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Evaluation of *Bacillus* spp. as plant growth-promoting rhizobacteria (PGPR) in broccoli (*Brassica oleracea* var. *italica*) and lettuce (*Lactuca sativa*)

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Abstract

Lettuce and broccoli are valuable agricultural products in Ecuador whose cultivation demands considerable quantities of mineral nutrients, usually obtained from chemical fertilizers. As the use of plant growth-promoting rhizobacteria (PGPR) has shown to be a biological alternative for cropping these vegetable species, several strains of *Bacillus* spp. were evaluated through laboratory and field tests. Biological nitrogen fixation was performed by a qualitative assessment in a free nitrogen culture medium; the indoleacetic acid production was carried out in a Tryptic Soy Broth (TSB) medium by quantifying its concentration using Salkowski's reagent; phosphate solubilization was established on an NBRIP medium and the transformed phosphorus concentration was quantified with the Mo-Blue reagent. The data obtained showed a significant difference between the treatments ($p < 0.01$) where *B. megaterium* and *B. licheniformis* showed a higher ability to fix nitrogen, produce auxins, and solubilize phosphate. Field tests achieved, on the one hand, an increase in height of 26.7 % and 13.7 % in lettuce and broccoli, respectively, with a weekly application of *B. licheniformis*. On the other hand, a weekly application of *B. megaterium* significantly increased the dry matter content, length, and root weight in lettuce as well as in broccoli. All comparisons were made against a control. These results show that the strains identified in this research could be considered as growth-promoting microorganisms and are a biological alternative to chemical fertilizers.

Keywords: auxins, biofertilizer, nitrogen fixation, phosphate solubilizing, rhizobacteria

Evaluación de *Bacillus* spp. como rizobacterias promotoras del crecimiento vegetal (RPCV) en brócoli *Brassica oleracea* var. *italica*) y lechuga (*Lactuca sativa*)

Resumen

La lechuga y el brócoli son productos de importancia agrícola en Ecuador, cuyo cultivo demanda considerables cantidades de nutrientes minerales obtenidos, generalmente, de fertilización química. Dado que el uso de rizobacterias promotoras de crecimiento vegetal (RPCV) es una alternativa biológica para el desarrollo de estas especies hortícolas, se estudiaron varias cepas de *Bacillus* spp. mediante pruebas en laboratorio y campo. La fijación biológica de nitrógeno se realizó mediante una evaluación cualitativa en medio de cultivo libre de nitrógeno; la producción de ácido indolacético se realizó en medio Tryptic Soy Broth (TSB) y se cuantificó la concentración de este con el reactivo de Salkowski; la solubilización de fosfatos se realizó en medio NBRIP, y se cuantificó la concentración de fósforo transformado con el reactivo Mo-Blue. Los datos obtenidos mostraron una diferencia significativa entre los tratamientos ($p < 0,01$), donde *B. megaterium* y *B. licheniformis* presentaron mayor capacidad para fijar nitrógeno, producir auxinas y solubilizar fosfatos. La evaluación de campo determinó, por una parte, un incremento en altura de 26,7 % y 13,7 % en lechuga y brócoli respectivamente, con la aplicación semanal de *B. licheniformis*. Por otra parte, la aplicación semanal de *B. megaterium* incrementó significativamente el contenido de materia seca,

longitud y peso de la raíz tanto en lechuga como en brócoli. Todas las comparaciones se hicieron frente a un testigo. Estos resultados demuestran que las cepas identificadas en la investigación pueden ser consideradas como rizobacterias promotoras de crecimiento vegetal y son una alternativa biológica a los fertilizantes de síntesis química.

Palabras clave: auxinas, biofertilizante, fijación de nitrógeno, rizobacterias, solubilización de fosfatos

Introduction

There are microorganisms in the soil capable of favorably increasing the capacity of plants to access essential nutrients for their development (Correa, 2016). This group of microorganisms is known as plant growth-promoting rhizobacteria (PGPR), comprised of several species of bacteria that can increase plant growth and productivity in different ways (González & Fuentes, 2017).

The bacteria associated with the rhizosphere of plants are capable of generating several mechanisms that positively influence plant growth, among which direct ones are identified, corresponding to the production of auxin- and gibberellin-type phytohormones (Moreno et al., 2018). Furthermore, rhizobacteria can affect the availability of nutrients by direct intervention in biogeochemical cycles, such as biological nitrogen fixation and phosphorus solubilization (Ramírez et al., 2017). Indirectly, they can contribute through the induction of systemic resistance to phytopathogens, the biological control of diseases, and the production of antibiotics and siderophores (Camelo et al., 2011). The best-known genres are *Azospirillum, Bacillus, Burkholderia, Rhizobium, Enterobacter, Azotobacter, Erwinia, Klebsiella, Xanthomonas*, and *Pseudomonas* (Bashan et al., 2012; Cassán et al., 2009).

In multiple investigations, the potential of the *Bacillus* genus bacteria as PGPR has been demonstrated. Besides, several of its species, such as *B. subtilis, B. megaterium, B. licheniformis,* and *B. pumilus*, are used for phosphate solubilization, production of plant growth-regulating hormones, biological fixation of nitrogen and production of substances that act as pathogen antagonists improving crop quality (Tejera et al., 2013).

Current agriculture is focused on implementing practices that ensure soil conservation, so the use of PGPR is presented as an alternative compatible with the microbiological activity that promotes plant nutrition (Grageda et al., 2012). Moreover, the implementation of PGPR is considered as a sustainable agriculture strategy in developing countries (Cassán et al., 2009).

Lettuce is one of the most important leafy vegetables worldwide. In Ecuador, there are 1,499 ha with an average yield of 19,432 t/ha (National Institute of Statistics and Censuses [INEC], 2016). The province with the highest production is Pichincha, with 15,575 t/ha cultivated on 924 ha, followed by the province of Chimborazo, with 1,905 t/ha produced on 232 ha (INEC, 2018).

Regarding broccoli, Ecuador is the seventh exporter country worldwide, and 98 % of its production is destined for export, mainly to the United States (INEC, 2016). Currently, the area planted with broccoli reaches 11,462 ha, with a production of 188,095 Mt, and the province with the highest production is Cotopaxi, with 10,199 ha planted with this vegetable (INEC, 2018).

Both broccoli and lettuce are horticultural species that occupy an important place in agricultural production in Ecuador, so the search for techniques that improve production without causing damage to the environment or human health is relevant.

The aim of this investigation was to evaluate the efficiency of *Bacillus* spp. as microorganisms that promote plant growth in vegetables through laboratory analyses and greenhouse trials. In this way, the application of biotechnological resources for the development of environmentally friendly agriculture and as an alternative to reduce the use of chemical fertilizers is expected.

Materials and methods

The *Bacillus* spp. strains used for the laboratory assays were cryopreserved in the strain repository of Centro de Investigación y Valoración de la Biodiversidad of the Laboratory of Life Sciences of Universidad Politécnica Salesiana.

Molecular identification of PGPR microorganisms

DNA extraction

Bacterial microorganisms were cultured in Tryptic Soy Broth (TSB) and incubated at 30 °C and 100 rpm. DNA was extracted from 24-hour cultures using the methodology described by Sambrook and Russell (2001).

The presence of DNA was established by electrophoresis. For this, 1 % agarose gel was used, and 5 µL of Sybr Safe was added for every 50 mL of agarose diluted in 1X TBE. A volume of 5 µL of DNA was mixed with 5 µL of Blue Juice 2X loading buffer, and samples were run at 90 V for 40 min in the Labnet electrophoresis chamber. The gel was developed and photographed on the Bio-Imaging Systems photo documenter.

Polymerase chain reaction (PCR)

For the molecular identification of the microorganisms, the 16S region was amplified with the primers 27F (5'-AGAGTTTGATCCTGGCTCA-3') and 1492R (5' GGTTACCTTGTTACGACTT-3') (Weisburg et al., 1991). In 0.2 mL Eppendorf tubes, 12.5 µL of Go Taq Master Mix from Promega, 0.5 µL of forward primer, 0.5 µL of reverse primer, 10 µL of nuclease-free water, and 1 µL DNA were added; a final volume of 25 µL was obtained for the reaction. PCR was performed in a Labnet Multigene brand thermal cycler under the following conditions: initial denaturation at 95 °C for 2 min, 24 denaturation cycles at 95 °C for 30 s, primer hybridization at 58 °C for 1 min, initial extension for 2 min at 72 °C, and final extension of 10 min at 72 °C, followed by maintenance at 4 °C. The PCR products were subjected

to Sanger sequencing and the sequence similarity search was carried out using the BLAST (N) option of the NCBI Genbank.

Biological fixation of atmospheric nitrogen

The strains were reactivated in Petri dishes with nutrient agar and incubated at 37 °C for 24 hours. For the preparation of the NFb culture medium, the formulation proposed by Ramírez et al. (2015) was used. Once the medium was obtained, the pH was adjusted to 6 with KOH (1 N) and sterilized at 121 °C for 15 min at 1-atmosphere pressure. Strains were cultured in NFB medium in Petri dishes and incubated at 37 °C for 48 hours.

Indoleacetic acid production (IAA)

The strains were reactivated in test tubes with nutrient broth and incubated at 37 °C for 48 hours. For this process, the methodology of Posada (2017) was used with the following modifications: 100 µL of the bacteria previously incubated in 6 mL of TSB medium supplemented with L-Tryptophan (1 mg/mL), were seeded. Subsequently, these were incubated at 30 °C and 100 rpm for four days.

The Salkowski reagent was employed to establish the IAA concentration; it was prepared using a 15 mL solution of $FeCl_3 \cdot 6\ H_2O$ (0.5 M) to which 300 mL of H_2SO_4 (98 %) and 500 mL of distilled water were added (Acuña et al., 2011). At the end of the incubation period, the tubes were centrifuged at 3,000 rpm for 15 min, and 1 mL of supernatant was taken and mixed with 4 mL of Salkowski reagent; then, it was left to stand at room temperature in the dark for 30 min. At the end of this time, the absorbance at 530 nm was measured in a JASCO V-730 spectrophotometer in plastic cells. Finally, a standard curve was designed from known concentrations, and the absorbance data obtained from the samples were replaced in the equation of the standard curve to establish the ppm of IAA produced by each strain.

Phosphate solubilization

The strains were reactivated in test tubes with nutrient broth and incubated at 37 °C for 48 hours. The NBRIP culture medium was prepared with the formulation used by Tejera et al. (2013) (Glucose, 10 g; Ca_3PO_4, 5 g; $MgCl_2\ 6H_2O$, 0.25 g; KCl, 0.2 g; $(NH_4)_2\ SO_4$, 0.1 g; distilled water, 1 L). The medium was sterilized at 121 °C for 15 min and 1-atmosphere pressure. The tricalcium phosphate was sterilized separately and added to the sterile medium NBRIP. A volume of 200 µL of the active bacteria was seeded in 100 mL flasks with 50 mL of NBRIP medium and incubated at 30 °C with shaking at 100 rpm for seven days (Rodríguez et al., 2013).

Likewise, the Mo-Blue reagent was prepared with solutions of sulfuric acid (H_2SO_4, 5 N), antimony and potassium tartrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$), ammonium molybdate ($(NH_4)_6Mo_7O_{24}$) and ascorbic acid ($C_6H_8O_6$, 0.1 M) using the methodology of Bobadilla and Rincón (2008).

After the incubation period, 2 mL of the sample was centrifuged at 5,000 rpm, and a volume of 500 µL of the supernatant was extracted and mixed with 400 µL of Mo-Blue reagent. Subsequently, it was adjusted to 2 mL with distilled water and measured by spectrophotometry at 880 nm with the JASCO V-730

equipment. The absorbance data obtained was replaced in the equation of the standard curve to determine the concentration (ppm) of phosphates solubilized by each strain.

Field assays

The field assay was carried out in the Tungurahua province, Ambato canton, and Izamba parish at an altitude of 2,590 meters above the sea level, with an average temperature of 14.6 °C and average humidity of 68.6 %.

From the two best bacterial strains obtained in the laboratory assays, five treatments were implemented, including one control, and three repetitions for each treatment, for a total of 15 experimental units per horticultural species. The treatments analyzed were T1: strain 1 with a single application; T2: strain 1 with a weekly application; T3: strain 2 with one application; T4: strain 2 with a weekly application; T5: control. Further, 338-hole germination trays were used, comprising each one experimental unit. For broccoli, the variety Avenger (Sakata) was used, and for lettuce, the variety Coolguard (PanDia Seeds) was employed. Biopreparations were made with the two selected strains based on the methodology described by Acurio et al. (2020) and applied to the germination trays at the concentration of $1x10^6$ CFU/mL with manual spray pumps.

The variables evaluated were stem length and thickness, dry matter, and root length and weight. The stem length and thickness data were registered every seven days using a Vernier caliper, and these were analyzed using the area under the curve methodology described by Navarro (2010). For dry matter, in week 6, the aerial part of the plant was separated and dried in paper bags in an oven at 72 °C for 48 hours. After drying, the weight was measured on an analytical balance. Similarly, in week 6, the variable root length was recorded with the aid of a Vernier caliper, and the root weight was measured with an analytical balance.

Statistical analysis

The data obtained were processed using an analysis of variance and Tukey's post hoc test ($\alpha = 0.05$) in the Infostat software version 2016.

Results and discussion

The sequences obtained were analyzed in NCBI Blast (N). Thus, the identification of the four strains used in the research was obtained (table 1).

Table 1. Molecular identification of bacterial species

Strain	Species	Identity percentage
IB10	*Bacillus licheniformis*	100 %
CT11	*Bacillus megaterium*	99 %
AB8	*Bacillus subtilis*	99 %
CT5	*Bacillus megaterium*	96 %

Source: elaborated by the authors

Bacterial capacity to promote *in vitro* plant growth

The colorimetric change of the NFB culture medium from green to blue occurred in all the Petri dishes after 24 hours and indicated the fixation of environmental nitrogen. Likewise, the growth of separate colonies was observed.

Posada (2017) established the presence of *nifU, nifS, nifV,* and *nifF* in *B. subtilis,* which are involved in the formation of clusters of the nitrogenase enzyme responsible for biological nitrogen fixation. Martínez et al. (2013), in their research, showed that strains of *Bacillus megaterium* and *Bacillus subtilis* grew in the NFB culture medium. On the other hand, Mota (2013) showed that *B. licheniformis* is potentially capable of fixing nitrogen; however, its growth is slow when glucose and malic acid are used as substrates, supporting the slow development of colonies that could be seen in the trials carried out.

For the quantification of indoleacetic acid, the standard curve was determined from known concentrations (table 2) and the equation of the curve y = 0.0157x - 0.0163 was obtained with an $R^2 = 0.9967$ (figure 1).

Table 2. Absorbances for the indoleacetic acid (IAA) calibration curve

PPM	ABS 530 nm ± sd
10	0.1588 ± 0.0001
15	0.1960 ± 0.0027
20	0.3022 ± 0.0076
25	0.3712 ± 0.0008
30	0.4545 ± 0.0012
35	0.5367 ± 0.0005
40	0.6277 ± 0.0018
45	0.6840 ± 0.0002
50	0.7680 ± 0.0019

Note: The data correspond to the average of three repetitions.

Source: elaborated by the authors

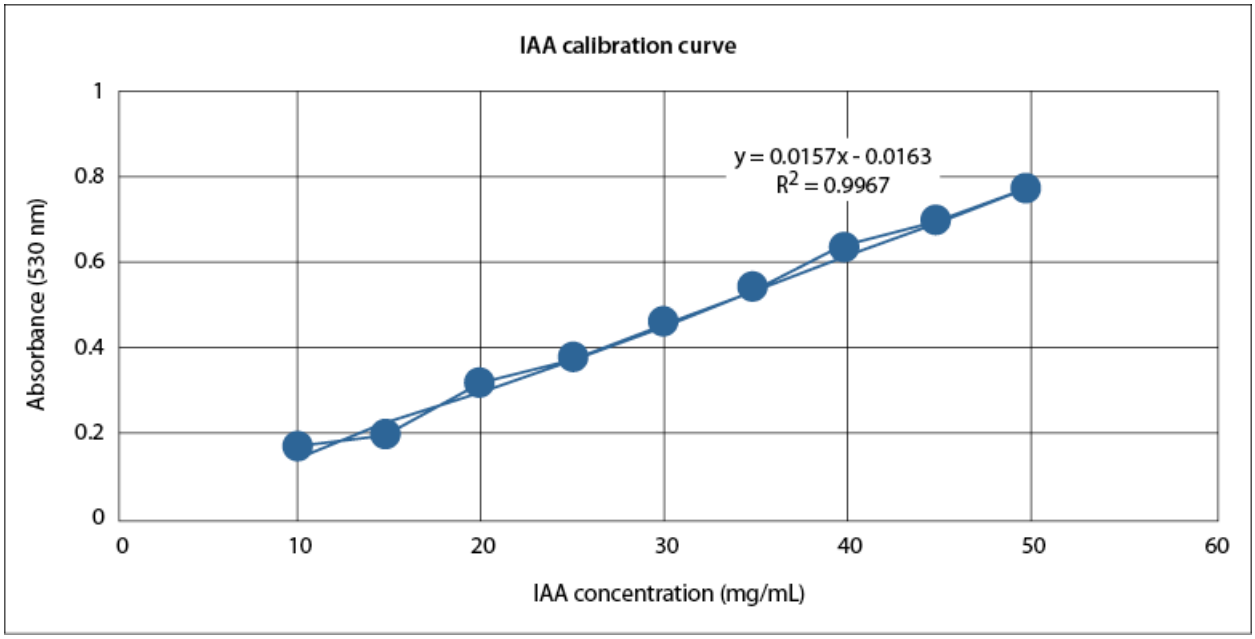

Figure 1. Indoleacetic acid (IAA) calibration curve at 530 nm.
Source: elaborated by the authors

Correspondingly, the standard curve for phosphate absorbance was established from known concentrations (table 3), and the equation of the curve y = 0.0651x - 0.119 was obtained with an R^2 = 0.9898 (figure 2).

Table 3. Absorbances for the phosphate calibration curve

PPM	ABS 880 nm ± sd
1	0.1582 ± 0.0002
2	0.2546 ± 0.0004
4	0.3960 ± 0.0021
6	0.5437 ± 0.0148
8	0.6184 ± 0.0042
10	0.7623 ± 0.0025

Note: The data correspond to the average of three repetitions.

Source: elaborated by the authors.

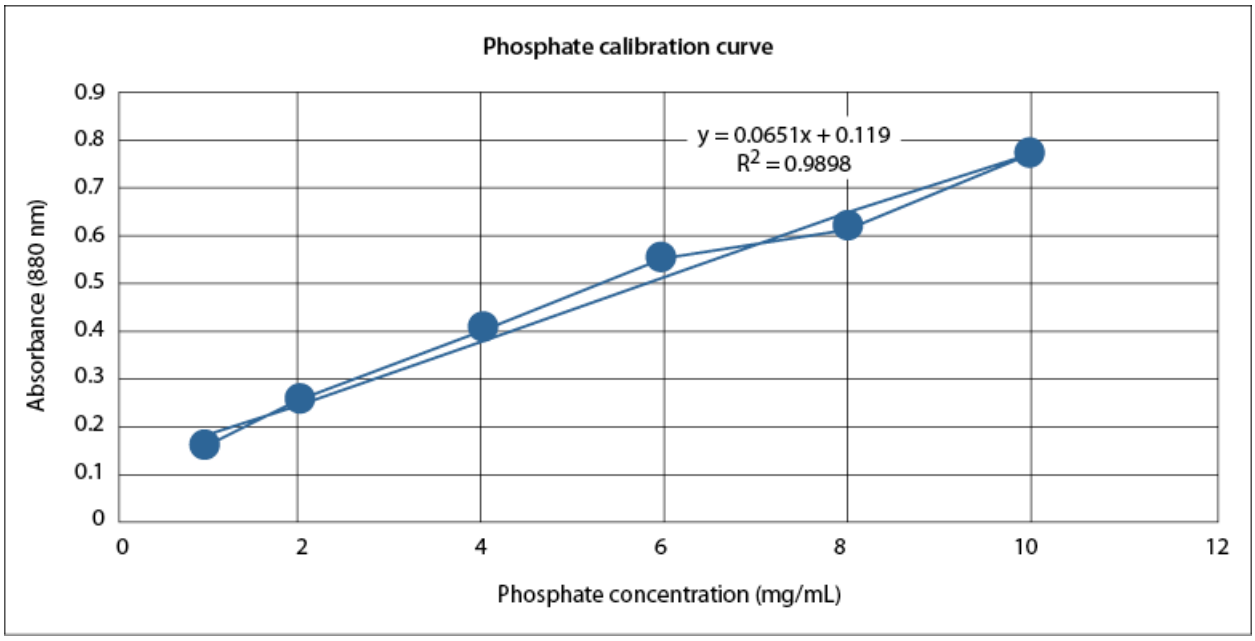

Figure 2. Phosphate calibration curve at 880 nm.
Source: elaborated by the authors

The studied strains were able to produce IAA and solubilize phosphates (table 4).

Table 4. *In vitro* plant growth-promoting bacterial capabilities

Strain	Production of indoles (µg/mL)	Strain	Phosphorous solubilization (µg/mL)
IB10	11.4050 ± 0.0879 a	CT11	43.2257 ± 0.0799 a
CT11	8.9040 ± 0.4550 b	IB10	41.7501 ± 1.7302 a
AB8	8.5503 ± 0.1904 b	CT5	35.9090 ± 0.8974 b
CT5	7.0426 ± 0.3695 c	AB8	35.2631 ± 1.6154 b
Control	1.2907 ± 0.0088 d	Control	1.9054 ± 0.1385 c

Means in the same column with a common letter are not significantly different, according to Tukey's test ($p < 0.05$). The data correspond to the mean of three repetitions with their respective standard deviation.

Source: elaborated by the authors

The analysis of variance showed that highly significant statistical differences were found for the production of indoles and phosphate solubilization ($p < 0.01$) with respect to the control. The *Bacillus licheniformis* strain IB10 and *Bacillus megaterium* strain CT11 registered the highest averages.

Various studies report the ability to produce auxins by *Bacillus* strains, such as the one conducted by Tejera et al. (2011). In this study, the authors quantified auxins using the Salkowski colorimetric method, with results ranging from 1 to 17 µg/mL for *Bacillus* spp. Specifically, Posada (2017) determined that *B. subtilis* produces total indoles at a concentration of 15 µg/mL using 500 mg/mL L-tryptophan. For *B. megaterium*, Chávez et al. (2016) reported concentrations of 6.3 to 7.1 mg/L in culture medium supplemented with 1 mg/mL of tryptophan; the *B. megaterium* strain used in this investigation obtained a higher value, i.e., 8.9 mg/mL. For *B. licheniformis*, concentrations of 35.15 µg/mL have been obtained in liquid culture medium with K-lactate, supplemented with tryptophan at 100 mg/L (Sánchez & Pérez, 2018). In contrast to the results obtained in this research, the *B. licheniformis* strain produced 11.4 µg/mL.

Regarding the solubilization of phosphates, Tejera et al. (2013) identified *Bacillus* strains with the ability to solubilize inorganic phosphates in liquid NBRIP medium, at soluble phosphate concentrations of 0.23 and 11.58 mg/L. On the other hand, Chávez et al. (2016) obtained in their research, soluble phosphorus concentrations ranging from 26.0 to 134.6 mg/L, where *Bacillus* strains grew. The values obtained in this test were 43.2 mg/L and 41.7 mg/L for *Bacillus megaterium* and *Bacillus linchenifromis*, respectively.

Field assays

The strains that obtained the best results in the laboratory assays and evaluated in the field were IB10 (*B. licheniformis*) and CT11 (*B. megaterium*) (table 5).

Table 5. Description of the applied treatments

Treatments		
T1	*B. licheniformis*	A single application when planting
T2	*B. licheniformis*	Weekly application
T3	*B. megaterium*	A single application when planting
T4	*B. megaterium*	Weekly application
T5	Control (Water)	

Source: elaborated by the authors

Evaluation of the response of lettuce (*Lactuca sativa*) to the inoculation with plant growth-promoting bacteria

At least one of the applied treatments presented a significant difference in seedling height and dry matter with a value of $p < 0.01$, while for thickness, there was no statistical difference (table 6).

Table 6. Area under the curve (AUC) for height and mean dry matter

Treatment	AUC mean for height ± sd	Treatment	Mean dry matter (g) ± sd
T2	1154.84 ± 44.90 a	T4	0.11 ± 0.002 a
T4	1149.78 ± 81.52 a	T3	0.09 ± 0.01 ab
T3	1083.62 ± 26.07 a	T2	0.09 ± 0.01 ab
T1	1014.17 ± 117.39 ab	T1	0.08 ± 0.002 b
T5	846.49 ± 121.88 b	T5	0.05 ± 0.005 c

Means in the same column with a common letter are not significantly different, according to Tukey's test ($p < 0.05$). The data correspond to the mean of three repetitions with their respective standard deviation.

Source: elaborated by the authors

In this investigation, 26.7 % more leaf growth was obtained compared to the control plants in lettuce. Martínez et al. (2013) mentioned that the production of IAA by PGPR promotes radical and vegetative development in lettuce seedlings, translated into higher seedling height.

In the research carried out by Stoll et al. (2018) in which bacterial strains were inoculated to lettuce seedlings, a significant increase in the fresh weight of the plant was observed in relation to the non-inoculated control since up to 30 % more weight was obtained due to its PGPR action. Sánchez et al. (2014) evaluated consortia of bacterial strains in lettuce (*L. sativa*) plants, a treatment that obtained 102 % more dry matter compared to the chemical treatment, due to better nutrition of seedlings as a result of higher root growth.

Maccarrone et al. (2016) showed higher growth and dry matter percentage compared to the control. Likewise, these authors demonstrated a higher increase in root development in plants treated with the bacterial suspension, by inoculating 1 x 10^7 CFU/mL of the *Bacillus* strain in lettuce seedlings in a 55-day trial. The application was made on the soil and leaves.

In the analysis of the root length, all the bacterial treatments compared to the control stood out, while for root weight, only treatment 4 stood out (table 7).

Table 7. Root length and weight

Treatment	Mean root length (mm) ± sd	Mean root weight (g) ± sd
T4	59.75 ± 3.17 a	0.28 ± 0.017 a
T3	58.73 ± 4.20 a	0.23 ± 0.019 b
T2	58.73 ± 4.20 a	0.23 ± 0.019 b
T1	54.18 ± 2.19 ab	0.06 ± 0.002 c
T5	47.67 ± 4.34 b	0.04 ± 0.015 c

Means in the same column with a common letter are not significantly different, according to Tukey's test ($p < 0.05$). The data correspond to the mean of three repetitions with their respective standard deviation.

Source: elaborated by the authors

Evaluation of the response of broccoli (*Brassica oleracea* var. *italica*) to the inoculation with plant growth-promoting rhizobacteria under greenhouse conditions

At least one of the applied treatments showed a significant difference in seedling height, thickness, and dry matter with a p-value < 0.01 (table 8).

Table 8. Area under the curve (AUC) for height and thickness and mean dry matter

Treatment	AUC mean height ± sd	AUC mean thickness (mm) ± sd	Treatment	Mean dry matter (g) ± sd
T2	1630.31 ± 72.367 a	21.23 ± 1.842 a	T2	0.13 ± 0.003 a
T4	1596.12 ± 49.947 ab	20.55 ± 0.579 a	T4	0.11 ± 0.046 ab
T1	1539.73 ± 106.557 abc	19.39 ± 0.577 a	T3	0.09 ± 0.005 abc
T3	1424.49 ± 12.536 bc	15.01 ± 2.415 b	T1	0.07 ± 0.004 bc
T5	1406.60 ± 53.465 c	14.40 ± 0.769 b	T5	0.05 ± 0.001 c

Means in the same column with a common letter are not significantly different, according to Tukey's test ($p < 0.05$). The data correspond to the mean of three repetitions with their respective standard deviation.

Source: elaborated by the authors

By inoculating seedlings with PGPR, these increase their length and thickness proportionally because the bacteria associated with the root produce phytohormones such as IAA, and increase nitrogen fixation and phosphate solubilization (Vivanco et al., 2016). These results were compared with the results obtained in the current investigation, where 13.7 % more leaf growth was obtained compared to the broccoli control plants.

Gutiérrez et al. (2019) determined that rhizobacteria induce sufficiency ranges in P, Ca, Fe, and Mn, which was demonstrated with the highest content of dry matter obtained compared to the control plants. This explains the ability of the applied strains to solubilize poorly mobile compounds in the soil, such as phosphates, thereby improving the assimilation of this macronutrient.

In the analysis of root length, treatments 2 and 4 stood out, meanwhile, treatment 4 was highlighted for its root weight, even though it is statistically similar to treatment T2, but with a probabilistic difference concerning the other treatments (table 9).

Table 9. Root length and weight

Treatment	Mean root length (mm) ± sd	Treatment	Mean root weight (g) ± sd
T4	92.70 ± 5.971 a	T4	0.21 ± 0.016 a
T2	92.18 ± 1.606 a	T2	0.20 ± 0.005 ab
T3	81.69 ± 0.451 b	T1	0.17 ± 0.019 bc
T1	80.66 ± 0.186 b	T3	0.17 ± 0.014 bc
T5	77.13 ± 4.206 b	T5	0.15 ± 0.008 c

Means in the same column with a common letter are not significantly different, according to Tukey's test ($p < 0.05$). The data correspond to the mean of three repetitions with their respective standard deviation.

Source: elaborated by the authors

According to Sánchez et al. (2014), inoculation with bacteria can improve the radical development of plants, by increasing the absorption capacity of water and nutrients in crops, through the production of growth regulators that are associated with the root interface. However, the efficiency of the microorganisms can be affected by some biotic conditions, such as synergistic and antagonistic responses or the phenological state of the plant (Bhattacharyya & Jhan, 2012). Furthermore, Yildirim et al. (2010) performed the isolation of *Bacillus* strains and produced a bacterial suspension at a concentration of 1 x 10^8 CFU/mL, which was applied directly to the broccoli seedling root. The authors demonstrated that the application of the suspension increases the total performance by 31 %, the performance of the head by 25 %, and the diameter of the head by 16 %. This is explained by the increase in nitrogen fixation and the production of phytohormones by *Bacillus* strains. Nonetheless, these authors also demonstrated that the efficiency in the application of the bacterial suspension increases if organic basal fertilization is also carried out since, during the reproductive period, the accumulated minerals will pass to the reproductive parts of the plant.

Conclusions

The molecular analysis of the four bacterial strains of the *Bacillus* genus determined that they belong to the species *B. licheniformis* (IB10)*, B. megaterium* (CT11)*, B. subtilis* (AB8), and *B. megaterium* (CT5). In the auxin production test, *B. licheniformis* obtained 11.40 µg/mL, followed by *B. megaterium* with 8.90 µg/mL. Regarding the phosphorus solubilization test, *B. megaterium* obtained a value of 43.22 µg/mL and *B. licheniformis* recorded a value of 41.75 µg/mL.

Weekly applications of *B. megaterium* in lettuce seedlings significantly influenced the increase in dry matter, root length, and root weight; on the other hand, in the height variable, better results were obtained with weekly applications of B. *licheniformis* compared to the control. In broccoli seedlings, the weekly application of *B. licheniformis* significantly influenced the increase in height, thickness, and dry matter, while with weekly applications of *B. megaterium*, the length and weight of the root showed higher values, in comparison with the control.

Finally, in both horticultural species, a positive effect on plant development was evidenced with the application of bacteria belonging to the *Bacillus* genus.

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Disclaimers

All the authors made significant contributions to the document, agree with its publication, and declare that there are no conflicts of interest in this study.

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