Rhizospheric plant growth-promoting bacteria (PGPR) in corn plants

Bacterias rizosféricas promotoras del crecimiento vegetal (PGPR) en plantas de maíz

Bactérias promotoras do crescimento vegetal rizosférico (PGPR) em plantas de milho

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Abstract

PGPR are considered a sustainable alternative to improve crop productivity, for its ability to biostimulate plant growth, induce systemic resistance, increasing tolerance to abiotic stress, among other benefits. The objective of the study was to evaluate the effect of plant growth-promoting rhizosphere bacteria (PGPR) on the germination and development of corn plants. Seven strains obtained from the Research Center of of the Santa Elena Peninsula State University, Ecuador, were reactivated, corn seeds were inoculated, and planted to evaluate germination and plant development in two stages (laboratory and nursery). The rhizobacteria significantly promoted germination by up to 17 %, emergence, and initial growth of corn, especially the species *Stenotrophomonas pavanii* and *Pantoea dispersa*. In addition, *P. dispersa* (b) species increased stomatal density on both leaf surfaces, which could be associated with better photosynthetic efficiency and water use. In conclusion, *S. pavanii* and *P. dispersa* strains promote germination and growth of Azor corn, the phylogenetic analysis indicates close groupings with reference isolates for their efficacy with significant potential such as (PGPR) with documented biotechnological capabilities for the genera *Pantoea* and *Stenotrophomonas*.

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Resumen

Las PGPR se consideran una alternativa sostenible para mejorar la productividad de los cultivos, por su capacidad de bioestimular el crecimiento vegetal, inducir resistencia sistémica, aumentar la tolerancia al estrés abiótico, entre otros beneficios. El objetivo del estudio consistió en evaluar el efecto de las bacterias rizosféricas promotoras del crecimiento vegetal (PGPR) sobre la germinación y el desarrollo de plantas de maíz. Se reactivaron siete cepas obtenidas del Centro de Investigación de la Universidad Estatal Península de Santa Elena, Ecuador, se inocularon semillas de maíz y fueron sembradas para evaluar la germinación y el desarrollo vegetal en dos etapas (laboratorio y vivero). Las rizobacterias promovieron significativamente la germinación hasta un 17 %, la emergencia y crecimiento inicial del maíz, especialmente las especies *Stenotrophomonas pavanii* y *Pantoea dispersa*. Además, la especie *P. dispersa* (b) aumentó la densidad estomática en ambas superficies foliares, lo que podría estar asociado con una mejor eficiencia fotosintética y un mejor uso del agua. Las cepas *S. pavanii* y *P. dispersa* promovieron la germinación y el crecimiento del maíz Azor, el análisis filogenético indicó agrupaciones cercanas con aislados de referencia por su eficacia con potencial significativo como (PGPR) con capacidades biotecnológicas documentadas para los géneros *Pantoea* y *Stenotrophomonas*.

Palabras clave: *Stenotrophomonas pavanii*, *Pantoea dispersa*, densidad estomática, *Zea mays*, pastos, rizobacterias.

Resumo

As PGPR são consideradas uma alternativa sustentável para melhorar a produtividade das culturas, por sua capacidade de bioestimular o crescimento vegetal, induzir resistência sistêmica, aumentar a tolerância ao estresse abiótico, entre outros benefícios. O objetivo do estudo consistiu em avaliar o efeito das bactérias rizosféricas promotoras do crescimento vegetal (PGPR) sobre a germinação e o desenvolvimento de plantas de milho. Sete cepas obtidas do Centro de Pesquisa da Universidade Estadual Península de Santa Elena, Equador, foram reativadas, inoculadas em sementes de milho e plantadas para avaliar a germinação e o desenvolvimento vegetal em duas etapas (laboratório e viveiro). As rizobactérias promoveram significativamente a germinação em até 17 %, a emergência e o crescimento inicial do milho, especialmente as espécies *Stenotrophomonas pavanii* e *Pantoea dispersa*. Além disso, as espécies *P. dispersa* (b) aumentaram a densidade estomática em ambas as superfícies foliares, o que pode estar associado a uma melhor eficiência fotossintética e uso de água. Em conclusão, as cepas *S. pavanii* e *P. dispersa* promovem a germinação e o crescimento do milho Azor, a análise filogenética indica agrupamentos próximos com isolados de referência por sua eficácia com potencial significativo, como (PGPR) com capacidades biotecnológicas documentadas para os gêneros *Pantoea* e *Stenotrophomonas*.

Palavras-chave: *Stenotrophomonas pavanii*, *Pantoea dispersa*, densidade estomática, *Zea mays*, pastagens, rizobactérias.

Introduction

The use of plant growth-promoting rhizosphere bacteria (PGPR) is considered a sustainable strategy to improve plant production (Bhardwaj *et al*., 2014). These bacterial strains biostimulate plant development producing several mechanisms that promote it, such as induced resistance (ISR), and the production of bioactive compounds (Backer *et al*., 2018), tolerance to abiotic stress (de Andrade *et al*., 2023), improvements in photosynthetic efficiency and antioxidant activity, as well as the bioproduction of siderophores and exopolysaccharides (Ferrante *et al*., 2024).

The most commonly reported PGPR microbial strains are genera of *Bacillus*, *Pseudomonas*, and *Enterobacter*, who's most investigated beneficial mechanisms include indoleacetic acid production, solubilize phosphorus and produce siderophores (Posada *et al*., 2021). These genera of native bacteria provide significant benefits to soils for agriculture, acting as natural biocontrol agents protecting crops from pathogens while promoting plant growth by solubilizing nutrients and producing plant hormones.

In tropical areas, several native PGPR species such as *Pantoea dispersa* and *Enterobacter asburiae*, rhizosphere isolations of sugarcane, increased height and weight, the manifestation of defense genes such as tolerance to extreme pH variations and osmotic imbalance (Singh *et al*., 2021). Regarding the use of microbial associations, studies by Alonazi *et al*. (2025) highlight positive effects of five native genera (*Azotobacter*, *Bacillus*, *Paenibacillus*, *Pantoea*, and *Pseudomonas*), which significantly increased productivity in maize plants.

Species such as *Stenotrophomonas maltophilia* (Upadhyay & Chauhan, 2022), and *Achromobacter piechaudii* (Danish *et al*., 2020), have been shown to generate high auxin production with the capacity to form biofilms, increase biomass and root growth in maize crops (Ríos-Ruiz *et al*., 2024); they also have shown be able to tolerate high levels of sodium (Peng *et al*., 2021), and grow well in soils with extreme pH (Wahab *et al*., 2024). An increasing number of rhizobacteria are being reported to be used in the creation of biocontrol agents and biofertilizers as a biotechnological strategy for agriculture (Rivera-Hernández *et al*., 2024).

However, phenotypic expressions induced by PGPR under stress conditions remain to be investigated, and a faster response would be the reduction of transpiration after closing their stomata (Bresson *et al*., 2013). Zhang *et al*. (2025) mention that stomata in maize Jingnongke 728 improve photosynthetic carbon assimilation and water efficiency, with a stomatal density ranging of 87.19 stomata.mm^{-2}. In this context, the aim of this study was to assess the influence of rhizospheric plant growth-promoting bacteria (PGPR) on maize plants. These bacteria could have great biotechnological potential in maize cultivation due to their enhanced biostimulant activity compared to conventional alternatives, potentially improving crop performance and development. In this context, the study aimed to evaluate the effect of plant growth-promoting rhizosphere bacteria (PGPR) on the germination and development of corn plants

Materials and methods

Bacterial strains and plant material

The bacterial species were obtained and selected for their PGPR characteristics from the microorganism strain bank of the Centro de Investigación Biotecnológica (CEB, according its acronyms in Spanish) of Universidad Estatal Península de Santa Elena, Ecuador, isolated from grass cultivars (*Pennisetum purpureum* cv. King Grass and *Panicum maximum* cv. Tanzania), as indicated in table 1. The genotype of seed used for tropical climate corresponded to the Azor corn hybrid from the Advanta® brand.

Table 1. Rhizospheric plant growth-promoting bacteria species selected for study.

Cultivate	Species	Identity percentage (%)	Accesion
Tanzania	*Pantoea dispersa* (a)	100.00	KF668475.1
Tanzania	*Enterobacter asburiae*	99.40	MT664184.1
Tanzania	*Stenotrophomonas pavanii*	99.13	PP703128.1
Tanzania	*Achromobacter xylosoxidans*	97.72	NR_113733.1
King grass	*Pantoea dispersa* (b)	100.00	MH675511.1
King grass	*Stenotrophomonas* sp.	100.00	OP389132.1
King grass	*Stenotrophomonas geniculata*	100.00	MT672503.1

Reactivation and growth of bacterial species

The isolates were reactivated in Yeast Mannitol Agar medium (YMA), by simple streaking and incubated (Memmert, model BE200, Germany) at 30.5 °C for 48 hours, then transferred to a test tube with 10 mL of Yeast Mannitol Broth (YMB), placing 1 uL of colony suspension for each strain and incubated under the same conditions and under agitation (Boeco, model MSH420, Germany) at 70 revolutions per minute (r.p.m.).

The preparation was centrifuged (THERMO SCIENTIFIC, MYSPIN6 model, Sweden) at 5.000 rpm for 24 h, where its absorbance was evaluated in the spectrophotometer (Boeco S-220 UV/VIS, Germany) at 600 nm; using the YMB without inoculating as a blank, followed by resuspension with distilled water until reaching a cell population of $1x10^6$ UFC.mL^{-1} using the Mac Farland scale (Rayyif *et al*., 2022).

Experimental design

The study consisted of two stages: (1) *in vitro* under laboratory conditions; and (2) *in situ* under nursery conditions. The experimental design was a completely randomized design (CRD) consisting of nine treatments with three replicates in the first stage. For each treatment, 72 seeds were inoculated, with one germination tray (Gardener's Supply Company, 24 wells) constituting an experimental unit. The treatments were designated as follows: T1 (*P. dispersa* (a)), T2 (*E. asburiae*), T3 (*S. pavanii*), T4 (*A. xylosoxidans*), T5 (*P. dispersa* (b)), T6 (*Stenotrophomonas* sp.), T7 (*S. geniculata*), T8 (distilled water control), and T9 (gibberellic acid control; NEW GIBERNED® from NEDERAGRO S.A.). In the second stage, 18 germinated seedlings were chosen from each of the six top-performing treatments, which included the controls from the *in vitro* phase.

***In vitro* stage**

Cleansing, disinfection and inoculation of seeds

The seeds were washed with distilled water to remove impurities. They were then disinfected for three minutes with 5 % sodium hypochlorite, rinsed with sterile water and finally immersed in 70 % ethanol for two minutes. A 25 % glucose solution was soaked in the seeds, then inoculated by immersion using 1.000 mL of the culture broth at a concentration ($1x10^6$ CFU.mL^{-1}) of each strain according to the treatments, for 10 minutes (Soto *et al*., 2016).

The inoculated seeds were placed in germination trays containing paper towels moistened with sterile distilled water and stored at 28 °C under constant darkness for seven days. After this first inoculation, the germination percentage (GP) of each treatment was evaluated, using the formula:

$$GP = \frac{\text{N° of germinates seeds}}{\text{Total N° of seeds}} \text{ X } 100$$

Ten seedlings were randomly evaluated from each replicate per treatment, in order to measure the height (cm) and stem diameter (cm).

***In situ* stage**

Sowing, re-inoculation and irrigation

Considering the seedling uniformity, three germinated seeds were sown per pot and repetition, which contained 1 kilogram of previously sterilized substrate. At 8 days after transplanting, the second inoculation was carried out, adding 1 mL of bacterial suspension ($1x10^6$ CFU.mL^{-1}) on each plant collar (Soto *et al*., 2016). 15 days after transplantation (dat), non-viable seedlings were removed from each experimental unit to preserve the best phenological characteristics, leaving one plant per pot. Irrigation was carried out every two days during both phases, to avoid waterlogging. During this stage, the variables were evaluated:

Measurement of stomatal frequency

A mature leaf was randomly selected from each experimental unit of the same physiological age to quantify the stomatal frequency on both sides of the leaves (adaxial and abaxial surface). A cut of the epidermis was made by taking a 1 cm^2 punch; a grid of the same size was placed on the 10X objective of the microscope (BOECO®, Germany). The total number of stomata within that square was counted by repeating in three sectors of each leaf, expressed as stomatal index (SI), under the formula $SI = \frac{100 \text{ x stomatal number}}{\text{stomatal number + cell number}}$ (Bresson *et al*., 2013).

Agronomic parameters of maize at 30 days after transplantation

The variables were evaluated: AL: Aerial length (cm); SD: Stem diameter (cm); NL: Number of leaves; RL: Root length (cm); AW: Aerial weight percentage (%); RW: Root weight percentage (%); AFW: Aerial fresh weight (g); ADW: Aerial dry weight (g); RFW: Root fresh weight (g); RDW: Root dry weight (g).

Phylogenetic analysis

The identification of the 7 species was carried out, the phylogenetic analysis based on fragments of the 16S rRNA gene hypervariable region V4, including 6 strains published as PGPR in corn and alfalfa crops, of reference sequences from the NCBI GenBank database. For sequence alignment, ClustalW and BlastN were employed. High-similarity sequences were selected for subsequent phylogenetic analysis of the 16S rRNA gene, conducted in MEGA X using the Neighbor-Joining method. This method provides an optimal balance of speed and reliability for classifying microorganisms at the genus and species level. Genetic divergence was calculated based on direct sequence differences, and the robustness of the phylogenetic tree was assessed via a bootstrap analysis with 1,000 replicates.

Statistical analysis

The results were subjected to an analysis of variance (ANOVA) using the F test at 0.05. The Shapiro-Wilk test was used to assess normality, and Levene's test was used to assess homogeneity of variances. Duncan's multiple range test was used to assess the differential effects between treatments, as it provides greater accuracy in detecting true differences with a p-value <0.05. The statistical software InfoStat version 2020 (Di Rienzo *et al*., 2020) was used for data analysis.

Results and discussion

Seed germination percentage

The statistical analysis revealed no significant differences among the treatments, as presented in table 2. However, it is worth mentioning

that some strains increased the germination percentage of maize seeds compared to controls by 10 to 17 %. A numerical disparity was presented in the T3 treatment (*S. pavanii*), with 86 % of germinated seeds.

Table 2. Effects of PGPR inoculation on germination rate, seedling diameter and height.

Treatments	Germination (%)	Seedling diameter (cm)	Seedling height (cm)
T1	80 a	2.04 ab	**9.67 a**
T2	75 a	1.54 d	6.15 bc
T3	**86 a**	**2.18 a**	**10.40 a**
T4	72 a	1.93 b	8.61b
T5	81 a	2.11 ab	**10.46 a**
T6	64 a	1.67 d	6.41bc
T7	51 a	1.71cd	3.97 c
T8	69 a	2.03 ab	**9.87 a**
T9	71 a	1.90 bc	7.91 b
S.E.	12.02	0.06	3.03
σ	20.83	0.11	6.91
F	0.77	11.36	7.83
p-value	<0.0001	<0.0001	0.0003
gl	26	26	26

T1 (*P. dispersa* (a)), T2 (*E. asburiae*), T3 (*S. pavanii*), T4 (*A. xylosoxidans*), T5 (*P. dispersa* (b)), T6 (*Stenotrophomonas* sp.), T7 (*S. geniculata*), T8 (distilled water control), T9 (gibberellic acid control). Values represent mean ± SE or σ. Different letters within a column signify statistically significant differences ($p<0.05$; Duncan's multiple range test).

The germination-promoting effect of rhizobacteria in various plant crops may be closely related to the production of phytohormones (cytokinins, auxins, gibberellines, etc.) information consistent with what indicated by Noumavo *et al*. (2013) and Eshaghi Gorgi *et al*. (2022) in corn seeds inoculated with species of *Enterobacter* and *Pantoea* genus.

Seedling height and diameter

The results for seedling height and diameter are shown in table 2. The ANOVA and the test of means indicate differences between treatments. For seedling diameter, the treatment that showed the best results was T3 (*S. pavanii*) at 2.18 cm, regarding seedling height, treatments T5 (*P. dispersa* (b)) and T3 (*S. pavanii*) showed the best values, with 10.46 and 10.40 cm, respectively.

Soto *et al*. (2016) in their evaluation regarding the inoculation of native rhizobacteria in two maize hybrids, showed a higher seedling height (13.60 cm) in seeds treated with a bacteria/fertilizer consortium. Amezquita-Aviles *et al*. (2022) reported that several isolated PGPR stood out for showing increases of 35-40 % in plant height, in seeds of Mexican Creole maize. Eshaghi *et al*. (2024) findings showed that PGPR are effective in enhancing maize plant diameter, this type of bacteria is potentially active. It can increase the availability of nutrients in the rhizosphere by capturing them (Waday *et al*., 2022).

Agronomic parameters of maize at 30 days after transplantation

Table 3 shows the results of a statistical analysis on agronomic parameters in maize cultivation 30 days after transplantation (dat). Statistical differences were observed in the parameters of aerial length (AL), treatment T5 (*P. dispersa* (b)) has the highest value and T9 (Gibberellic acid (Control)) the lowest. In stem diameter (SD), it is observed that the treatment T3 (*S. pavanii*) obtained the best average with 8.00 cm. The parameter number of leaves (NL) indicates that the treatments T3 (*S. pavanii*) and T5 (*P. dispersa* (b)) obtained the best averages with 5 leaves present in both cases. However, the variables root length, percentage of aerial weight, percentage of root weight, aerial fresh weight, aerial dry weight, root fresh weight and root dry weight did not show statistical differences between treatments. Despite this, the improvement of the averages in those treatments inoculated with PGPR rhizobacteria is notable.

Table 3. Averages in agronomic parameters of maize cultivation at 30 days after transplantation.

Treatments	Aerial length (cm)	Stem diameter (cm)	Number of leaves
T1	52.67 ab	6.33 bc	4.33 ab
T3	55.00 ab	8.00 a	5.00 a
T5	60.33 a	6.67 bc	5.00 a
T7	57.33ab	6.67 bc	4.67 ab
T8	55.33 ab	7.67 b	4.67 ab
T9	46.33 b	6.00 c	4.00 b
S.E.	3.49	0.48	0.07
σ	6.04	0.83	0.14

T1 (*Pantoea dispersa* (a)), T3 (*Stenotrophomonas pavanii*), T5 (*Pantoea dispersa* (b)), T7 (*Stenotrophomonas geniculata*), T8 (distilled water control), T9 (gibberellic acid control). Data are presented as mean ± standard error (SE) or standard deviation (σ). Within a row, mean values followed by different lowercase letters are not significantly different according to Duncan's multiple range test ($p>0.05$).

The data obtained in this work are consistent with those described by different authors, they confirm that the application of rhizobacteria biostimulates the development of corn crops (Vera *et al*., 2025). Thus, Soto *et al*. (2016) reports a greater development in aerial length (64.23 cm) at 30 days of cultivation, inoculated with native rhizobia strains. Pereira *et al*. (2020) evaluated various inoculation concentrations of single strains and consortia, which were found to improve biomass (fresh and dry) in maize under controlled conditions. According to Bouremani *et al*. (2023), they mention that the inoculation of plants with PGPR at early stages promotes root and sprout growth, resulting in significant increases in leaf and radical biomass in crops such as maize.

Stomatal density

The data obtained in the stomatal count submitted to the analysis of variance; they present statistically significant differences between the treatment being the T5 (*P. dispersa* (b)) which presented a greater number of stomata with 77 stomata.cm^2 adaxial and 86 stomas.cm^2 abaxial, for other treatments (Figure 1).

The results obtained in this work agree with what has been described by different authors, who corroborate that the inoculation of rhizobacteria promotes plant growth and augmentation in maize cultivation (Vera *et al*., 2025). Thus, Soto *et al*. (2016) report greater development in aerial length (64.23 cm) at 30 days of cultivation, inoculated with native rhizobia strains. Pereira *et al*. (2020) also evaluated different inoculation concentrations, single strains and consortia, and found enhancements in biomass (fresh and dry) in maize under controlled conditions. According to Bouremani *et al*. (2023), inoculation of plants with PGPR at early stages promotes root and shoot growth, resulting in significant increases in leaf and root biomass in crops such as maize.

Several authors such as Bresson *et al*. (2013) mention that one of PGPR inoculation effects in maize seedlings was a greater stomatal control in the closure of stomas to water stress, but without changes in stomatal density.

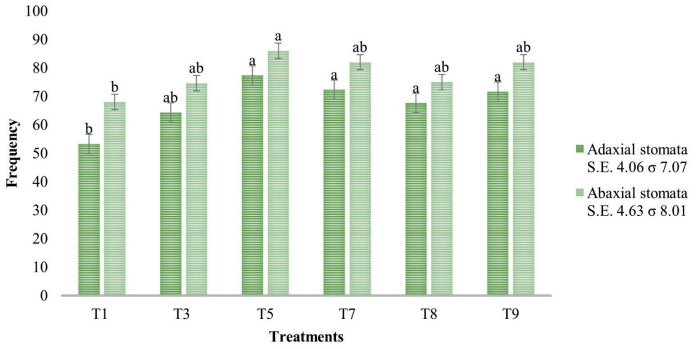

Figure 1. Stomatal density in maize leaves following inoculation with plant growth-promoting rhizobacteria (PGPR). T1 (*Pantoea dispersa* (a)), T3 (*Stenotrophomonas pavanii*), T5 (*P. dispersa* (b)), T7 (*S. geniculata*), T8 (distilled water control), T9 (gibberellic acid control). Data are presented as mean ± standard error. Bars sharing the same lowercase letter are not significantly different according to Duncan's multiple range test (p>0.05).

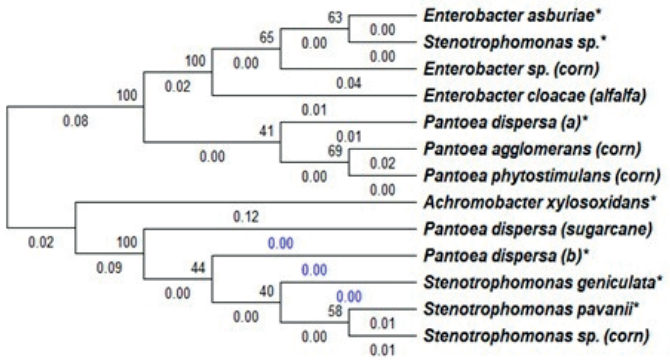

Figure 2. Phylogenetic tree based on 16S rRNA gene sequences of the studied plant growth-promoting rhizobacteria (PGPR) under study (*) and 6 strains published as PGPR in corn and alfalfa crops.

Liu *et al*. (2023), in their study on deficient irrigation with *Bacillus pumilus* in maize, found that stomatal density was not affected by inoculation; however, it was influenced by soil water status and the PGPR-moisture interaction. This indicates that the PGPR effect on stomata often depends on the environmental context (water stress, salinity, etc.). However, Serna (2022), in his work on stomatal response in maize to climate change, severe water deficit has been reported to decrease stomatal size, aperture, and density, possibly associated with the need for transpiration cooling in maize (Yuan *et al*., 2023). However, this work shows evidence that three of the six inoculated strains presented higher number of stomata in both foliar surfaces, with respect to water control; with significant results of *P. dispersa* (b) strain as effect of rhizobacteria, in the increase of stomatal frequency.

PGPR optimize plant physiology by hormonally regulating stomatal density, for example, by increasing leaf coverage and adjusting gas exchange to prevent dehydration. This bacterial mediation increases enzymatic activity and chlorophyll levels, thereby enhancing photosynthetic efficiency and carbon fixation.

Phylogenetic Analysis

Figure 2 shows the 16SrRNA gene sequences of the 7 strains inoculated in this work, together with the strains *P. dispersa* AA7 accession no. (MT557017.1), obtained from sugarcane; *P. agglomerans* (GQ225111), isolated from corn; *P. phytostimulans* (PQ300029.1), isolated from corn; *Stenotrophomonas* sp. (MT780855.1), isolated from corn roots; *Enterobacter* sp. (HM355806) and the *E. cloacae* strain (KJ668861.1), isolated from alfalfa crops. Interestingly, a tiny distance (0.01) is observed between the strains of *P. dispersa* (b), *S. pavaii* and *S. geniculata*; very close with *P. dispersa* and *Stenotrophomonas* sp., isolated from the rhizosphere of sugarcane and corn coincidentally reported with very good results in both cases.

The placement of the study strains in the phylogenetic tree alongside known reference sequences, such as *P. dispersa* (MT557017.1) and *Stenotrophomonas* sp. (MT780855.1), is particularly relevant. These reference species were isolated from the rhizosphere of important crops (sugarcane and corn) and have been reported to have very good results as PGPR (Mareque & Battistoni, 2025; Quach *et al*., 2025).

Pérez-Pérez *et al*. (2020) isolated and identified *S. pavanii* from the rhizosphere of maize, concluding that this species possesses characteristics that promote plant growth, biological nitrogen fixation, mineral solubilization, and phytohormone production. While *P. dispersa* has the ability to synthesize indoles, produce siderophores and solubilize phosphates (Amezquita-Aviles *et al*., 2022). This agrees with Guevara-López *et al*. (2025) microbial inoculants cause an increase in stomatal conductance, which is correlated with the consumption of intercellular carbon, leading to greater photosynthesis in the leaves and consequently a greater production of biomass in the corn crop.

Conclusions

The findings from both *in vitro* and *in situ* germination and vegetative growth assays demonstrate that the *Stenotrophomonas pavanii and Pantoea dispersa* strains, isolated from local agricultural soils and applied as seed inoculants to Azor hybrid maize, exert a pronounced biostimulant effect. Compared to the control, these treatments increased seed germination rates by up to 17 %, plant height by 6 %, and stem diameter by 7 %. Furthermore, inoculation with these strains resulted in increased foliar stomatal density-a PGPR-mediated response that is not widely documented in the scientific literature.

The robust phylogenetic clustering of these Santa Elena pasture isolates with effective reference strains, supported by their observed efficacy, strongly indicates that these native bacterial species hold significant promise as plant growth promoters. This finding is consistent with the documented biotechnological potential of the *Pantoea* and *Stenotrophomonas* genera.

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