2019). Antioxidant capacity was determined as described in Brand-Williams et al. (1995), using DPPH as an oxidizing agent.

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Statistical analysis

Using the SAS 9.4 software, the analysis of variance (Anova) was performed for each variable and when finding significant differences, Tukey mean test was performed ($p< 0.05$).

Results and discussion

Plant growth and biomass

The results show that the applications of PGPR promoted the growth and accumulation of plant biomass (Table 2). For plant height, *P. paralactis* and *A. radioresistens* increased it by 17.08 and 14.6%, respectively, compared to control.

Table 2. Growth and biomass of cucumber plants.

Treatment	PH (cm)	SD (mm)	RL (cm)	DAB (g)
S. meliloti	158.26 ab*	5.22 ab	16.85 ab	40.86 a
P. paralactis	174.53 a	5.51 a	26.2 a	24.26 b
A. radioresistens	170.83 a	5.26 a	13.1 bc	43.5 a
Control	149.06 b	4.88 b	5.45 c	16.13 c

PH= plant height; SD= stem diameter; RL= root length; DAB= dry aerial biomass; * = different letters indicate significant differences between treatments (Tukey, $p< 0.05$).

For stem diameter, *P. paralactis* and *A. radioresistens* were again superior to the control, showing increases of 12.9 and 7.7%, respectively. As for root length, *S. meliloti* and *P. paralactis* increased it by 209.1 and 380.7%, respectively, compared to control. For dry aerial biomass, all treatments with PGPR exceeded the control, showing increases of 153.3, 48.8, and 169.6% for *S. Meliloti*, *P. paralactis*, and *A. radioresistens*, respectively.

Fruit growth and yield

Yield increased with the application of PGPR (Table 3). Fruit length was positively modified using PGPR. *S. meliloti* and *A. radioresistens* outperformed the control by 29.6 and 27.7%, respectively. For stem diameter, all PGPR treatments were superior to the control, with *A. radioresistens* showing the highest increase with 22.7%. Finally, in terms of yield, the treatments with PGPR were also superior to the control, with *A. radioresistens* being the one that increased it to a greater extent (37.7%).

Table 3. Growth and yield of cucumber fruits.

Treatment	Fruit length (cm)	Stem diameter (mm)	Yield (kg plant^{-1})
S. meliloti	23.33 a*	54.43 a	4.26 b
P. paralactis	21 ab	52 a	4.17 b
A. radioresistens	23 a	56.52 a	4.85 a
Control	18 b	46.03 b	3.52 c

* = different letters indicate significant difference between treatments (Tukey, $p< 0.05$).

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Priming seeds with beneficial bacterial inocula is a promising alternative to improve seed germination rate and early seedling growth, which greatly impacts crop yields. The results of this experiment show that the use of PGPR improved the growth, biomass, and yield of cucumber plants.

Similar results were reported by Lastochkina *et al*. (2020) in wheat crops, where priming seeds with *Bacillus subtilis* improved early seedling growth under standard growing conditions and drought stress. Miljakovi# *et al*. (2022) evaluated soybean seed priming with *Bradyrhizobium japonicum* and *Bacillus megaterium* and indicated that germination percentage, shoot length, root length, dry root weight, and seedling vigor index were significantly improved.

It has been reported that just hydrating the seeds before sowing reactivates the metabolism and initiates the germination process; now, treating the seeds with bacterial strains allows the entry of microorganisms through the testa, which triggers different responses mediated by the microorganisms (Fiodor *et al*., 2023).

These responses include an increase in germination speed and superior early growth of seedlings, mainly triggered by hormones (auxins, cytokinins, and gibberellins) produced by microorganisms, which increases cell division and elongation (Pérez-García *et al*., 2023). This practice of biopriming causes such marked effects that they are reflected in better crop yields despite only making applications on the seeds.

In the experiment, PGPR were also applied in the form of drench, which may have potentiated the effect. These microorganisms, by acting in the rhizosphere, are capable of improving the solubilization and assimilation of nutrients, such as N, P, K, Zn and Fe, which are key nutrients in plant growth and development (Mahmud *et al*., 2020; Nitu *et al*., 2020; Rawat *et al*., 2021). Plants, by acting in symbiosis with these microorganisms, provide them with different exudates (sugars, amino acids, and organic acids) that help improve soil microbial activity and diversity (Lei *et al*., 2023).

Bacteria of the genus *Pseudomonas* have the ability to fix N_2 and are producers of antibiotics, auxins, siderophores, cellulolytic enzymes, and organic acids for phosphorus solubilization and promotion of induced systemic resistance against phytopathogens, which makes them ideal in agricultural production for either biocontrol or biofertilization (Sánchez-Carillo and Guerra-Ramírez, 2022).

Bacteria of the genus *Sinorhizobium* fix N_2, are producers of siderophores, hormones, solubilizers of P, and have antagonistic and deaminase activity (Toro-Ipanaqué *et al*., 2020). Bacteria of the genus *Acinetobacter* are capable of fixing N_2, solubilizing P and S, producing siderophores and hormones, and improving the availability of magnesium (Mg) to plants (Ramírez-Cariño *et al*., 2023).

Bioactive compounds and soluble proteins from fruits

Bioactive compounds were increased in fruits with the use of PGPR (Figure 1). For phenols, flavonoids and ascorbic acid, *P. Paralactis* was the strain that improved their accumulation to a greater extent, with increases of 47.8, 55.5 and 133.9%, respectively, compared to control. Antioxidant capacity was increased with *P. paralactis* and *S. Meliloti* by 19.1 and 35.3%, respectively, compared to control. Soluble proteins were increased with *P. paralactis* and *S. meliloti* by 37.16 and 55.4%, respectively, compared to control (Figure 2).

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Figure 1. Bioactive compounds in cucumber fruits. Different letters indicate significant differences between treatments (Tukey, *p*< 0.05).

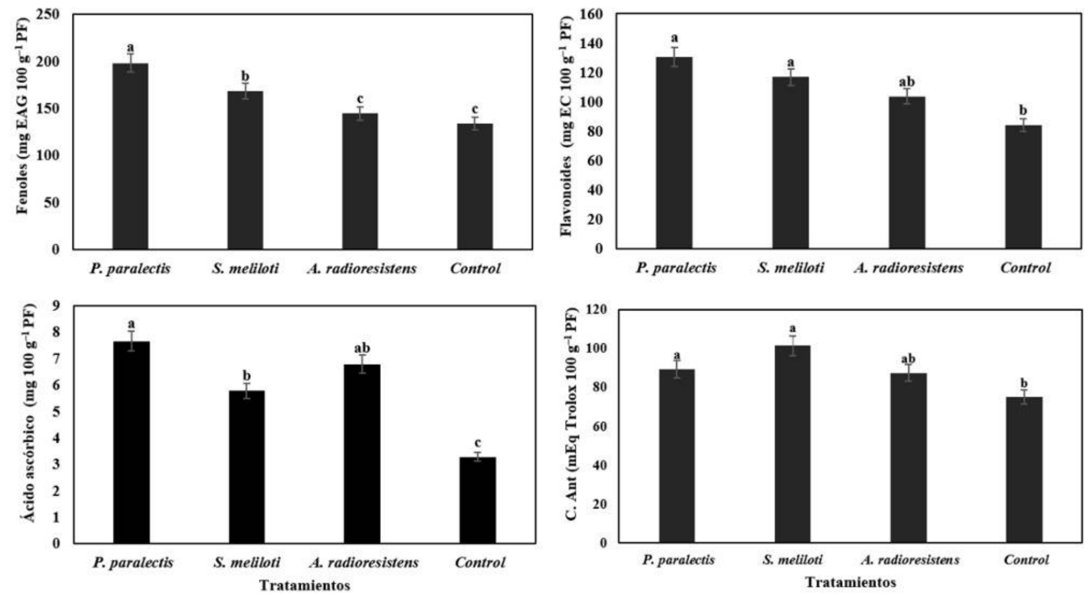
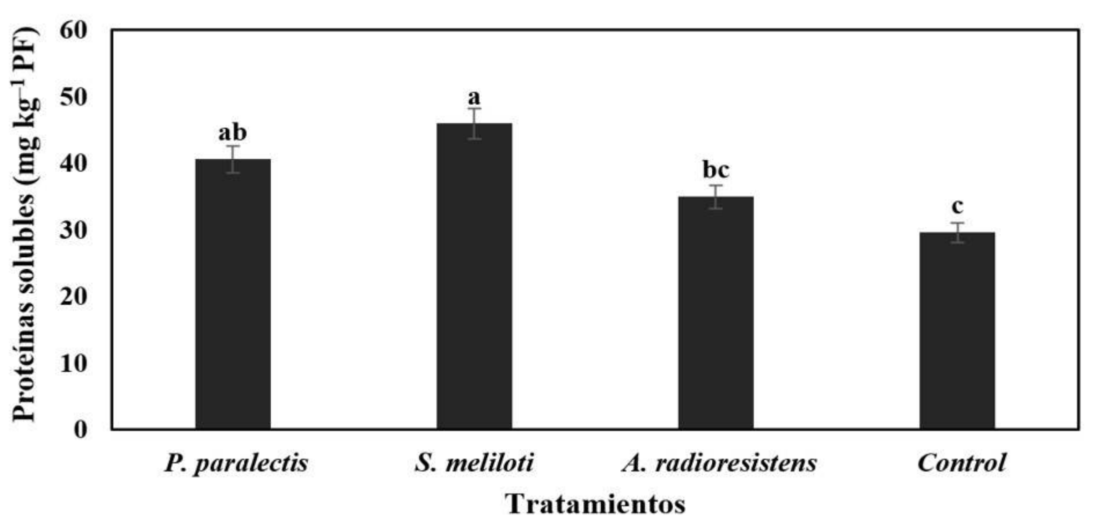


Figure 2. Soluble proteins in cucumber fruits. Different letters indicate significant differences between treatments (Tukey, *p*< 0.05).



The results of the present study show that the use of the bacterial strains improved the accumulation of bioactive compounds in cucumber fruits. Similar results are shown by Pérez-García *et al*. (2023) with the same strains used in this experiment, where they found an increase in total phenols, flavonoids, and antioxidant capacity in cucumber seedlings.

Biopriming, being a technique used on seeds before sowing, causes a radical change in seeds that involves a metabolic modification that can improve plant germination, growth, and yield (Rajendra-Prasad *et al*., 2020). These metabolic changes involve increased synthesis of primary metabolites

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(nucleic acids, carbohydrates, and lipids) and secondary metabolites (phenolic compounds, ascorbic acids, and reduced glutathione) by the action of eustress caused by microorganisms (Chakraborti *et al*., 2022).

This eustress or positive stress causes increases in antioxidant levels in plants without harming yields and in some cases, such as the one in this study, it increases them significantly. When microorganisms enter seeds through the testa, they cause an increase in the production of reactive oxygen species (ROS) to plant-tolerant levels; nevertheless, these ROS act as signaling molecules and induce the synthesis of antioxidants for their subsequent reduction and metabolic homeostasis (Irshad *et al*., 2023).

The application via drench produces other responses compared to priming, which are related to an improvement in the biological, physical, and chemical properties of the soil due to the action of PGPR (Orozco-Mosqueda *et al*., 2021). These improvements increase the availability of nutrients for plants, growth phytohormones are released, and the root system is protected from pathogen attack, which brings increases in nutritional quality and bioactive compounds (Gómez-Godínez *et al*., 2023; Shao *et al*., 2023).

Conclusions

Biopriming and drenching with PGPR improved plant growth parameters and yield and bioactive compounds of cucumber fruits, mainly because PGPR, through different mechanisms, positively modulate physiological and metabolic processes that improve the aforementioned variables.

This type of biostimulation modulates the physiology of photosystems by increasing quantum absorption and energy flow in plants. By improving these aspects related to the photosynthetic process, the production of photoassimilates is increased and therefore the yields and quality of the harvested products are increased. For this reason, it would be appropriate to perform analyses related to the photosynthetic process, such as pigment concentration and gene expression, in subsequent experiments.

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