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Enterobacter sp. DBA51 produces ACC deaminase and promotes the growth of tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotiana tabacum* L.) plants under greenhouse condition

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ABSTRACT

Bacterial isolated from rhizospheric soil associated with the semi-desertic plant *Coronilla juncea* L. were screened for 1-aminocyclopropane-1-carboxylate deaminase (ACCD) activity, a common trait for plant-growth-promoting rhizobacteria (PGPR). Among bacterial isolates, strain DBA51 showed phosphate solubilizing index (PSI), producing indole acetic acid (IAA), and with the hemolysis-negative test. Sequencing and analysis of the 16S rDNA gene identified DBA51 as *Enterobacter*. DBA51 did not show antagonistic activity *in vitro* against bacterial (*Clavibacter michiganensis, Pseudomonas syringae* pv. *tomato* DC3000 and *Pectobacterium cacticidum* FHLGJ22) and fungal phytopathogens (*Alternaria* sp., *Fusarium oxysporum* fsp. *lycopersici, Fusarium oxysporum* fsp. *cubense* M5, and *Rhizoctonia* sp.). Root inoculations with DBA51 in tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotiana tabacum* L.) plants were performed under greenhouse conditions. Plant height (20 %) and root biomass (40 %) were significantly enhanced in tomato plants inoculated with DBA51 compared to non-inoculated plants, although for tobacco plants, only root biomass (27 %) showed significant differences with DBA51. In addition, physiological parameters such as photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), and transpiration rate (mmol H_2O m^{-2} s^{-1}) were also evaluated, and no differences were detected between DBA51-inoculated and control treatment in tomato and tobacco leaves. The observed results indicate that the DBA51 strain could be used as a biofertilizer to improve yields of horticultural crops.

1. Introduction

In the recent past, beneficial microorganisms have been turned to special attention in modern agriculture. Plant growth-promoting rhizobacteria (PGPR) are being used to reduce (a)biotic stress in agronomic crops to counteract the great challenges in crops. In this context, the broad diversity of PGPRs associated with the rhizosphere of host plants includes *Acetobacter, Acinetobacter, Alcaligenes, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Beijerinckia, Burkholderia, Derxia, Enterobacter, Erwinia, Herbaspirillum Flavobacterium, Gluconacetobacter, Klebsiella, Ochrobactrum, Paenibacillus, Pantoea, Pseudomonas, Rhizobium, Rhodococcus,* and *Serratia*, among others (Vega-Celedón et al., 2021). The plant growth-promoting (PGP) traits for beneficial rhizobacteria included plant growth and enhanced plant tolerance for biotic and abiotic stress by potassium and phosphate solubilization, nitrogen fixation, siderophore production, indole-3-acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) activity, antagonism against phytopathogens, and many more mechanisms (Backer et al., 2018). In this sense, PGPR represents a sustainable alternative to enhancing soil fertility or promoting the growth of crops (Sharma et al., 2016).

Among PGPRs, *Enterobacter* species can have positive and negative effects on plant growth depending on various factors, including the specific strain of *Enterobacter* and the plant species involved.

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Enterobacter spp. are commonly found in the rhizosphere, which is the soil region surrounding plant roots, and they can influence plant growth in several ways, *i.e.*, nitrogen fixation for *Enterobacter asburiae* BY4 (Singh et al., 2021); phosphate solubilization for *E. cloacae* ZNP-3, *E. cloacae* ZNP-4, *E. ludwigii* AFFR02, and *E. cloacae* AS10 (Singh et al., 2017, 2022; Kang et al., 2021; Ghosh et al., 2022); IAA production and ACCD activity for *E. cloacae* ZNP-3, *E. cloacae* ZNP-4, *E. asburiae* BY4, and *E. cloacae* AS10 (Singh et al., 2017, 2021, 2022; Ghosh et al., 2022); plant growth-promotion and tolerance to stress abiotic presented by *Enterobacter* sp. 16i, *E. cloacae* ZNP-3, *E. cloacae* ZNP-4, *E. asburiae* BY4, *E. cloacae* AS10, *E. hormaechei* MF957335, and *E. ludwigii* AFFR02 (Jochum et al., 2019; Singh et al., 2017, 2021, 2022; Ghosh et al., 2022; Ranawat et al., 2021; Kang et al., 2021). Therefore, PGPR strains of *Enterobacter* nowadays are considered bio-fertilizers and represent an alternative for horticultural crops and could mitigate the climate change effects.

Recently, global warming has severely affected crop production, involving several abiotic stressors. Many practices have been implemented to address this challenge in agriculture to counteract these plant abiotic stressors, including PGPR application (Singh et al., 2017). Certain PGPRs readily colonize the rhizosphere of host plants and establish a close association with roots (Gray and Smith, 2005). However, when crop plants are exposed to (a)biotic stress, ethylene accumulation is presented, occasioning delays in the proliferation of roots and shoots and promoting senescence in crop plants (Ma et al., 2003; Sheehy et al., 1991). The stress ethylene is reduced by PGPR treatment due to ACC-Deaminase activity by hydrolysis of the precursor of ethylene ACC to ammonia and α-ketobutyrate (Penrose and Glick, 2003). Therefore, using ACCD-producing rhizobacteria is more environmentally friendly and economical than transgenic plants (Glick 2012).

The research presented here aimed to bioprospect rhizobacteria from soil samples of semi-desert weeds in Coahuila, México, based on ACCD activity. The molecular identification was based on the barcode 16S rDNA sequence, and other PGP traits were assessed, including the growth promotion in tomato plants (*Solanum lycopersicum* L.) and tobacco plants (*Nicotiana tabacum* L.) under greenhouse conditions.

2. Materials and methods

2.1. Soil sample collection

The rhizosphere soil was collected from weeds in the open field located at field experimental (25° 39′ N and 101° 06′ O, 1193 masl) at the Las Encinas of Centro de Investigación en Química Aplicada (CIQA), Ramos Arizpe, Coahuila, Mexico. The soil collected was around 1 kg, placed in soil sampling bags in refrigeration conditions, and stored until use at 4 °C.

2.2. Screening for bacterial isolates with ACC deaminase

The isolation of rhizobacteria displaying ACCD activity was performed according to Penrose and Glick (2003). From 5 different soil samples were weighed, 1 g of soil was added to a flask containing 50 ml of LB medium (Luria Bertani) and later agitated in an incubator at 200 rpm to dissolve the soil for 24 h at 28 °C. After that, 1 ml of culture was inoculated in a DF medium (Dworkin and Foster, 1958) supplemented with $(NH_4)_2SO_4$ for 24 h, 200 rpm, and 28 °C. Later, 1 ml of DF culture was inoculated in a DF medium supplemented with 1-aminocyclopropane-1-carboxylate (ACC) as a unique source of N and was incubated at 28 °C, 200 rpm for 24 h. Serial dilutions were performed, and 100 μl from each dilution was inoculated in Petri dishes with DF with ACC added; plates were incubated at 28 °C for 3 days. From DF-ACC$^+$ plates, two separated colonies isolated from 5 different soil samples were selected for future evaluations. Ten isolates were inoculated in LB and KB (King's Base) plates and incubated at 28 °C for 24 h, and all isolates were cultivated in LB broth at the same conditions. The pellet was obtained by centrifugation at 12,000 rpm, re-suspended in glycerol at 17 %, and stored at -80 °C.

2.3. Phosphate solubilizing test

Ten isolates with ACCD positives were evaluated for their capacity to solubilize phosphate tricalcium $Ca_3(PO4)_2$ in Pikovaskya's (PVK) medium, according to Nautiyal (1999). All isolates were cultivated overnight in LB broth and were inoculated in PVK plates with a drop of 10 μl of bacterial suspension thrice, PVK plates were incubated in an oven (Thermo Scientific, USA) at 28 °C for 4 days. Later, the phosphate solubilization index (PSI) was determined by measuring the diameter of the halo around the colonies using the formula: $PSI = D_h / D_c$

D_h = The average diameter of the halos around the colonies.

D_c = The average diameter of its respective colonies.

PSI < 2 is considered low, medium if 2 ≤ PSI < 3, and high with PSI ≥ 3.

2.4. Antagonistic test with bacterial and fungal phytopathogens

Ten bacterial isolates were tested in KB plates previously inoculated with *Clavibacter michiganensis* (CM), *Pseudomonas syringae* pv. *tomato* DC3000 (DC3000) and *Pectobacterium cacticidum* FHLGJ22 (FHLGJ22), all isolates were added in four drops separated in KB plates and incubated at 28 °C for 5 days. In addition, bacterial isolates were inoculated with four drops in PDA (Potato Dextrose Agar) plates previously inoculated with *Alternaria* sp. (ALT), *Fusarium oxysporum* fsp. *lycopersici* (FOL), *Fusarium oxysporum* fsp. *cubense* M5 (M5) (Maldonado-Bonilla et al., 2019), and *Rhizoctonia* sp. (RIZ). The PDA plates were incubated at 25 °C for 7 days.

2.5. Production of indole acetic acid and hemolysis test

Four isolated bacteria were inoculated into tubes containing 5 ml of Tryptic Soy Broth (TSB) medium supplemented with L-tryptophan as pre-inoculum and were incubated in a shaker at 28 °C ± 2 °C and 160–180 rpm for 24–48 h. Adjust pre-inoculum at OD_{600nm} to 0.1 in triplicated into new tubes containing TSB medium supplemented with L-tryptophan up to a final volume of 5 ml. Later, incubate the tubes in the dark (covered with aluminum foil) in a shaker at 28 °C ± 2 °C and 160–180 rpm for 24–48 h. After that, transfer 1.5 ml of each bacterial culture to 2.0 ml microtubes and centrifuge at 5000 x g for 10 min at room temperature, and transfer 500 μl of the supernatant to a clean spectrophotometer cuvette and add 500 μl of Salkowsky's reagent to each cuvette. The cuvettes were incubated at room temperature in the dark for 30 min; the absorbance was measured in the spectrophotometer at 530 nm. Finally, the concentration of IAA produced by isolating bacteria was calculated using the standard calibration curve with known amounts of IAA (from 0 to 250 μg/ml) (Batista et al., 2021).

Pathogenicity test was performed with four isolated inoculated in Blood Agar Base medium with sterile 5 % defibrillated blood. Each isolated was plated in triplicate at 28 °C for 96 h. The hemolytic activity of each bacterium was observed by forming a clear zone surrounding the growth zone of the bacteria, which corresponds to beta-hemolysis (Denaya et al., 2021).

2.6. Molecular identification

For bacterial identification, DBA51 was selected as a rhizobacteria as it displays more plant growth promotion traits. From an overnight culture of DBA51, DNA extraction and purification were performed with *Quick*-DNA™ Fungal/Bacterial Miniprep Kit (ZYMO RESEARCH, USA). Amplification of the 16S rDNA gene was performed using the forward, 27f (5′-AGAGTTTGATC(A/C)TGGCTCAG-3′) and reverse, 1492r (5′-TACCTTGTTACGACTT-3′) primer combination (Frank et al., 2008).

Table 1
Distribution of all treatments in tomato and tobacco plants under greenhouse conditions ($n = 5$).

	Treatments									
	Control	DBA11	DBA12	DBA21	DBA22	DBA41	DBA42	DBA51	DBA52	LPM1
Experiment #1 (tomato)	5	5	5	5	5	5	5	5	5	-
Experiment #2 (tomato)	5	-	-	-	-	-	-	5	-	5
Experiment #3 (tobacco)	5	-	-	-	-	-	-	5	-	5

5 replicates, - no evaluated.

End-Point PCR amplification was achieved using a DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The PCR conditions of denaturing, annealing, and extension were 95 °C for 1 min, 47 °C for 1 min, and 72 °C for 2 min, respectively. The amplicon expected of ~1523 bp size was separated by electrophoresis in 1 % agarose gels stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, USA). The PCR product was cloned and sequenced by capillary electrophoresis using the service of Macrogen (Korea). This sequence was later used as a query to search for the most similar sequence in the database of 16S-type sequences of the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) using the BLASTN algorithm. The sequence of the 16S rDNA gene from *Enterobacter* sp. strain DBA51 is available in the GenBank under Accession Number OR591255. The 16S rDNA sequence of DBA51 together with sequences of type strains of Enterobacteriaceae, the PGPR *Enterobacter cloacae* ZNP-3 (Singh et al., 2017) and *Enterobacter cloacae* AS10 (Ghosh et al., 2022), and *Pseudomonas fluorescens* as outgroup were aligned by using CLUSTALW algorithm to later construct a phylogeny with the maximum likelihood method combined with the Tamura-Nei substitution model and 1000 bootstrap replicates. Alignment and phylogeny were conducted in MEGA11 (Tamura et al., 2021).

2.7. Greenhouse assays to growth promotion by rhizobacteria

The plant-growth promotion assays were performed under greenhouse conditions with *Enterobacter* sp. strain DBA51. Two experimental independents were performed with tomato plants (*Solanum lycopersicum* L.), and one experiment was performed with tobacco plants (*Nicotiana tabacum* L.). Seedlings of tomato and tobacco were inoculated with 10 ml during transplanting with a bacterial suspension of 1×10^7 CFU/ml (DBA51), *Bacillus subtilis* strain LPM1 (LPM1) was used as a positive control, and a bacterial suspension of 1×10^8 CFU/ml was used for inoculation. Control plants were non-inoculated with rhizobacteria. Tomato and tobacco plants were grown in 1.5 L pots with peat moss/perlite (70/30 v/v) and fertilized with 20N-20P-20 K (FertiDrip, Agrodelta, Monterrey, Mexico) once a week and watered twice a week. The experiments were distributed according to the Table 1.

2.8. Biometric and physiological parameters

Biometric traits were measured at 20 DAI (Days After Inoculation) in tomato and tobacco plants; plant size was obtained with a measured tape (TRUPER, Gripper), stem diameter was measured using a Vernier (KARLEN, Digital Caliper), fresh shoot, and root biomass were measured utilizing of a precision balance (OAHUS). Physiological parameters were evaluated at 20 DAI in fully exposed leaves (third leaf from the top) from 5 plants per treatment and were measured for leaf infrared gas exchange analysis (IRGA): CO_2 fixation and H_2O transpiration rates, stomatal conductance (LI-COR 64000XT LI-COR, Inc. Lincoln, NE, USA). The measurements were achieved under natural air temperatures between 12:00 to 15:00 h, on clear days (300 μmol photons m^{-2} s^{-1} PAR in the greenhouse), and IRGA set to 400 ppm reference CO_2 and an airflow of 500 μmol s^{-1}.

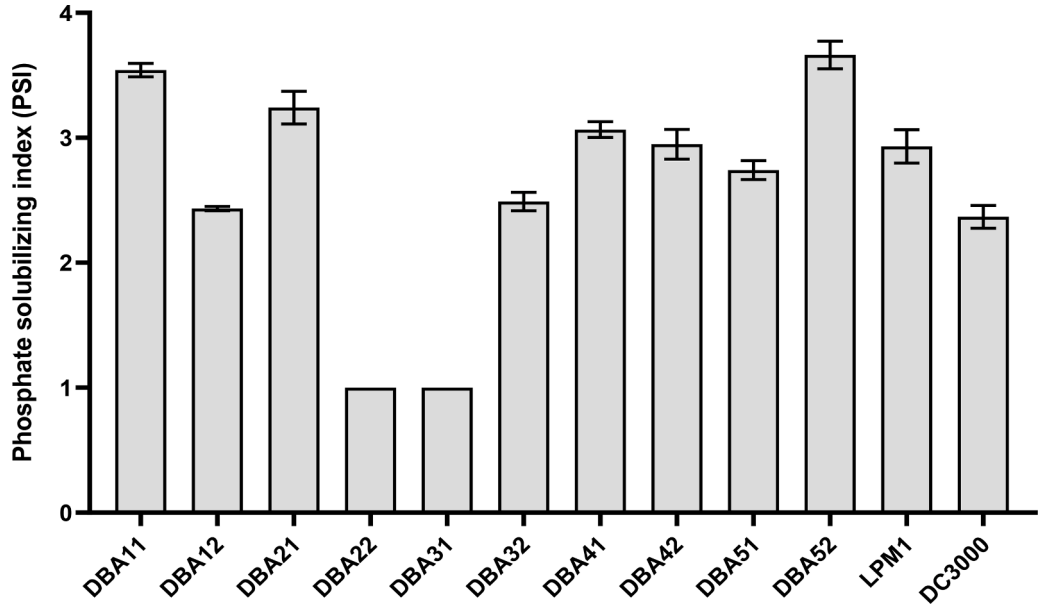

Fig. 1. Phosphate solubilizing index test with Pikovaskya's (PVK) medium. (DBA11 to DBA52) ten bacterial isolates, (LPM1) *Bacillus subtilis* LPM1, and (DC3000) *Pseudomonas syringae* pv. *tomato* DC3000.

Table 2
Indole acetic acid basal production and hemolysis test in isolated bacteria.

Isolate	Hemolysis	IAA production (μg/ml)
DBA12	++	ND
DBA42	–	1.5862 ± 0.08
DBA51	–	1.7916 ± 0.57
DBA52	–	1.1547 ± 0.22

– negative, ++ positive; ± standard deviation; ND no determinate.

2.9. Statistical analysis

All results obtained from biometric and physiological parameters were analyzed to compare the means and significant differences by analysis of variance (ANOVA) at $p \leq 0.05$, followed by Tukey's multiple comparison test. The graphics and analysis were performed using GradPad Prism version 8 for Windows, GradPad Software, La Jolla, California, USA.

3. Results and discussion

3.1. Isolation and preliminary screening based on ACC-Deaminase and phosphate solubilizing of rhizobacteria

Initially, this study focused on selecting weeds plants to explore those bacterial associations in the rhizosphere. Although weeds are undesired plants in crops, their growth in adverse environmental conditions and survival could be attributed to beneficial microorganisms associated with the rhizosphere, which can also play beneficial roles in agronomic crops (Sarathambal et al., 2014). In this sense, soil samples were collected from the rhizosphere of five semi-desert weeds in Coahuila and evaluated for ACCD-producing bacteria screening. Among these, two different bacteria colonies were chosen from each soil sample; ten bacterial colonies were obtained by growing on plates with DF medium supplemented with ACC as a unique nitrogen source (Penrose and Glick, 2003). Later, the phosphate solubilizing was determined by the PSI test and for incubation *in vitro* on PVK medium for ten isolated bacteria, resulting in eight isolates with PSI positives (Fig. 1). However, the range of PSI was between 2.4 for DBA12 and DBA52 with 3.7, only DBA22 and DBA31 isolated phosphate solubilizing was absent in both isolates. Those isolates were selected due to the capacity of phosphate tricalcium solubilization, shown in PVK plates that presented a halo of activity. These values of PSI were compared to other PGPRs, *Enterobacter ludwigii* AFFR02, which displayed a 2.3 PSI (Kang et al., 2021), and *Lysinibacillus pakistanensis* PCPSMR15 presented the highest PSI of 4.0 (Lelapalli et al., 2021). In this study, isolate DBA52 showed the highest PSI with 3.7 compared to the rest of the isolates and strain controls (LPM1 and DC3000); isolate DBA52 could represent an alternative to future bioassays in crop plants to solubilize phosphoric fertilizers and increase phosphorus uptake by the plant as biofertilizer.

3.2. Quantification of indole-3-acetic acid (IAA) and hemolysis test from isolates

Among other PGP traits, the basal levels of IAA production were evaluated in DBA42, DBA51, and DBA52 isolates. For the DBA51 isolate, phytohormone production was 1.7916 ± 0.57 μg ml^{-1}, resulting higher than DBA42 with 1.5862 ± 0.08 μg ml^{-1} and DBA52 with 1.1547 ± 0.22 μg ml^{-1} (Table 2). The IAA basal levels in other *Enterobacter* PGPR strains were reported with 0.364± 0.02 μg ml¯1 for *E. cloacae* ZNP-4 (Singh et al., 2022), 3.117 ± 0.20 μg ml^{-1} for *E. cloacae* ZNP-3 (Singh et al., 2017), and 29.59 ± 0.19 μg ml^{-1} for *E. asburiae* BY4 (Singh et al., 2021). However, the hemolysis test was performed to discard those isolates that presumably could be pathogenic for humans; therefore, the DBA12 isolate was positive and removed for further studies (Table 2).

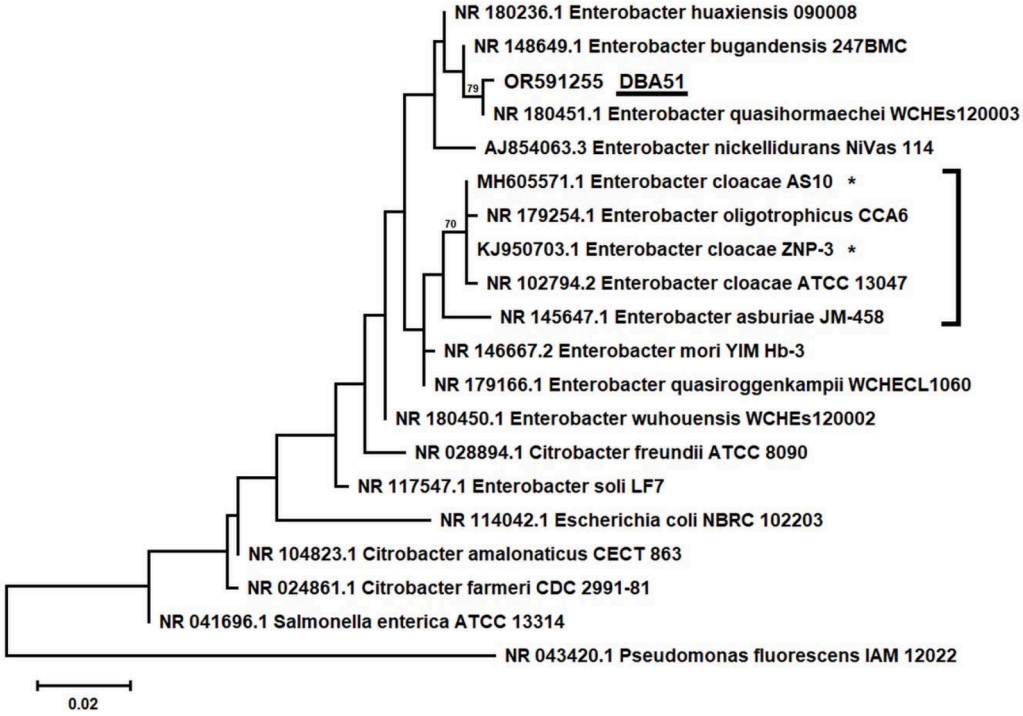

Fig. 2. The maximum likelihood phylogenetic tree with 1000 bootstrap replicates of the 16S rDNA sequences of Enterobacteriaceae illustrates that *Enterobacter quasihormaechei* WCHEs120002 is the most related to the strain DBA51 (underlined). The sequence of *Pseudomonas fluorescens* IAM 12022 were included as an external group. Accession numbers of each sequence are presented at the left of each name. The bracket encompasses strains of the *Enterobacter cloacae* complex, which contains the previously reported PGPR strains AS10 and ZNP-3 marked with an asterisk (*). Support values of 70 or higher are indicated over the corresponding nodes.

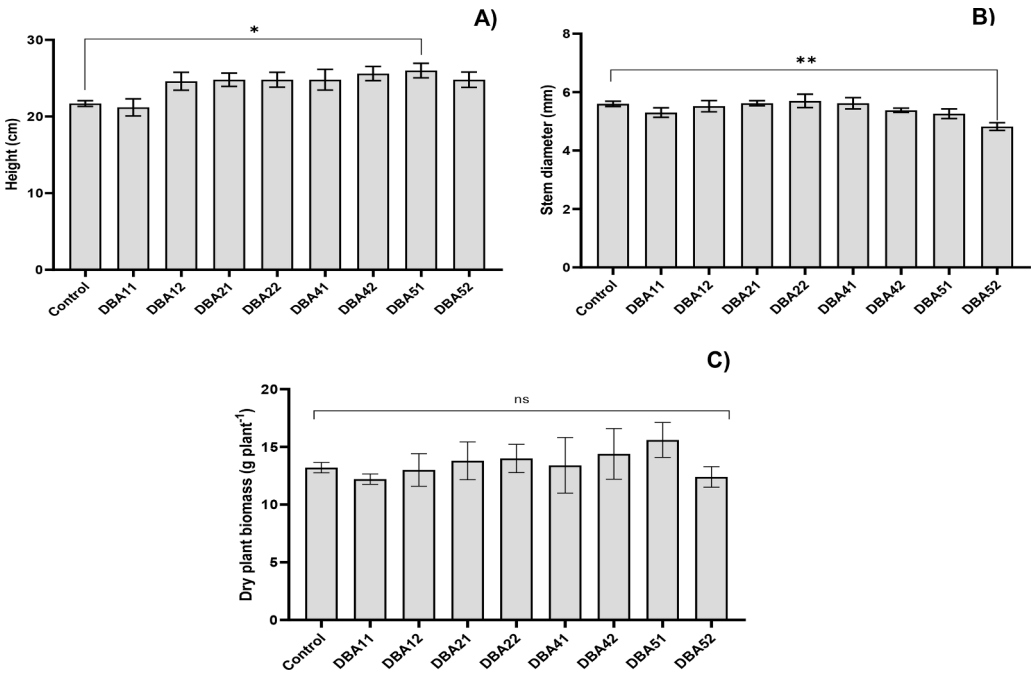

Fig. 3. Biometric parameters evaluated in tomato plants at 20 DAI treated with bacterial isolates. (**A**) Plant size in cm, (**B**) Stem diameter in mm, (**C**) Dry plant biomass in g. Level of significance: *, $p \leq 0.05$; **, $p \leq 0.01$; ns, no significance. One-way ANOVA, Tukey's multiple comparison test.

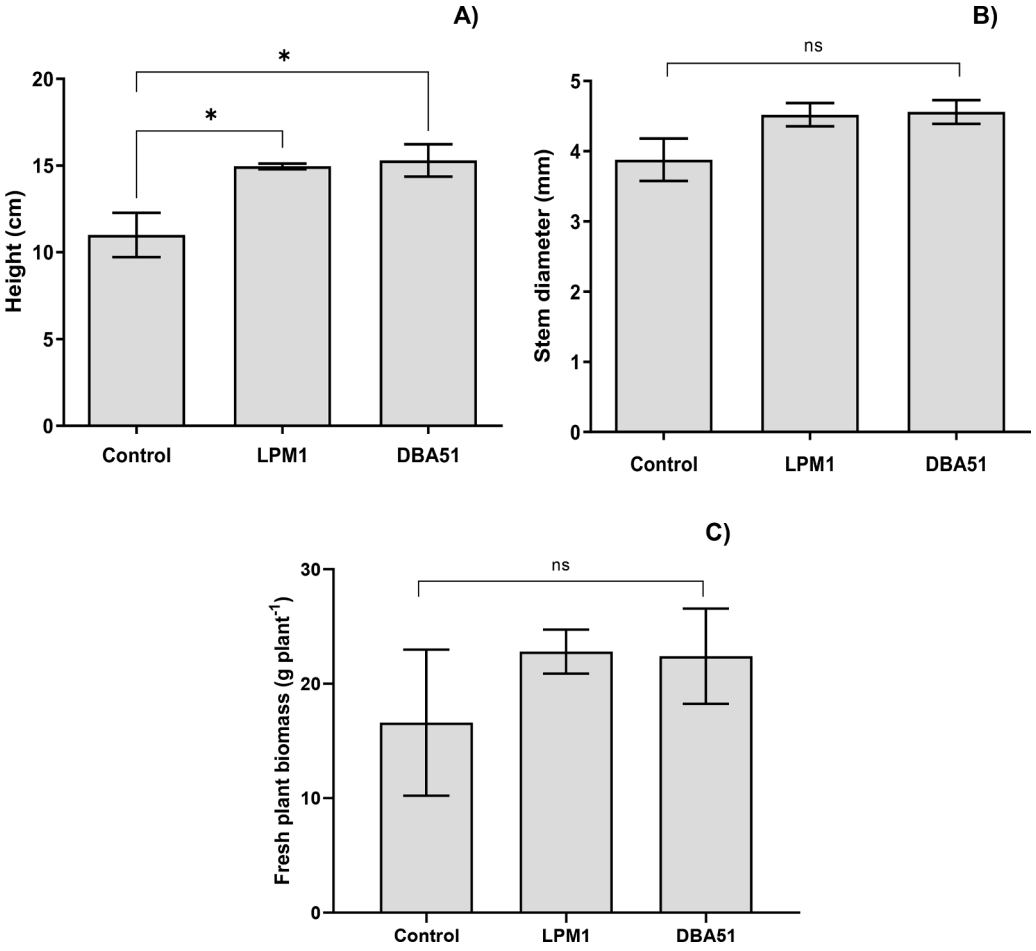

Fig. 4. Biometric parameters evaluated in tomato plants at 20 DAI. **Control**, no-inoculated plants; **LPM1**, *Bacillus subtilis* LPM1; and **DBA51**, *Enterobacter* sp. DBA51. (**A**) Plant size in cm, (**B**) Stem diameter in mm, (**C**) Fresh plant biomass in g. Level of significance: *, $p \leq 0.05$; ns, no significance. One-way ANOVA, Tukey's multiple comparison test.

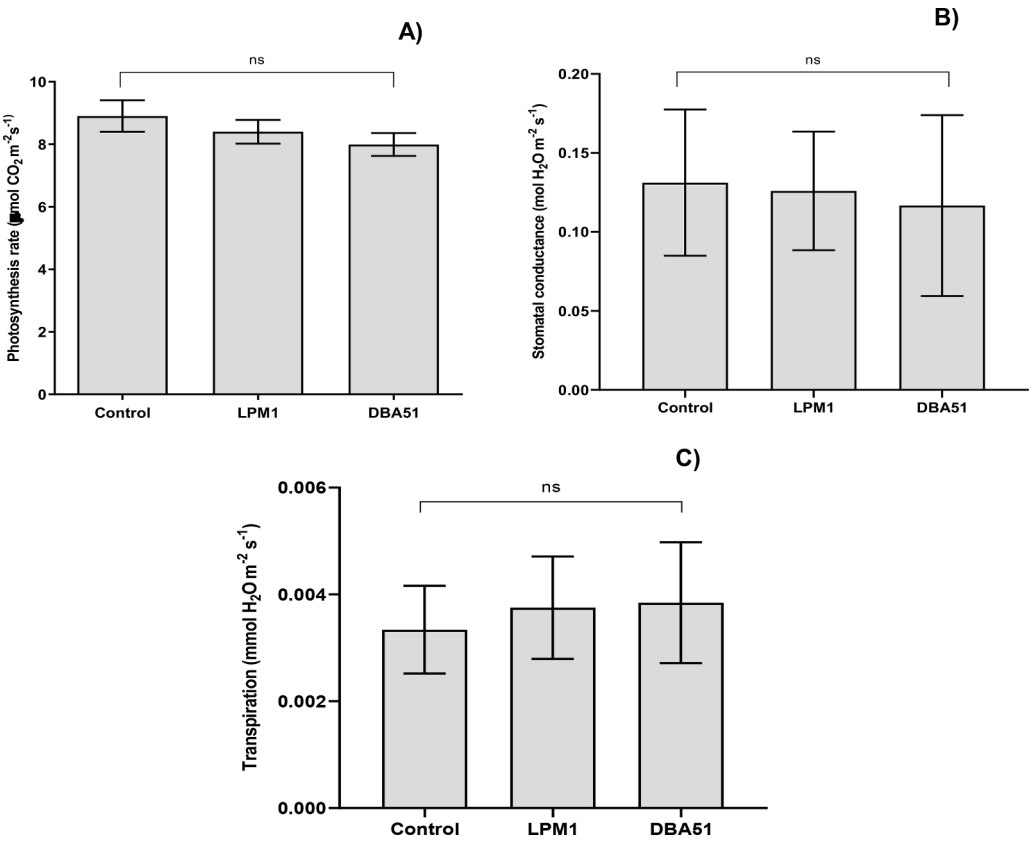

Fig. 5. Physiological parameters evaluated in tomato plants at 20 DAI. **Control**, no-inoculated plants; **LPM1**, *Bacillus subtilis* LPM1; and **DBA51**, *Enterobacter* sp. DBA51. (**A**) Photosynthesis rate, (**B**) Stomatal conductance, (**C**) Transpiration rate. Level of significance: ns, no significance at $p \leq 0.05$. One-way ANOVA, Tukey's multiple comparison test.

3.3. Antagonism activity against bacterial and fungal phytopathogens

To evaluate the possible characteristics of biocontrol of DBA51 isolate, several bioassays were performed *in vitro* with plates with LB agar and PDA. DBA51 showed no biocontrol activity against all phytopathogens evaluated (CM, DC3000, FHLGJ22, ALT, FOL, M5, and RIZ). For the PGPRs, the induced systemic resistance (ISR) is well known, but this was not characterized in this study. In this sense, *E. hormaechei* MF957335 promoted growth in tomato plants and yield and induced disease tolerance (Ranawat et al., 2021). Although, the possibility of evaluating other phytopathogens at *in vitro* conditions is still being considered. However, certain PGPR strains cannot show antagonism activity for several microorganisms; for example, *E. cloacae* ZNP-3 presented positive antimicrobial activity for *Escherichia coli, Erwinia carotovora, F. oxysporum, F. graminearum, Aspergillus flavus*, and *Penicillium citrium*; but not for *Staphylococcus aureus, Bacillus cereus*, and *F. moniliforme* (Singh et al., 2017).

3.4. Identification of the selected DBA51 isolate

Once obtained, the 16S rDNA sequence of DBA51 was used as a query to search for related sequences in GeneBank, the sequences of Enterobacteriaceae the ones of higher identity (data not shown). Sequences of type strains of Enterobacteriaceae and the PGPR *Enterobacter cloacae* ZNP-3 (Singh et al., 2017) and *Enterobacter cloacae* AS10 (Ghosh et al., 2022) were retrieved to construct a maximum likelihood phylogeny (Fig. 2). DBA51 and *Enterobacter quasihormaechei* strain WCHEs120003 form a subcluster independent of the sequences of the *Enterobacter cloacae* complex (Annavajhala et al., 2019) that includes the PGPR mentioned above which cause beneficial effects in monocot plants. As DBA51 was isolated from rhizospheric soil associated with a dicot plant and promotes growth in dicot plants as well, future work will be focused on elucidating whether there is a correlation between the lineage of *Enterobacter* and host plant benefited by the bacterial activity. Additional molecular criteria are necessary to clear identification at level species, and the DBA51 isolate was proposed to report as *Enterobacter* sp. strain DBA51, whose sequence was deposited in GenBank with Accession Number OR591255. However, in the future, molecular analysis will be achieved to explore genomic characterization and compare it to PGPR *Enterobacter* strains with other PGP traits. In this sense, the phenotypical attributes evaluated here could confirm that the DBA51 strain can act as PGPR.

3.5. Plant growth-promotion and physiological analysis in tomato and tobacco plants by DBA51 inoculation

A preliminary bioassay was performed in tomato plants with eight isolates under greenhouse conditions to explore growth promotion (Fig. 3). All biometric parameters evaluated in this study were performed at 20 DAI, the time was selected according to development stage in tomato plants with physiologically active; in other study reported that 21 days was sufficient to detect growth promotion in tomato plants previously inoculated with the PGPR strain of *B. subtilis* BEB-DN (Valenzuela-Soto et al., 2010). Here, DBA51 showed significant differences in plant size with 20 % higher, and dry plant biomass was not significant (Fig. 3A and C). However, the dry root biomass was 62 % more than the control non-inoculated at vegetative stages (data not shown). Contrastingly, the DBA52 isolate showed a reduced stem diameter and significantly differed from the control non-inoculated (Fig. 3B). Therefore, the following greenhouse experiments were performed using the DBA51 strain.

In order to evaluate the beneficial effects of DBA51 treatment,

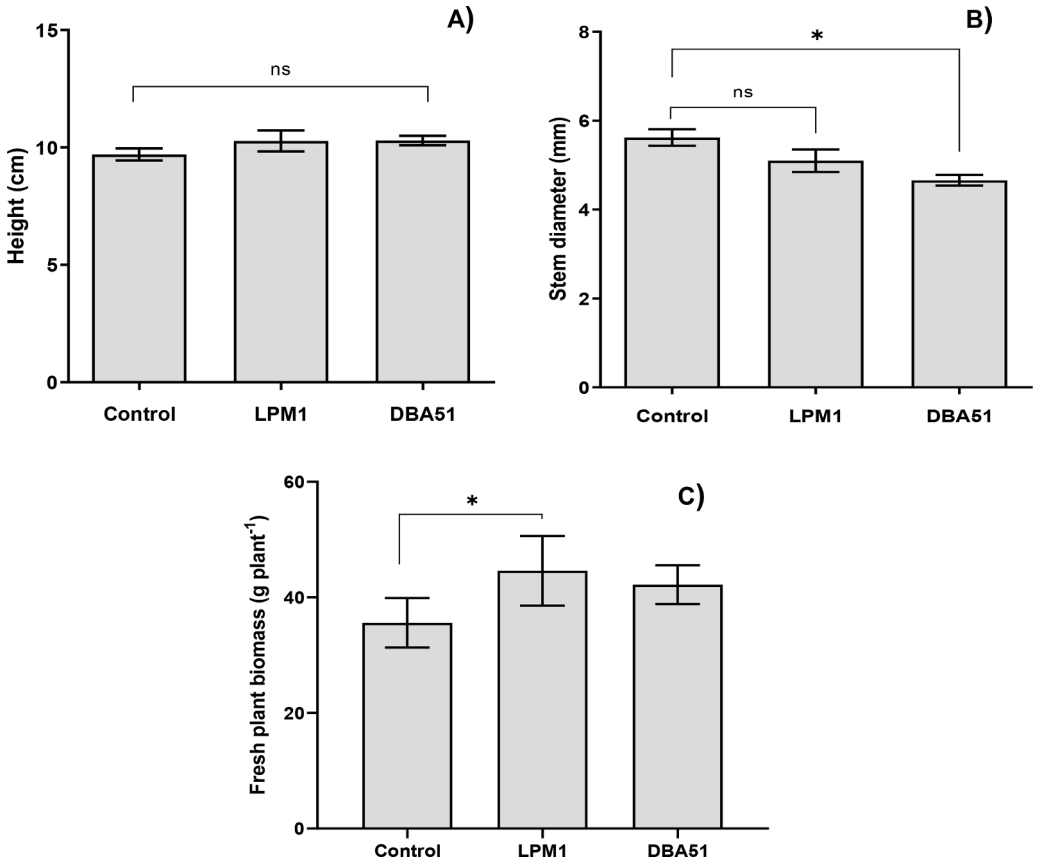

Fig. 6. Biometric parameters evaluated in tobacco plants at 20 DAI. **Control**, no-inoculated plants; **LPM1**, *Bacillus subtilis* LPM1; and **DBA51**, *Enterobacter* sp. DBA51. (**A**) Plant size in cm, (**B**) Stem diameter in mm, (**C**) Fresh plant biomass in g. Level of significance: *, $p \leq 0.05$; ns, no significance. One-way ANOVA, Tukey's multiple comparison test.

greenhouse bioassays were performed on tomato (*S. lycopersicum* L.) and tobacco (*N. tabacum* L.) plants compared to the LPM1 strain previously reported as PGPR (Chávez-Betancourt et al., 2006). DBA51 and LPM1 inoculation on tomato plants showed significant differences in plant size, with 36 % higher for LPM1 and 39 % higher for DBA51 (Fig. 4A) and stem diameter. Fresh plant biomass was not significant (Fig. 4B and C), although the fresh root biomass with 42 % more for LPM1 and 40 % more for DBA51 (data not shown), compared to control non-inoculated. These results showed a similar tendency to the preliminary experiment performed in tomato plants, indicating that DBA51 improves plant size and root biomass at the vegetative stages (20 DAI). Singh et al. (2021) reported that *E. asburiae* BY4 significantly promoted the growth in two varieties of sugarcane

(*Saccharum* spp. interspecific hybrids) and was evident with parameters of plant height and root weight at 30, 60, and 90 DAI compared to control. However, when physiological parameters were evaluated at this phenological stage, no differences were detected by any treatments of photosynthesis rate, stomatal conductance, and transpiration (Fig. 5).

Although differences were detected in biometric parameters at 20 DAI but not for physiological variables, we suggest that more than 30 DAI in tomato or tobacco plants could detect possible differences in photosynthesis rate similar to sugarcane evaluated at 30, 60, and 90 DAI at greenhouse conditions (Singh et al., 2021). In bell pepper plants previously inoculated with *B. subtilis* LPM1 presented significant differences in photosynthesis rate compared to control non-inoculated at 30 and 60 DAI performed under shade-house (Cárdenas-Flores et al., 2023).

To explore the growth promotion by DBA51 in tobacco plants, a bioassay was performed at the greenhouse conditions. Similarly, biometrical parameters were evaluated at 20 DAI. The fresh plant biomass showed significant differences in LPM1 with 37 % more and DBA51 with 18 %. Still, no significant differences were detected compared to control non-inoculated (Fig. 6C). Contrastingly, DBA51 showed a reduced stem diameter, significantly different from the control non-inoculated (Fig. 6B). In this sense, Kumar et al. (2016) reported that *Paenibacillus lentimorbus* B-30488 (B-30488) enhanced growth promotion in tobacco plants and was evident for shoot length, root length, shoot thickness, fresh weight, and dry weight for 28 DAI at greenhouse conditions. However, the photosynthesis rate was reduced in DBA51 and was significantly different compared to the control non-inoculated (Fig. 7A); meanwhile, with stomatal conductance and transpiration, no differences were detected at 20 DAI (Fig. 7B and C). Tobacco plants previously inoculated with strain B-30488 showed significant differences in photosynthesis rate at 28 DAI compared to control non-inoculated (Kumar et al., 2016); this could indicate that 20 DAI was not sufficient to detect differences in some of the parameters evaluated in tomato and tobacco plants.

In this study, DBA51 promoted growth in tomato and tobacco plants at vegetative stages (20 DAI), and this promotion was evident in root biomass for both plants. This could be explained by ACCD presence in the DBA51 strain, which could influence plant growth promotion under greenhouse conditions. However, the ACCD activity was enhanced in other plants exposed to stress conditions (stress by ethylene) (Penrose and Glick, 2003). Like other *Enterobacter* strains, DBA51 also presented the production of IAA that induces root proliferation (Singh et al., 2017). Other studies have reported that IAA and ACCD-producing *Enterobacter* sp. are associated with plants such as *Citrus reticulate* (Thokchom et al., 2014), *Populus* (Taghavi et al., 2009), *Piper nigrum* (Jasim et al., 2013), *Triticum aestivum* L. (Singh et al., 2017, 2022), and *Saccharum* spp. (Singh et al., 2021). However, it is important to note that not all

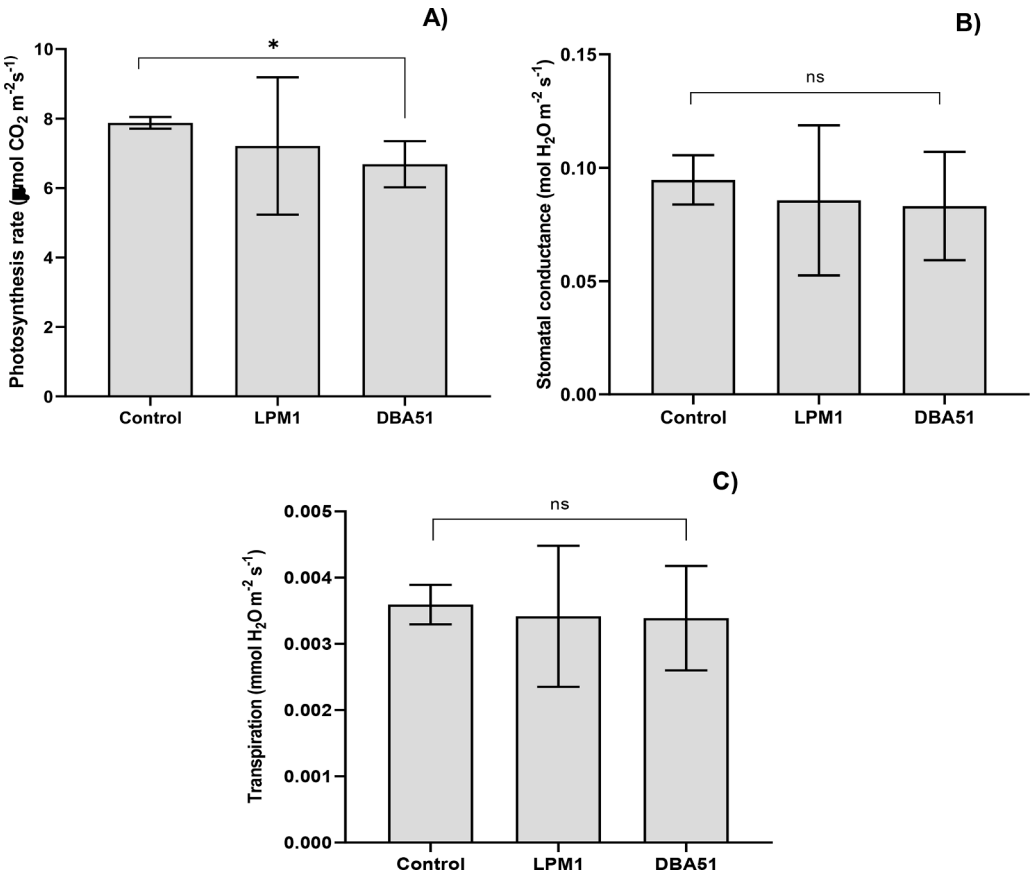

Fig. 7. Physiological parameters evaluated in tobacco plants at 20 DAI. **Control**, no-inoculated plants; **LPM1**, *Bacillus subtilis* LPM1; and **DBA51**, *Enterobacter* sp. DBA51. (**A**) Photosynthesis rate, (**B**) Stomatal conductance, (**C**) Transpiration rate. Level of significance: *, $p \leq 0.05$; ns, no significance. One-way ANOVA, Tukey's multiple comparison test.

Enterobacter strains positively affect plant growth. Some *Enterobacter* strains can be plant pathogens and cause diseases, although, in the present study, the DBA51 strain was hemolysis-negative, indicating that it can be used as a biofertilizer safely. Therefore, the specific attributes and impact of *Enterobacter* on plant growth can vary widely depending on the strain and the environmental conditions.

4. Conclusion

The present work is the first report of *Enterobacter* sp. as PGPR isolated from semi-desert weeds in Coahuila. *Enterobacter* sp. strain DBA51 presented an ACCD and can use 1-aminocyclopropane-1-carboxylate as the unique nitrogen source, producing IAA and solubilizing phosphate as PGPR traits. In addition, the DBA51 strain promoted growth in tomato and tobacco plants by significantly increasing the root biomass under greenhouse conditions. Therefore, the DBA51 strain is a promising candidate for evaluating crop yields under-field applications.

CRediT authorship contribution statement

YOO and LGSL performed all *in vitro* measurements; JVL and ABP performed greenhouse experiments and photosynthesis measurements; LDMB performed molecular identification and phylogenetic analysis; AFO and JHVS planned the work; JHVS performed the bacterial isolation and wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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