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Strategies to facilitate the bioprospection of properties of agronomic interest in microbial culture collections and a successful case of selection to mitigate drought stress in maize

Natalia Caetano Vasques[1,2], Paula Cerezini[1,3], Adalgisa Ribeiro Torres[1,3], Marco Antonio Nogueira[1,2] and Mariangela Hungria[1,2*]

Abstract

Background The replacement of synthetic molecules with biological solutions has been a global goal with high priority in agriculture. Worldwide, there are several well-organized microbial collections holding high biodiversity and biotechnological potential. This study was based on the hypothesis that in vitro analyses can guide the selection of promising strains for subsequent in vivo evaluation. We selected 100 strains representative of the "Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja", Brazil, to be evaluated in vitro for proteolytic and cellulolytic activities, production of 1-aminociclopropano-1-carboxilato deaminase (ACC-deaminase), siderophore, indolic compounds (indole-3-acetic acid, IAA), exopolysaccharides (EPS), biofilm, solubilization of nutrients, and ability to grow in medium with reduced water activity and high temperature. The 100 strains were also evaluated in a greenhouse on maize growing in sterile substrate to assess their ability to promote tolerance to drought.

Results Hydrolytic and proteolytic activities were highlighted in *Paenibacillus*, *Pantoea,* and *Bacillus*, and ACC-deaminase was widespread in 38 strains of several genera. Tolerance to drought and high temperature (40 ± 2 °C) was highly present in *Bacillus*. Outstanding results were obtained with *Azospirillum* for EPS, in *Paraburkholderia*, *Pseudomonas*, and *Bacillus* for biofilm, and in *Chromobacterium* for IAA. Regarding properties that could putatively help the uptake of nutrients, 30 strains synthesized siderophores, but only seven were able to solubilize calcium phosphate, five of which were classified as *Pseudomonas*. A high correlation was found between the ability to grow in vitro in medium with reduced water activity and tolerance to drought in vivo. From this initial greenhouse experiment, 15 strains were selected to confirm their potential to mitigate drought in a greenhouse experiment with non-sterile soil. Three outstanding strains, *Bacillus velezensis* CNPSo 2384, *Bacillus subtilis* CNPSo 2606, and *Bacillus*

*Correspondence:
Mariangela Hungria
mariangela.hungria@embrapa.br; mariangelahungria@hotmail.com

Full list of author information is available at the end of the article

sp. CNPSo 2723 were identified as promising candidates to compose future bio-inputs aimed at increasing plant tolerance to drought.

Conclusion Speeding up strain selection programs is highly relevant, and certain properties of agronomic interest can be found more easily in specific bacterial genera. Additionally, the proof of concept for a preliminary in vitro evaluation was confirmed in vivo for plant tolerance to drought, thereby stimulating the validation of other important microbial properties.

Keywords Plant growth-promoting bacteria, *Bacillus*, *Pseudomonas*, IAA, EPS, Biofilm, Solubilization of nutrients, ACC-deaminase, Siderophores

Background

Biological collections are outstanding components of a nation's scientific and national sovereignty, being considered essential for its innovation infrastructure [1]. Therefore, microbial collections represent a rich reservoir for the survey of potential new technologies. Worldwide, there are important microbial culture collections (MCC), many also known as Biological Resource Centers (BRC), acting as centers of excellence in *ex-situ* conservation and microbial taxonomy, such as ATCC (United States of America), BCCM (Belgium), DSMZ (Germany), and WDCM (China). In 2022, the World Federation for Culture Collections (WFCC) – the leading organization that synchronizes activities of the MCC – listed 820 collections worldwide [2]. In Brazil, a mega-diverse country that holds around 25% of global biodiversity [3], the existing system of collections receives increasing recognition in governmental policies as part of efforts to organize germplasms of economic interest, including microbial collections.

Microbial collections typically contain hundreds or thousands of strains or isolates. For example, our culture collection located in Londrina, State of Paraná, Brazil, "Diazotrophic and Plant Growth Promoting Bacteria Culture Collection of Embrapa Soja" (WFCC Collection # 1213, WDCM Collection # 1054), currently includes 4,800 strains obtained over more than four decades. Many of these strains were obtained from field collections of legumes' nodules, others from a variety of legumes and non-legume species, from natural or agricultural areas, in studies of biodiversity [4, 5], ecology [6–8], or bioprospection for agricultural purposes [9–11].

As a result of the agricultural sector's growing awareness of biological inputs (bio-inputs), there has been a significant increase in the search for solutions based on microorganisms [12]. Soil health collapses in poorly managed soils, biodiversity faces the sixth mass extinction, and crop yields is reaching a plateau. Against this critical narrative, a call for regenerative agriculture emerges, one that goes beyond sustainability, focusing on the recovery of soil health and fertility [13]. In response to this scenario, a governmental program to stimulate the production And use of bio-inputs was established in Brazil in 2020 [14], reinforcing the country´s vocation for the use of bio-inputs in agriculture.

Plant growth-promoting bacteria (PGPB) have been increasingly applied in agriculture as a sustainable practice to enhance crop yields [15]. PGPB can stimulate plant growth based on several properties, including the synthesis of growth-regulating molecules [16], siderophores [17, 18], 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) [19], exopolysaccharides (EPS) and biofilms [20], hydrolytic enzymes such as cellulases and proteases [21, 22]. Growth promotion may also be achieved by increasing plant tolerance to abiotic stresses, such as drought [23, 24]. In addition, some bacteria have properties that can enhance the plant uptake of nutrients [25], such as the capacity for solubilize inorganic phosphate [26, 27] or associated with the biological N_2 fixation (BNF) [28]. Finally, we can cite mechanisms of growth-promotion related to the direct biocontrol of pests and diseases, including induced systemic resistance (ISR) and the synthesis of antimicrobial molecules, among others [29, 30].

In the highly dynamic microenvironment of the plant-soil system, microorganisms interact with roots, exudates, and the native microbiota, directly influencing the colonization and activity of introduced strains. Species such as *Azospirillum brasilense*, *Pseudomonas fluorescens*, and various rhizobia, present in the Embrapa Soja collection, have already demonstrated successful associations with the rhizosphere of different crops, highlighting their potential for adaptation to agricultural environments [9–11]. These bacteria not only express plant growth-promoting traits but are also influenced by factors such as soil type, host crop, and cultivation conditions, including water deficit. Therefore, studies on the occurrence and ecological behavior of these strains in the plant-soil environment under different climatic conditions contribute to the development of formulations with greater persistence and positive interaction with plants under field conditions.

The search for biological solutions in agriculture, with an emphasis on microorganisms, has been enormous, so that the growth rate of bio-inputs use far exceeds that of chemicals, and further increases are expected over the next decades [31]. However, the search for elite microbial strains can be a laborious task, especially due to the large number of microorganisms whose functionality has not yet been fully investigated.

The objective of this study was to evaluate whether in vitro analyses can guide the selection of strains with agronomic potential for subsequent in vivo validation. For that, a careful selection of 100 representative strains based on the main genera isolation site, and indications of relevant properties from previous studies was made for the analysis of in vitro traits that might indicate agronomic potential (e.g., ability to increase the uptake of nutrients by plants, drought tolerance, production of phytohormones), and further evaluation of tolerance to drought in vivo in maize plants. The hypothesis tested was that traits evaluated under in vitro conditions would be predictive of the strains' performance in plants under water stress. As main results, we identified genera in which certain properties might be predominant, facilitating bioprospection in culture collections, and we also confirmed a correlation between in vitroand in vivo properties that confer tolerance to drought in maize plants.

Methods

Bacterial strains

A total of 100 strains, representative of a collection containing 4,800 strains, were initially investigated in vitro for properties putatively indicative of their biotechnological potential of agronomic traits of interest. The strains are deposited at the "Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja" (WFCC Collection #1213, WDCM Collection # 1054) in Londrina, State of Paraná, Brazil. The selection of the 100 strains was aimed at including representative strains of the main genera, site of isolation, and indications of relevant traits from previous studies. The selected strains and corresponding culture medium used for their maintenance are shown in Supplementary Table S1 [32, 33].

Plant growth-promoting traits in vitro

Proteolytic activity

For the evaluation of the synthesis of proteolytic enzymes, the strains were grown in a culture medium containing skim milk, as described by Gerhardt et al. [33]. After incubation for 7 days at 28 ± 2 °C, casein hydrolysis was evaluated by the formation of a translucent haloes of degradation around the colony, as shown in Supplementary Figure S1.

Cellulolytic activity

To evaluate the cellulose degradation capacity of the strains, the method described by Gerhardt et al. [34] was employed, which was carried out using a culture medium containing carboxymethyl cellulose (CMC) as carbon source. After incubation for 5 days at 28 °C, the cellulose degradation haloes were revealed by adding 2 mL of 1 Mol L-1 NaCl solution on the medium surface, And after 5 min, the saline solution was removed, and 2 mL of a 0.1% Congo red solution was added. After 30 min, distilled water was applied to wash off the dye solution, revealing haloes around colonies showing of positive cellulolytic activity (Figure S1).

Production of 1-aminocyclopropane-1-carboxylate deaminase

The ability of some strains to metabolize ACC as the sole source of nitrogen (N) in the medium, due to the action of the enzyme ACC-deaminase, was evaluated by adapting the methodology of Glick et al. [35] and Lucon et al. [36]. Initially, a N-free culture medium (NF) and a buffer solution (containing KH_2PO_4 and Na_2HPO_4) were prepared. After sterilization in an autoclave, the buffer solution was added to the culture medium, and the mixture was enriched with a liquid solution containing ACC (0.03%), which had been previously sterilized using a bacteriological filter. The same NF medium was prepared without ACC, therefore without any source of N. The strains were inoculated on plates with both media and incubated at 28 °C for 8 days. Growth in both conditions was then compared. Strains that showed more pronounced growth in the ACC-containing medium compared to the NF medium were considered capable of using ACC as an ACC-deaminase producer (Figure S1).

Siderophore production

To assess siderophore production, the isolates were grown on King B agar culture medium [37] containing chromazurol S (CAS) [38]. The medium was inoculated with 10 μL of bacterial culture suspension, and after 24 and 72 h of incubation at 28°C, in the dark, the evaluations were carried out. The ability to produce siderophores was considered positive when an orange or pink halo was observed around the colony, and its extent was then measured using a pachymeter (King Tools, São Paulo, Brazil) in both evaluations to obtain the halo/colony ratio (Figure S1).

Production of indolic compounds

To quantify the production of indole-3-acetic acid (IAA) in vitro [39], each bacterial strain was inoculated in the corresponding liquid culture medium for each genus (Table S1) and enriched with tryptophan (100 μg mL^{-1}), and the flasks incubated under shaking (100 rpm) for 7 days at 28 °C. The growth broth was then transferred to microtubes and centrifuged at 10,000 rpm for 10 min (Eppendorf -Centrifuge 5804 R). Following, 1 mL of the supernatant was transferred to new microtubes, and

750 µL of the Salkowski's reagent (1 mL of $FeCl_3.6H_2O$ (0.5 Mol L−1), 50 mL of $HClO_4$ (35%) was added. After 30 min of reaction in the dark at room temperature, the staining intensity was evaluated in a spectrophotometer (Genesys™ 10S UV–VIS spectrophotometer) at a wavelength of 540 nm. The results were expressed in µg mL^{-1} of IAA in the medium based on a calibration curve with synthetic IAA (0, 50, 100, 200, 300, 400, 500, 600, 800, 1000 µg mL^{-1}) (Figure S1).

Production of exopolysaccharides

The assessment for EPS production was conducted based on the method proposed by de Meneses et al. [40] and Castellane et al. [41], with some adaptations. Aliquots of 2 mL of culture broth incubated for 72 h at 28 °C were placed in microtubes and centrifuged at 14,000 rpm (Eppendorf—Centrifuge 5804 R) for 12 min at 4 °C. The cell pellet was discarded, and 50 µL of the supernatant were transferred to a new microtube containing 150 µL of ice-cold ethanol. The microtubes were centrifuged again at 14,000 rpm at 4 °C for 10 min. The microtubes were then transferred to a concentrator (Eppendorf—Concentration Plus) at 45 °C for 1 h or until dry, and then 200 µL of ultrapure water were added and homogenized in a vortex. For quantification, 200 µL of 5% phenol and 1 mL of concentrated H_2SO_4 were added and homogenized in a vortex, using ultrapure water, phenol, and H_2SO_4 as blank. After 15 min of reaction at room temperature, spectrophotometric analysis was carried out (Genesys™ 10S UV–VIS spectrophotometer), reading the O.D. at 485 nm. The results were obtained according to a calibration curve based on the Phenol–Sulfuric method described by DuBois et al. [42] for measuring total carbohydrates, using glucose (0, 10, 20, 40, 60, 80, 100, 200 µL mL^{-1}) as the standard substrate for the calibration curve (Figure S1).

Production of biofilm

The assessment of biofilm production was carried out according to the method proposed by Lima et al. [43], with some adaptations. Bacterial growth was assessed in microtubes (Eppendorf—2 mL) in the respective culture medium for each strain (Table S1). In this analysis, there is no standard comparative concentration, considering only the differences among the strains based on optical density (O.D.), where higher readings indicate greater biofilm production (Figure S1).

Phosphate solubilization

The capacity for phosphate solubilization was evaluated using the Sylvester-Bradley et al. [44] method. After autoclaving (121 °C for 20 min), the medium was separately supplemented with inorganic calcium phosphate ($CaHPO_4{\cdot}2H_2O$), which was previously prepared and sterilized. The cultures were incubated at 28 °C, and the presence of a solubilization halo was assessed at 3, 7, and 12 days after inoculation (DAI). The Solubilization Index was determined by the ratio between the halo diameter and the colony diameter, using a pachymeter (King Tools, São Paulo, Brazil) (Figure S1).

Ability to grow in a culture medium with reduced water activity and high temperature (40 °C)

To evaluate the development of the strains in a medium with reduced water activity (Aw), Aw was tested at 0.919 and 0.897, as proposed by Hallsworth et al. [45]. After incubation at 40 ± 2 °C for seven days in Petri dishes, the strains that exhibited visible growth were considered positive (Figure S1), while those that failed to grow in the medium with a high solute concentration and incubated at high temperatures were considered negative.

Phenotypic clustering

The in vitro evaluation data of 100 PGPB strains for agronomically relevant traits were used for phenotypic clustering. A binary matrix was generated based on the presence or absence of these traits, and the isolates were grouped using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm with the aid of Bionumerics® software version 7.6.3 (Applied Mathematics, Sint-Martens-Latem, Belgium) [46]. Clustering distinction was established at a 70% similarity threshold.

Numerical data were also normalized and used to generate a heatmap in the R environment (v. 4.5.0), supported by RStudio (v. 2024.12.1) and the packages ggplot2 (v. 3.5.2), reshape2 (v. 1.4.4), and ggtext (v. 0.1.2).

Evaluation of the bacterial strains on conferring plant tolerance to drought under greenhouse

First experiment: evaluation of 100 strains in maize grown in sterile substrate

Substrate preparation Plants were grown in 1 L pots (12 cm in diameter), filled with approximately 1 kg of a substrate composed of coarse sand and ground coal in a 1:1 (v:v) ratio. The substrate was previously homogenized in trays and sterilized in an autoclave at 121 °C for 40 min.

Inoculum preparation The inocula were prepared by cultivating each bacterial strain in the corresponding liquid medium (Table S1), ensuring an equivalent concentration of approximately 10^8 CFU mL^{-1} for each strain.

Conduction of the experiment The treatments consisted of the 100 selected strains and two non-inoculated controls: one subjected to water restriction similar to the inoculated plants, and another maintained under adequate watering conditions. The experiment was laid out in a completely randomized design with three replicates.

Maize (*Zea mays* L.) seeds of hybrid Pioneer 30F53 PRO 3 were surface-disinfested by immersion in 70% ethanol for 1 min, 0.4% sodium hypochlorite for 5 min, and six consecutive rinses in sterile distilled water.

The inoculation was carried out directly in the pot. The seeds were inoculated with 1 mL of the bacterial suspension cultivated in liquid medium, without removing the culture medium. This volume was applied to each seed, ensuring complete coverage of the seed by the inoculum. Immediately afterward, the seeds were covered with substrate, guaranteeing full coverage and proper contact with the soil.

The greenhouse experiment was carried out at the Experimental Station facilities of Embrapa Soja in Londrina, Paraná State, southern Brazil (23°11′ S, 51°11′ W). Plants were supplied with sterilized Hoagland and Arnon's nutrient solution [47] at 50% of the N concetration, in a greenhouse with forced ventilation, natural photoperiod, and a temperature of 29± 2 °C (day) and 17± 2 °C (night). Plants were submitted to water restriction at the V3 stage, 10 days after emergence (DAE), when they had three fully developed leaves, for 12 days.

Induction of water stress The water deficit condition was monitored by randomly weighing selected pots to track mass loss, ensuring that the plants did not reach the permanent wilting point but remained stressed throughout the period of water restriction. The amount of nutrient solution for replenishment was established daily and distributed equally among the treatments. A view of the experiment is shown in Figure S2.

Data collection

Plants were collected 22 DAE, 12 days after the water restriction was imposed. Roots and shoots were separated, and the following traits were assessed: on the day before harvesting (21 DAE), plant height was measured from the soil surface to the base of the first mature leaf. Chlorophyll was determined using a chlorophyll meter (SPAD 502, Konica Minolta Sensing, Inc., Osaka, Japan) according to the calibration described by Kaschuk et al. [48]. At harvest, fresh and dry shoot biomass, and root system volume were recorded according to Rondina et al. [49].

Second experiment: evaluation of selected strains in maize grown in non-sterile soil

Substrate and inoculant preparations and conduction of the experiment Fifteen bacterial strains that most promoted plant growth under water restriction in the first greenhouse experiment, as well as positive results in most in vitro tests, were selected for confirmation in a second greenhouse trial. This second trial followed similar inocula preparation And sowing procedures, except for the substrate, consisting of 5 kg of non-sterile soil per pot (24 cm in diameter). The soil was classified as Typic Acrudox [50] taken at 0–20 cm topsoil layer from a commercial farm located in Ponta Grossa, Paraná State, Brazil, and presenting the following characteristics: pH ($CaCl_2$) = 5.14; organic matter = 18.24 g dm^{-3}; available P = 2.85 mg dm^{-3}; exchangeable K = 0.10 $cmol_c$ dm^{-3}; Ca = 3.47 $cmol_c$ dm^{-3}; Mg = 1.10 $cmol_c$ dm^{-3}; soluble N = 2.5 mg dm^{-3}; H + Al = 4.12 $cmol_c$ dm^{-3}; Cation Exchange Capacity (CEC) = 8.79 $cmol_c$ dm^{-3}; granulometry: sand = 238 g kg^{-1}, silt = 30 g kg^{-1}, and clay = 732 g kg^{-1}.

Preparation of inoculants and procedures of inoculation were the same as described for the first experiment The experiment was also carried out at the Experimental Station facilities of Embrapa Soja, under greenhouse conditions, with an average photoperiod of 13 h (day) and 11 h (night) ± 28 min and a temperature of 24± 3.1 °C (day) and 20± 2 °C (night). During the trial, the average day/night relative humidity was 52.5 to 92.7%, respectively. The experiment was conducted following a completely randomized design, with five replicates per treatment.

Water stress induction For the adjustment of soil moisture, the water-holding capacity was determined using a tension table and Richards's extractor device, resulting in a water-retention curve that correlates the water content with the soil water potential (ψw). During the first 22 DAE, all plants received water to maintain the ψw at 70% of the water-holding capacity. After 22 DAE, at V5 stage, when the plants had five fully developed leaves, they were submitted to water restriction. For that, the pots under water restriction were maintained at 30% of water-holding capacity, while non-inoculated control plants were kept with adequate water supply (70% water-holding capacity). Soil moisture was monitored daily by weighing each pot on an electronic scale, and adjustments of moisture were made in the morning (between 9 And 11 a.m.). We considered the fresh mass of plants at well-watered conditions at 22 DAE from extra pots to correct the effect of plant weight on the water reposition in pots containing plants subjected to water restriction. An overview of the experiment is shown in Figure S3.

Data collection

On the 3rd, 5th and 9th days of water restriction, physiological parameters were recorded in both stressed and non-stressed plants with a portable gas exchange meter, model LI-6400 (Li-Cor, Biosciences Inc., Nebraska, USA). Determinations included net photosynthetic (A) and transpiration (E) rates, stomatal conductance (gs), intercellular CO_2 concentration (Ci), and temperature of leaves. Gas exchanges were assessed in the central leaf

Table 1 In vitro evaluation of protease, cellulase, growth under reduced water activity, 1-aminociclopropano-1-carboxilato deaminase(ACC)-deaminase, phosphate solubilization, biofilm and EPS production, IAA (indole-3-acetic acid) and siderophore synthesis of 100 bacterial strains of the Microbial Culture Collection of Embrapa Soja

Strain ID	Protease	Cellulase	Water activity		ACC deaminase	Phosphate solubilization	Biofilm (O.D)	EPS (μg mL^{-1})	IAA (μg mL^{-1})	Siderophore (Halo/colony)	
			0.919	0.897						24H	72H
Achromobacter sp. CNPSo 2660					+		0.122d [a]	0.00i [a]	29.97 g [a]		
Agrobacterium deltaense. CNPSo 2707	+						0.337d	25.13 g	88.14d	0.00i [a]	1.45i [a]
Agrobacterium fabacearum CNPSo 675							0.489c	47.86e	83.01d		
Agrobacterium pusenese CNPSo 3315					+		0.428d	43.0e	86.97d	1.39 g	2.07 g
Agrobacterium pusense CNPSo 3348							0.221d	56.85d	73.87e		
Agrobacterium sp. CNPSo 1235					+		0.486c	66.40d	86.83d		
Agrobacterium sp. CNPSo 1668					+		0.714c	15.37 h	63.22e		
Agrobacterium sp. CNPSo 4041					+		0.596c	71.31c	63.40e	0.00i	1.73 h
Agrobacterium sp. CNPSo 4045							0.35d	74.04c	92.91d		
Azoarcus indigens CNPSo 2541	+						0.515c	29.38f	15.95 h	0.00i	1.73 h
Azorhizobium caulinodans CNPSo 139							0.297d	3.52i	29.16 g		
Azospirillum brasilense CNPSo 2083	+				+		0.053d	10.66 h	48.53f		
Azospirillum brasilense CNPSo 2084	+				+		0.078d	129.25a	30.25 g		
Azospirillum halopraeferens CNPSo 3601							0.049d	9.89 h	23.01 h		
Azospirillum rugosum CNPSo 3757	+						0.173d	131.38a	14.68 h		
Azospirillum thiophilum CNPSo 2786							0.314d	11.93 h	77.16d	2.04c	3.37d
Bacillus aryabhatai CNPSo 2603			+		+		0.070d	44.30e	83.64d		
Bacillus sp. CNPSo 2383							0.742c	0.68i	7.86 h		
Bacillus sp. CNPSo 2658		+	+				0.088d	43.93e	20.86 h		
Bacillus sp. CNPSo 2723	+	+	+				0.273d	41.70e	47.17f		
Bacillus sp. CNPSo 2725	+	+	+				0,442d	42.16e	6.22e		
Bacillus sp. CNPSo 3218	+	+	+	+			0.206d	44.77e	89.31d		
Bacillus subtilis CNPSo 2605		+	+	+			0.334d	45.32e	85.47d		
Bacillus subtilis CNPSo 2606	+	+	+	+			0.940b	43.30e	99.35d		
Bacillus subtilis. CNPSo 2620	+	+	+	+	+		0.999b	49.19e	51.47f	1.56f	1.54 h
Bacillus velezensis CNPSo 2384		+	+	+	+		1.249a	2.08i	7.78 h		
Bacillus velezensis CNPSo 2657	+	+	+		+		0.147d	45.95e	5.76f	0.00i	4.07b
Bradyrhizobium frederickii CNPSo 3443					+		0.360d	10.91 h	2.41 h	1.42 g	1.66 h
Bradyrhizobium diazoefficiens CNPSo 6							0.144d	6.29i	2.00 h		
Bradyrhizobium elkanii CNPSo 14							0.042d	0.00i	8.74 h		
Bradyrhizobium elkanii CNPSo 9							0.238d	6.40i	3.09 h		
Bradyrhizobium frederickii CNPSo 3426					+		0.066d	13.10 h	4.45 h		
Bradyrhizobium japonicum CNPSo 7							0.445d	0.00i	4.60 h		
Bradyrhizobium pachyrhizi CNPSo 2259					+		0.176d	4.11i	3.27 h		
Bradyrhizobium sp. CNPSo 2907	+				+		0.210d	11.86 h	15.81 h		
Bradyrhizobium yuanmingense CNPSo 3084	+						0.075d	1.13i	1.54 h	1.27 h	1.31i

Table 1 (continued)

Strain ID	**Protease**	**Cellulase**	**Water activity**		**ACC deaminase**	**Phosphate solubilization**	**Biofilm (O.D)**	**EPS (μg mL^{-1})**	**IAA (μg mL^{-1})**	**Siderophore (Halo/colony)**	
			0.919	**0.897**						**24H**	**72H**
Chromobacterium violaceum CNPSO 1947	+						0.083d	30.14f	17.25 h	1.75e	2.39f
Chromobacterium violaceum CNPSo 1950					+		0.139d	29.78f	22.97 h	1.92d	3.27d
Chromobacterium violaceum CNPSo 1952					+		0.063d	92.34b	22.97 h		
Chromobacterium violaceum CNPSo 1954							0.012d	37.85f	206.58a	0.00i	2.89e
Chromobacterium violaceum CNPSo 1958					+		0.053d	25.69 g	11.49 h		
Chromobacterium violaceum CNPSo 1963							0.092d	28.80f	21.33 h		
Delftia sp. CNPSo 3288					+		0.086d	10.92 h	20.37 h	1.25 h	1.39i
Ensifer (= *Sinorhizobium*) *mexicanus* CNPSo 2067					+		0.116d	10.57 h	44.68f		
Enterobacter sp. CNPSo 3867							0.195d	35.95f	7.63 h		
Gluconacetobacter azotocaptans CNPSo 2783	+					+	0.006d	22.50 g	9.88 h		
Methylobacterium sp. CNPSo 989		+			+		0.044d	1.72i	3.00 h		
Microbacterium sp. CNPSo 3287	+						0.051d	6.91i	40.26f		
Microbacterium sp. CNPSo 3855							0.330d	30.60f	12.53 h		
Neorhizobium (= *Rhizobium*) *huautlense* CNPSo 206							0.066d	34.30f	77.70d		
Neorhizobium galegae CNPSo 2061	+				+		0.223d	11.74 h	50.42f		
Niveispirillum irakense CNPSo 3756							0.579c	10.65 h	52.15f		
Ochrobactrum oryzae CNPSo 2784	+						0.352d	0.00i	56.79e		
Paenibacillus polymyxa CNPSo 2227		+			+		0.533c	17.36 h	43.20f		
Paenibacillus sp. CNPSo 3221		+					0.418d	16.08 h	49.81f		
Paenibacillus sp. CNPSo 3309		+			+		0.060d	4.70i	79.48d	0.00i	1,21i
Paenibacillus sp. CNPSo 3854	+	+					0.265d	8.80 h	28.83 g		
Pantoea agglomerans CNPSo 2602	+					+	0.129d	52.23e	19.05 h		
Pantoea ananatis CNPSo 2797		+			+		0.357d	7.54 h	81.48d		
Pantoea ananatis CNPSo 2798		+			+		0.568c	77.25c	114.77c		
Pantoea ananatis CNPSo 3282	+	+					0.208d	19.52 g	90.71d		
Pantoea sp. CNPSo 2344							0.049d	12.15 h	18.44 h		
Pantoea sp. CNPSo 2493	+		+		+		0.236d	58.11d	117.04c		
Paraburkholderia atlantica CNPSo 3155		+					0.262d	10.16 h	7.63 h		
Paraburkholderia franconis CNPSo 3157							0.142d	13.04 h	7.51 h		
Paraburkholderia guartelaensis CNPSo 2995							0.620c	0.00i	2.17 h		
Paraburkholderia guartelaensis CNPSo 3008	+				+		0.548c	0.00i	2.45 h	1.26 h	2.14 g
Paraburkholderia nodosa CNPSo 1204							1.404a	19.76 g	6.63 h		
Paraburkholderia nodosa CNPSo 1213							0.565c	3.17i	3.46 h		
Paraburkholderia nodosa CNPSo 1294							0.389d	10.40 h	6.87 h		
Paraburkholderia nodosa CNPSo 1299							1.458a	0.00i	30.29 g		
Paraburkholderia nodosa CNPSo 1301							0.489c	0.74i	4.77 h		
Paraburkholderia nodosa CNPSo 1307							0.962b	3.21i	59.78e		

Table 1 (continued)

Strain ID	Protease	Cellulase	Water activity		ACC deaminase	Phosphate solubilization	Biofilm (O.D)	EPS ($\mu g\ mL^{-1}$)	IAA ($\mu g\ mL^{-1}$)	Siderophore (Halo/colony)	
			0.919	**0.897**						**24H**	**72H**
Paraburkholderia sabiae CNPSo 3136	+				+		0.220d	11.67 h	87.84d	0.00i	1.44i
Paracocus sp. CNPSo 3707							0.042d	45.23e	97.18d		
Pseudomonas fluorescens CNPSo 2224					+		0.547c	91.11b	58.95e	1.21 h	1.32i
Pseudomonas fluorescens CNPSo 2799					+		0.188d	62.55d	37.17 g	1.39 g	1.71 h
Pseudomonas lurida CNPSo 2218					+		1.166a	50.51e	75.90e	1.33 g	1.91 g
Pseudomonas fluorescens CNPSo 2719	+					+	0.677c	47.44e	31.73 g	1.79e	3.27d
Pseudomonas soli CNPSo 1987	+						0.159d	45.61e	70.37e	1.25 h	1.47i
Pseudomonas sp. CNPSo 2220					+		0.919b	47.80e	140.27b	1.33 g	1.96 g
Pseudomonas sp. CNPSo 2222						+	0.568c	41.36e	124.36c		
Pseudomonas sp. CNPSo 2604	+					+	0.288d	62.88d	47.48f		
Pseudomonas sp. CNPSo 2625	+						0.635c	52.16e	28.12 g	2.62a	3.57c
Pseudomonas sp. CNPSo 2835							0.252d	51.50e	44.88f	1.55f	2.92e
Pseudomonas sp. CNPSo 2844	+						0.328d	78.26c	46.32f		
Pseudomonas sp. CNPSo 2851	+						0.509c	88.92b	70.81e		
Pseudomonas sp. CNPSo 2856							0.167d	87.60b	46.37f		
Pseudomonas sp. CNPSo 2864	+						0.134d	94.05b	84.45d	1.29 h	1.37i
Pseudomonas sp. CNPSo 2878	+					+	0.827c	78.79c	66.12e	2.40b	3.55c
Pseudomonas sp. CNPSo 2887								102.06b	47.49f		
Pseudomonas sp. CNPSo 4132	+				+		0.429d	50.23e	71.22e	1.38 g	1.33i
Pseudomonas sp. CNPSo 4140					+	+	0.531c	31.23f	55.85e	1.42 g	1.26i
Rhizobium giardinii CNPSo 171					+		0.111d	10.74 h	6.74 h	2.69a	4.34a
Rhizobium leucaenae CNPSo 224							0.057d	14.73 h	63.83e		
Rhizobium leucaenae CNPSo 229					+		0.299d	15.94 h	18.84 h		
Rhizobium sp. CNPSo 1627							0.814c	96.94b	14.09 h		
Rhizobium sp. CNPSo 3610							0.098d	21.16 g	39.55f	1.32 g	1.73 h
Rhizobium tropici CNPSo 1018					+		0.492c	57.76d	106.54d		
Rhizobium tropici CNPSo 103							0.152d	84.43c	63.87e		

[a] Values represent the mean of three biological replicates and when followed by the same letter are not statistically different according to the Scott-Knott test ($p < 0.05$)

in the morning (9–11 a.m.). Plants were collected at 35 DAE, when roots and shoots were separated. The following traits were assessed: shoot fresh weight, root mean volume, shoot dry weight, root dry weight. Plant height was evaluated before harvesting as in Experiment I, and root length was obtained according to Tennant [51].

Statistical analysis

For all experiments, the data analysis was structured to assess the significance of treatments and to explore relationships between the assessed variables. Initially, a one-way analysis of variance (ANOVA) was performed, followed by the Scott-Knott test at a 5% of significance to group similar treatments. For all ANOVA tests, the normality of residuals and the homogeneity of variances were assessed prior to analysis.

To evaluate the relationship between in vitro and in vivo results from the first greenhouse experimental trial, Pearson's correlation coefficient (PCC) was applied using R-Statistics to determine the strength and direction of the linear relationship between the data. Statistical significance was determined at $p \leq 0.05$.

Additionally, principal component analysis (PCA) explored the variability and relationships among the in vivo data assessed in the first greenhouse experiment. The PCA analysis was conducted using SPSS software (version 22.0) to identify the main components that explain the variability in the dataset. This multivariate analysis allowed the summarization of the data structure and identification of key patterns associated with the treatments.

Results and discussion

In vitro plant growth-promoting traits

The selected 100 PGPB strains were evaluated in vitro for ten putative plant growth-promoting traits. Protease production was confirmed in 33 strains by the formation of a typical degradation halo around the colonies on a culture medium containing skim milk (Table 1). Additionally, cellulase production was observed in 18 strains, eight of them exhibiting the production of both enzymes, belonging to the genera *Bacillus* (6), *Paenibacillus* (1), and *Pantoea* (1) (Table 1). The genus *Bacillus* has been reported as highly efficient in producing hydrolytic enzymes [52–54]. The protease activity has great potential to help in the biocontrol of pests and diseases in agriculture. For example, Khedher et al. [55] reported the effect of this enzyme produced by some species of *Bacillus* on the rupture and deformation of fungal hyphae. Proteases can impair the structure and some cellular functions of pathogenic fungi due to vacuolization, protoplast leakage, and cracking of mycelia [55]. An important advantage of biocontrol using microbial hydrolytic enzymes to replace chemical fungicides is the lack of damage to plant tissues [56].

Thirty-eight strains demonstrated ACC-deaminase activity, indicated by robust growth in medium containing ACC as the sole N source. This trait was distributed across 15 genera: *Achromobacter* (1), *Agrobacterium* (4), *Azospirillum* (2), *Bacillus* (4), *Bradyrhizobium* (4), *Chromobacterium* (3), *Delftia* (1), *Ensifer* (= *Sinorhizobium*) (1), *Methylobacterium* (1), *Neorhizobium* (1), *Paenibacillus* (2), *Pantoea* (3), *Paraburkholderia* (2), *Pseudomonas* (6), and *Rhizobium* (3) (Table 1).

The production of ACC-deaminase is related to the regulation in the levels of ethylene due to mineralization of precursor molecules, releasing ammonia, which may contribute to plant nutrition to a small extent [57, 58]. Therefore, the ACC-deaminase-producing strains help to reduce the negative response to ethylene produced under stressing conditions, such as water deficit, and also to reduce the senescence, a widespread physiological strategy under stress [59]. The induction of stress tolerance promoted by ACC-deaminase-producing PGPB has been broadly reported in various crops, including improvements in plant growth, nutrient content, and antioxidant properties in wheat (*Triticum aestivum* L.) associated with strains of the genera *Variovorax*, *Pseudomonas*, *Achromobacter*, and *Ochrobactrum* [60]. In maize associated with *Achromobacter xylosoxidans*, the ACC-deaminase activity increased photosynthetic rate, stomatal conductance, total chlorophyll and carotenoid contents, and grain yield [61]. In soybean (*Glycine max* (L.) Merr.), inoculation with *Curtobacterium* sp. relieved the salt stress and stimulated plant growth [62]. Interestingly, in cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*), the inoculation of an ACC-deaminase-producing strain *Leclercia adecarboxylata* promoted both growth and tolerance to salt stress [63], highlighting the biotechnological potential of ACC-deaminase-producing strains such as those identified in our study.

Siderophore production was observed in 30 strains, spanning 11 genera: *Agrobacterium* (3), *Azoarcus* (1), *Azospirillum* (1), *Bacillus* (2), *Bradyrhizobium* (2), *Chromobacterium* (3), *Delftia* (1), *Paenibacillus* (1), *Paraburkholderia* (2), *Pseudomonas* (12), and *Rhizobium* (2) (Table 1). To better assess the efficiency of siderophore production among strains with different growth rates, the assay was conducted at two distinct time points (after 24 And 72 h of incubation). This approach was necessary because halo development was not synchronized across all strains. By including an additional reading, it was possible to capture the full expression of siderophore production in slower-growing strains and to account for differences in halo/colony ratios, which varied significantly among the isolates. This metric allowed a more accurate comparison of siderophore production capacity, beyond just the presence or absence of a halo. After 72 h of incubation, *Rhizobium giardini* CNPSo 171 and *Pseudomonas* sp. CNPSo 2625 showed the highest halo-to-colony ratios, indicating high efficiency in siderophore production relative to colony growth.

The 30 siderophore-producing strains identified in our study may be of biotechnological interest due to their ability to increase nutrient availability and inhibit pathogen growth through iron (Fe) complexation [64, 65]. Among them, *Pseudomonas* was the most prevalent genus (12 strains), as also reported by Tian et al. [66]. The genus is widely recognized for enhancing Fe uptake in plants grown in Fe-deficient soils [67]. In maize, siderophore production has been associated with improved Fe transport to stalks, leaves, and seeds, which is relevant for the nutritional quality of food and feed [68]. Moreover, by restricting Fe availability in the rhizosphere, siderophores contribute to the suppression of phytopathogens, offering an eco-friendly alternative to synthetic fungicides [69].

Regarding the synthesis of phytohormones, *Chromobacterium violaceum* strain CNPSo 1954 stood out in a tryptophan-supplemented culture medium, producing the highest amount of IAA, 206.58 μg mL^{-1} (Table 1). Despite being quite variable, all strains were able to synthesize IAA when supplemented with tryptophan, ranging from 1.54 to 206.58 μg mL^{-1}; tryptophan is the precursor of IAA. *C. violaceum* is known for the synthesis of a secondary metabolite named violacein, which gives the purple color to the colonies [70]. Although violacein is not essential for bacterial growth and survival, it is involved in the synthesis of tryptophan [71]. Other genera also synthesized high concentrations of IAA, such as *Pseudomonas*, which has been widely recognized in previous studies as an important producer of indolic compounds [72, 73], and also *Pantoea*. IAA stimulates longer roots with an increased number of root hairs and roots branching, which are crucial for nutrient uptake [74]. Additionally, depending on the concentration, IAA may increase the length of the primary root and enhance tolerance to salt and drought stress by driving the roots away from regions of elevated salinity or towards regions with more available water [75, 76].

Regarding the high biotechnological potential of *C. violaceum* CNPSo 1954 in the synthesis of IAA, it is worth mentioning that when searching for strains to be used as bio-inputs, they must also be investigated for pathogenicity to humans, animals, and plants. *C. violaceum* rarely causes diseases in humans; it is abundantly found in the rivers of the Amazon, where strain CNPSo 1954 was obtained from [77], but as pathogenicity has been reported [78], the strains should always be investigated. If pathogenicity is confirmed, alternatives can be found, such as the use of secondary metabolites as phytostimulants [79].

Regarding EPS production, the strains *Azospirillum rugosum* CNPSo 3757 and *Azospirillum brasilense* CNPSo 2084 (= Ab-V6) stood out, with average values of 131.38 μg mL^{-1} And 129.25 μg mL^{-1}, respectively (Table 1). The production of EPS is fundamental for microbial life, as it provides an ideal environment for their survival, favoring chemical reactions and the provision of nutrients, and can also benefit plant growth by increasing soil physical conditions and facilitating the aggregation of particles, in addition to reports on mitigation of environmental stresses, such as salinity and drought [80, 81]. In this study, although two strains of *Azospirillum* stood out for EPS production, *Pseudomonas* accounted for five out of nine strains with the greatest potential. *Pseudomonas* has been reported for its plenty production of EPS under water restriction, creating a microenvironment that promotes water maintenance, thereby protecting the microorganism and the associated plant roots against dehydration [82, 83].

EPS is part of the structural matrix of biofilms; however, in this study, there was no direct relationship between the production of EPS and biofilm formation, as the maximum production of biofilm in vitro was obtained by strains of *Paraburkholderia*. Among the 100 strains assessed for biofilm production (SD = 17%), *Paraburkholderia nodosa* CNPSo 1299 (O.D. 1.458), *P. nodosa* CNPSo 1204 (O.D. 1.404), *Bacillus velezensis* CNPSo 2384 (O.D. 1.249), and *Pseudomonas lurida* CNPSo 2218 (O.D. 1.166) stood out, showing the highest optical density values, highlighting superior biofilm capacity (Table 1). This can be explained not only by the capacity of EPS synthesis but also on factors such as the structure and physiology of the microbial cell and quorum-sensing mechanisms. Biofilm production can also be changed during the process of association between the microorganism and the host plant, when other interfering factors may occur, such as interactions with other microorganisms, root exudates, microbial competition, physicochemical characteristics, and soil organic matter content, among others [84].

A very low number of strains was able to solubilize calcium phosphate, five of each belonging to the genus *Pseudomonas* (CNPSo 2222, CNPSo 2604, CNPSo 2719, CNPSo 2878 And CNPSo 4140), in addition to *Pantoea agglomerans* CNPSo 2602 and *Gluconacetobacter azotocaptans* CNPSo 2783 (Table 1 and Fig. 1), corroborating the data from Sánchez López et al. [85]. These strains can contribute with the increasing P-limiting conditions reported in agriculture [86], commonly found in Brazilian soils [87]. The benefits and importance of making P available by PGPB are reinforced by the finite availability of phosphate rocks for P-fertilizer production, increasing production costs, and the global low levels of soil available P to support agricultural production [88, 89].

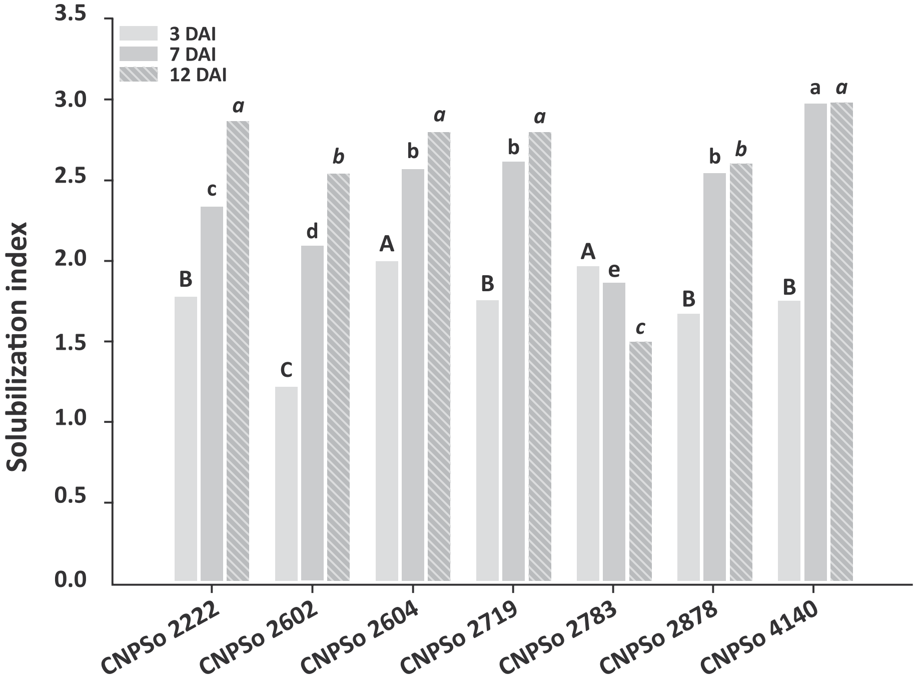

Fig. 1 Solubilization index of the strains for calcium phosphate solubilizing activity. Capital letters compare the halo/colony ratio assessed at 3 days; lower case letters compare the ratio at 7 days; italic lowercase letters compare the ratio at 12 days after inoculation. Values represent the means of three biological replicates and when followed by the same letter are not statistically different according to the Scott-Knott test ($p \leq 0.05$)

Only 11 strains grew at reduced water activity in the culture medium with an Aw of 0.919 and temperature of 40 °C, and only five had satisfactory growth under even more restrictive conditions, with Aw of 0.897 (Table 1). The five strains are species of *Bacillus*, highlighting the adaptability of this genus to environments with restricted water availability and elevated temperatures. This resilience is evidenced by the ability of these Gram-positive bacteria to form endospores, which makes the genus more tolerant to life-threatening abiotic conditions such as extremes of temperature, pH, and radiation [90, 91]. This combination of physiological and genetic traits allows *Bacillus* strains not only to survive but also to play important roles in challenging environments, such as those affected by water restriction and higher temperatures, a scenario ever more frequent as a result of the global warming.

Studies have shown that plants colonized by *Bacillus* spp. exhibit enhanced water uptake, which plays a crucial role in protecting them from drought-induced damage [92]. Furthermore, under water restriction, the absorption of nutrients typically decreases. However, when treated with *Bacillus* spp., plants demonstrate improved efficiency in nutrient uptake, even under drought conditions [93]. Importantly, our results provide new evidence that bacterial strains can play a major role in the agriculture of the future, where water constraints will be more frequent, with species of the genus *Bacillus* has been outstanding compared to the other strains evaluated.

Phenotypic clustering

The dendrogram analysis, considering potential plant growth-promoting traits among the 100 strains assessed in vitro, identified five clusters at a similarity level of 70% (Fig. 2). Cluster 3 had the highest number of isolates, 39, with the genera broadly distributed, followed by clusters 5 (predominantly *Pseudomonas*) And 4, with 22 And 15 isolates, respectively. Cluster 1, comprising only *Bacillus*, had nine isolates.

The heatmap analysis confirmed distinct patterns among bacterial genera regarding the quantitative expression of in vitro traits associated with plant growth-promotion (Fig. 3). Strains of the genus *Paraburkholderia* exhibited high capacity for biofilm formation, while *Azospirillum* stood out for exopolysaccharide production. IAA synthesis was particularly high in a *Chromobacterium* strain. In contrast, *Pseudomonas* strains showed consistent performance across multiple tests, indicating a multifunctional profile, while *Bacillus* displayed high variability, with results depending on the strain.

Assessment of plant tolerance to drought under greenhouse conditions

First screening for tolerance to drought

In the first experiment, in which 100 bacterial strains were assessed in sterile substrate on the growth of maize, inoculated plants showed superior performance in some parameters compared to control, indicating promising effects of certain strains on the physiological variables

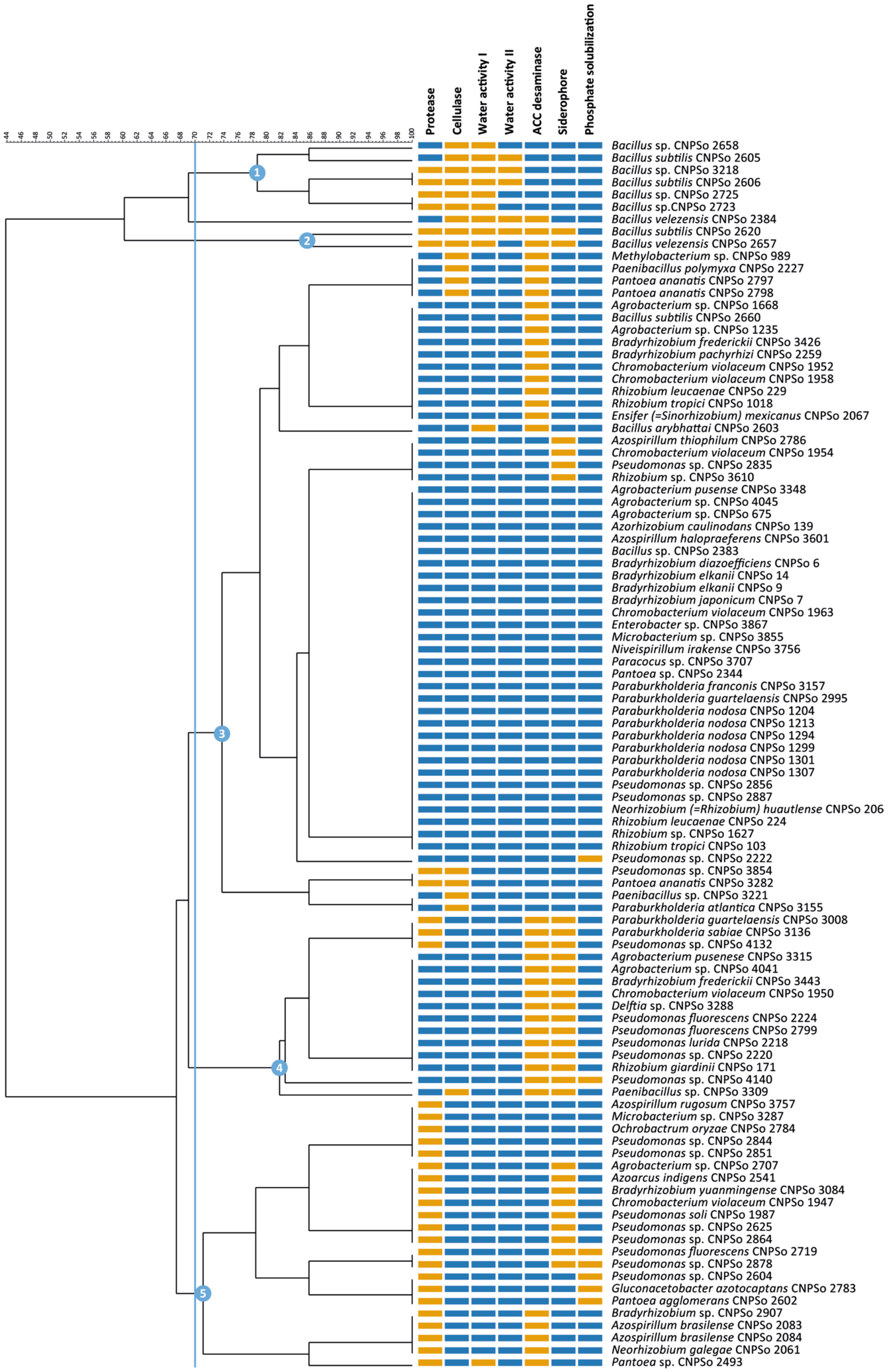

Fig. 2 Phenotypic dendrogram based on putative plant growth-promoting traits of 100 bacterial strains of the Microbial Culture Collection of Embrapa Soja. Dendrogram built on Bionumerics software (v.7.6.3) using the UPGMA algorithm. The clusters were obtained considering the similarity level of 70%. Positive traits are represented in yellow, while negative traits are shown in blue

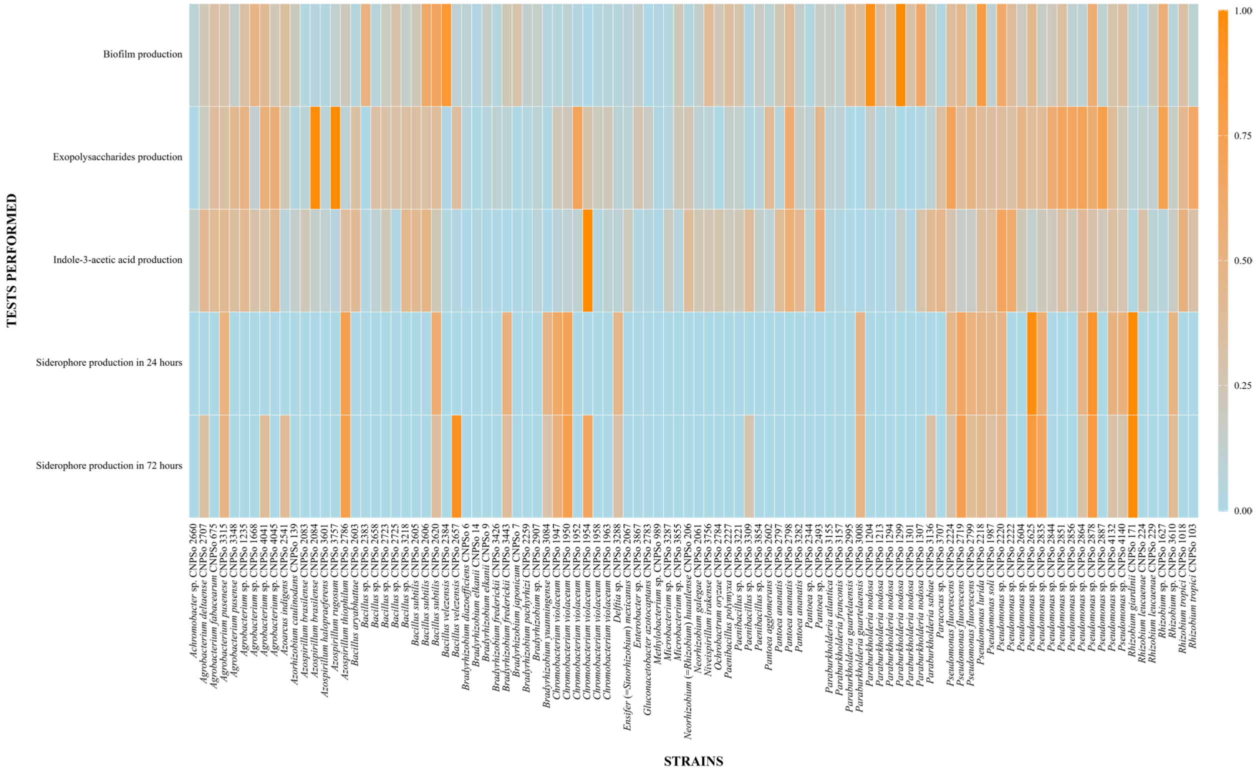

Fig. 3 Heatmap of plant growth-promoting traits of 100 bacterial strains from the Microbial Culture Collection of Embrapa Soja. Data were normalized on a scale from 0 to 1, with a value of 1 assigned to strains with the highest performance for each evaluated trait. These are represented by orange coloration, while lower values follow a gradient from lighter orange to blue, with blue indicating values closer to 0

assessed in this study. It is worth commenting that seeds were inoculated with 1 mL of bacterial suspensions obtained from liquid cultures with different compositions. However, we saw no differences in seed germination rates and vigor that could be attributed to differences in nutrient composition and therefore, we consider that plant growth was not affected by nutrients carried with the inoculant; furthermore, plants were always supplied with nutrient solution. For plants under water restriction, 11 strains stood out: *Achromobacter sp.* CNPSo 2660, *Azoarcus indigens* CNPSo 2541, *Bacillus aryabhatai* CNPSo 2603, *B. velezensis* CNPSo 2384, *Bacillus* sp. CNPSo 2725 And CNPSo 2383, *Bradyrhizobium pachyrizi* CNPSo 2259, *C. violaceum* CNPSo 1954, *Pantoea agglomerans* CNPSo 2602, *Pantoea* sp. CNPSo 2344 And CNPSo 2493. These strains significantly increased shoot fresh and dry weight, root volume, plant size, and chlorophyll content compared with the non-inoculated plants (Control I) (Table 2). Inoculation with 20 strains significantly increased chlorophyll content, with *Bacillus velezensis* CNPSo 2657 standing out by promoting a 27% increase compared with plants grown under well-watered conditions (Control II) (Table 2). The performance of two promising strains promoting drought tolerance in maize is shown in Figure S4.

Very important, the inoculation of 42 strains resulted in shoot dry weight of plants kept under water restriction statistically similar to plants kept under well-watered conditions, belonging to the genera: *Bacillus* (9), *Paraburkholderia* (6), *Pseudomonas* (7), *Chromobacterium* (5), *Pantoea* (4), *Paenibacillus* (1), *Agrobacterium* (2), *Bradyrhizobium* (2), *Rhizobium* (2), *Achromobacter* (1), *Azoarcus* (1), *Azospirillum* (1), and *Gluconacetobacter* (1) (Table 2).

The average root volume ranged from 4.12 to 13.86 mL per plant, with the greatest value observed in plants inoculated with *B. velezensis* CNPSo 2384, which was statistically different from all the other treatments, including the non-inoculated plants grown under well-watered conditions (Control II) (Table 2).

For 21 strains, plant size under water restriction was comparable to well-watered plants (Control II). These strains included *Chromobacterium violaceum* CNPSo 1954, CNPSo 1963 And CNPSo 1958; *Psudomonas lurida* CNPSo 2218, *Pseudomonas soli* CNPSo 1987 And CNPSo 2220; *Bacillus subtilis* CNPSo 2620, *B. velezensis* CNPSo 2657, *B. aryabhatai* CNPSo 2603, *Bacillus* sp. CNPSo 2725 And CNPSo 2383; *Azoarcus indigens* CNPSo 2541; *Gluconacetobacter azotocaptans* CNPSo 2783; *Pantoea agglomerans* CNPSo 2602, *Pantoea* sp. CNPSo 2344 And CNPSo 2493; *Bradyrhizobium*

Table 2 Growth parameters[a] of maize hybrid Pioneer 30F53 PRO 3 inoculated with 100 bacterial strains of the Microbial Culture Collection of Embrapa Soja and grown in sterile substrate under water restriction

Treatment	**SFW (g pl^{-1})**	**SDW (g pl^{-1})**	**RV (mL pl^{-1})**	**PS (cm pl^{-1})**	**CC (µg cm^{-2})**
Control I (non-inoculated with water restriction)	4.17d[b]	0.720b[b]	5.31e[b]	33.5c[b]	4.80d[b]
Control II (non-inoculated without water restriction)	8.20a	1.223a	11.92b	40.8a	8.14b
Achromobacter sp. CNPSo 2660	6.08b	0.763a	5.53e	41.0a	8.10b
Agrobacterium sp. CNPSo 1235	4.89c	0.773a	5.82e	36.2c	7.67b
Agrobacterium sp. CNPSo 1668	4.93c	0.703b	6.33e	38.0b	8.68a
Agrobacterium sp. CNPSo 2707	5.64c	0.773a	6.66d	37.8b	8.95a
Agrobacterium pusenese CNPSo 3315	2.39e	0.473b	5.95e	32.7c	4.05d
Agrobacterium sp. CNPSo 3348	3.18e	0.607b	6.64d	35.0c	5.69d
Agrobacterium sp. CNPSo 4041	4.53d	0.690b	6.25e	37.3c	7.55b
Agrobacterium sp. CNPSo 4045	3.32e	0.497b	6.32e	33.3c	5.20d
Agrobacterium sp. CNPSo 675	4.04d	0.640b	5.84e	34.3c	8.52a
Azoarcus indigens CNPSo 2541	7.02b	1.037a	7.47d	44.0a	7.16b
Azorhizobium caulinodans CNPSo 139	2.76e	0.457b	5.45e	33.7c	8.58a
Azospirillum brasilense CNPSo 2083 = Ab-V5	2.91e	0.403b	10.04c	32.0c	6.04c
Azospirillum brasilense CNPSo 2084 = Ab-V6	3.74d	0.497b	9.85c	37.7b	5.60d
Azospirillum halopraeferens CNPSo 3601	3.17e	0.597b	7.43d	36.0c	6.43c
Azospirillum rugosum CNPSo3757	3.76d	0.633b	6.04e	33.3c	8.11b
Azospirillum thiophilum CNPSo 2786	5.17c	0.767a	9.40c	39.2b	4.43d
Bacillus velezensis CNPSo 2384	6.40b	0.913a	13.86a	38.7b	7.13b
Bacillus aryabhatai CNPSo 2603	6.11b	0.907a	11.74b	42.8a	9.71a
Bacillus sp. CNPSo 2383	6.47b	0.853a	10.70b	42.3a	7.75b
Bacillus sp. CNPSo 2658	5.76c	0.770a	5.95e	39.8b	9.19a
Bacillus sp. CNPSo 2723	5.32c	0.673b	6.97d	38.5b	7.20b
Bacillus sp. CNPSo 2725	6.24b	0.870a	7.64d	42.0a	7.32b
Bacillus sp. CNPSo 3218	5.07c	0.790a	9.11c	40.0b	6.76c
Bacillus subtilis CNPSo 2605	5.14c	0.873a	5.88e	40.5b	8.39a
Bacillus subtilis CNPSo 2606	5.55c	0.897a	5.54e	39.7b	7.44b
Bacillus subtilis CNPSo 2620	5.64c	0.903a	7.54d	44.3a	9.26a
Bacillus velezensis CNPSo 2657	5.36c	0.707b	6.87d	41.7a	10.38a
Bradyrhizobium diazoefficiens CNPSo 6	3.04e	0.600b	8.76c	35.5c	5.76d
Bradyrhizobium elkanii CNPSo 14	3.15e	0.533b	4.68e	36.8c	5.72d
Bradyrhizobium elkanii CNPSo 9	2.56e	0.467b	6.99d	32.8c	6.43c
Bradyrhizobium frederickii CNPSo 3426	3.17e	0.603b	6.44e	34.2c	5.57d
Bradyrhizobium frederickii CNPSo 3443	3.39e	0.653b	7.05d	37.2c	7.93b
Bradyrhizobium japonicum CNPSo 7	3.30e	0.563b	8.41c	34.0c	7.29b
Bradyrhizobium pachyrhizi CNPSo 2259	6.57b	0.883a	9.41c	41.8a	8.79a
Bradyrhizobium sp. CNPSo 2907	3.80d	0.567b	8.77c	34.7c	4.94d
Bradyrhizobium yuanmingense CNPSo 3084	4.95c	0.753a	8.66c	37.3c	6.20c
Chromobacterium violaceum CNPSo 1947	5.19c	0.753a	5.91e	39.7b	7.65b
Chromobacterium violaceum CNPSo 1950	5.42c	0.797a	6.24e	39.7b	7.74b
Chromobacterium violaceum CNPSo 1952	4.97c	0.727a	6.00e	39.3b	6.14c
Chromobacterium violaceum CNPSo 1954	5.96b	0.953a	5.97e	45.0a	9.29a
Chromobacterium violaceum CNPSo 1958	4.28d	0.653b	6.50e	41.7a	6.67c
Chromobacterium violaceum CNPSo 1963	4.67c	0.787a	6.53e	42.5a	6.50c
Delftia sp. CNPSo 3288	2.64e	0.510b	6.11e	32.2c	6.62c
Ensifer (= Sinorhizobium) mexicanus CNPSo 2067	3.99d	0.543b	6.48e	34.7c	6.39c
Enterobacter sp. CNPSo 3867	3.86d	0.613b	5.68e	35.0c	8.77a
Gluconacetobacter azotocaptans CNPSo 2783	5.49c	0.767a	6.98d	42.2a	6.95c
Methylobacterium sp. CNPSo 989	3.81d	0.570b	6.45e	36.2c	7.51b
Microbacterium sp. CNPSo 3287	2.50e	0.513b	6.20e	32.0c	6.58c
Microbacterium sp. CNPSo 3855	4.10d	0.690b	6.84d	36.7c	7.65b
Neorhizobium (= *Rhizobium*) *huautlense* CNPSo 206	2.86e	0.513b	5.30e	33.7c	7.72b

Table 2 (continued)

Treatment	SFW ($g\ pl^{-1}$)	SDW ($g\ pl^{-1}$)	RV ($mL\ pl^{-1}$)	PS ($cm\ pl^{-1}$)	CC ($\mu g\ cm^{-2}$)
Neorhizobium galegae CNPSo 2061	4.11d	0.683b	6.71d	37.0c	5.69d
Niveispirillum irakense CNPSo 3756	3.46e	0.590b	6.06e	35.5c	7.51b
Ochrobactrum oryzae CNPSo 2784	3.94d	0.550b	6.14e	38.8b	4.71d
Paenibacillus polymyxa CNPSo 2227	5.54c	0.657b	10.64b	41.8a	6.62c
Paenibacillus sp. CNPSo 3221	5.09c	0.847a	8.89c	40.0b	7.93b
Paenibacillus sp. CNPSo 3309	2.73e	0.520b	7.41d	32.0c	4.61d
Paenibacillus sp. CNPSo 3854	3.78d	0.657b	5.91e	35.7c	5.24d
Pantoea agglomerans CNPSo 2602	6.10b	0.993a	9.21c	42.0a	8.81a
Pantoea ananatis CNPSo 2797	5.17c	0.717b	10.02c	39.5b	5.04d
Pantoea ananatis CNPSo 2798	3.26e	0.530b	10.81b	34.3c	4.80d
Pantoea sp. CNPSo 2344	6.63b	0.903a	10.19c	41.2a	8.00b
Pantoea sp. CNPSo 2493	6.41b	0.970a	9.39c	41.0a	7.39b
Pantoea sp. CNPSo 3282	5.03c	0.817a	10.50b	38.3b	6.64c
Paraburkholderia atlantica CNPSo 3155	5.49c	0.867a	10.19c	38.8b	8.48a
Paraburkholderia franconis CNPSo 3157	5.67c	0.920a	8.53c	40.8a	7.23b
Paraburkholderia guartelaensis CNPSo 2995	3.83d	0.670b	7.77d	32.7c	6.66c
Paraburkholderia guartelaensis CNPSo 3008	3.66d	0.610b	9.56c	31.8c	5.78d
Paraburkholderia nodosa CNPS0 1307	4.96c	0.730a	7.03d	39.2b	6.74c
Paraburkholderia nodosa CNPSo 1204	5.51c	0.847a	6.68d	40.8a	8.58a
Paraburkholderia nodosa CNPSo 1213	4.10d	0.570a	6.44e	36.3c	6.76c
Paraburkholderia nodosa CNPSo 1294	3.67d	0.553b	6.52e	35.2c	7.37b
Paraburkholderia nodosa CNPso 1299	4.43d	0.640b	6.30e	39.0b	6.29c
Paraburkholderia nodosa CNPSo 1301	4.41d	0.670b	6.79d	39.2b	9.49a
Paraburkholderia sabiae CNPSo 3136	5.45c	0.830a	10.35b	37.8b	7.90b
Paracocus sp. CNPSo 3707	3.69d	0.600b	6.38e	37.3c	8.86a
Pseudomonas fluorescens CNPSo 2224	4.92c	0.710b	11.29b	38.3b	6.18c
Pseudomonas fluorescens CNPSo 2799	3.17e	0.507b	10.43b	34.7c	6.36c
Pseudomonas lurida CNPSo 2218	4.68c	0.763a	7.36d	44.7a	8.58a
Pseudomonas soli CNPSo 1987	5.31c	0.903a	6.30e	44.0a	7.65b
Pseudomonas sp. CNPSo 2220	4.65c	0.827a	10.50b	41.7a	7.67b
Pseudomonas sp. CNPSo 2222	4.02d	0.610b	10.05c	38.0b	6.89c
Pseudomonas sp. CNPSo 2604	4.91c	0.773a	6.25e	37.0c	8.04b
Pseudomonas sp. CNPSo 2625	4.92c	0.757a	6.79d	40.3b	9.61a
Pseudomonas fluorescens CNPSo 2719	5.29c	0.683b	6.56d	37.8b	7.03c
Pseudomonas sp. CNPSo 2835	4.06d	0.677b	8.15d	39.3b	6.80c
Pseudomonas sp. CNPSo 2844	3.55d	0.623b	8.59c	37.7b	5.90c
Pseudomonas sp. CNPSo 2851	4.32d	0.697b	9.58c	38.2b	5.50d
Pseudomonas sp. CNPSo 2856	3.90d	0.573b	9.98c	35.7c	5.48d
Pseudomonas sp. CNPSo 2864	4.12d	0.617b	7.59d	36.7c	6.98c
Pseudomonas sp. CNPSo 2878	4.18d	0.733a	8.09d	35.2c	6.92c
Pseudomonas sp. CNPSo 2887	4.23d	0.680b	9.43c	33.7c	6.64c
Pseudomonas sp. CNPSo 4132	3.69d	0.557b	5.75e	36.5c	8.84a
Pseudomonas sp. CNPSo 4140	4.88c	0.803a	5.64e	40.3b	8.28b
Rhizobium giardinii CNPSo 171	2.73e	0.530b	4.12e	34.3c	7.34b
Rhizobium leucaenae CNPSo 224	3.87d	0.710b	6.59d	34.8c	7.95b
Rhizobium leucaenae CNPSo 229	3.88d	0.630b	6.75d	35.3c	6.51c
Rhizobium sp. CNPSo 1627	4.59c	0.830a	6.01e	38.0b	7.16b
Rhizobium sp. CNPSo 3610	3.16e	0.543b	5.91e	33.2c	6.32c
Rhizobium tropici CNPSo 1018	5.01c	0.837a	7.37d	37.7b	7.34b
Rhizobium tropici CNPSo 103	3.92d	0.690b	5.31e	38.3b	7.44b

[a] Shoot Fresh Weight (SFW); Shoot Dry Weight (SDW); Root Volume (RV); Plant Size (PS); and Chlorophyll Content (CC) of maize plants inoculated with PGPB strains under water restriction

[b] Values represent the mean of three replicates and when followed by the same letter are not statistically different according to the Scott-Knott test ($p \leq 0.05$)

pachyrhizi CNPSo 2259; *Paenibacillus polymyxa* CNPSo 2227;; *Achromobacter* sp. CNPSo 2660; *Paraburkholderia franconis* CNPSo 3157, and *P. nodosa* CNPSo 1204 (Table 2).

The Pearson's correlation coefficient analysis between the traits assessed in vitro and the results from the first experiment in greenhouse revealed positive correlations among the traits of the strains grown in vitro under reduced water activity (Aw 0.919 and 0.897), at 40 °C, and the ability to promote shoot fresh weight, shoot dry weight, and chlorophyll content of maize (Fig. 4). These results can highly speed up strain selection programs towards the development of bio-inputs aiming at the new challenges of climate changes faced by agriculture. Therefore, our initial hypothesis was confirmed for drought tolerance, while the validation of other key traits, such as phosphate solubilization, remains to be addressed.

A slightly weaker relationship was found between traits of the strains grown under reduced water activity (Aw 0.919 And 0.897), at 40 °C, and biofilm production in the in vitro analyses (Fig. 4). Although other plant growth-promoting properties, such as EPS and biofilm production, have been reported in mitigating water stress [94], we did not observe a correlation with the in vivo plant performance in our study. For both root volume and chlorophyll content, plants inoculated with *Bacillus* spp. reached the highest averages, values even higher than those of plants that were not subjected to water deficit (Table 2). The potential of *Bacillus* spp. in increasing chlorophyll content was also verified in cotton plants (*Gossypium hirsutum* L.) by Diaz [95]. Plants with higher

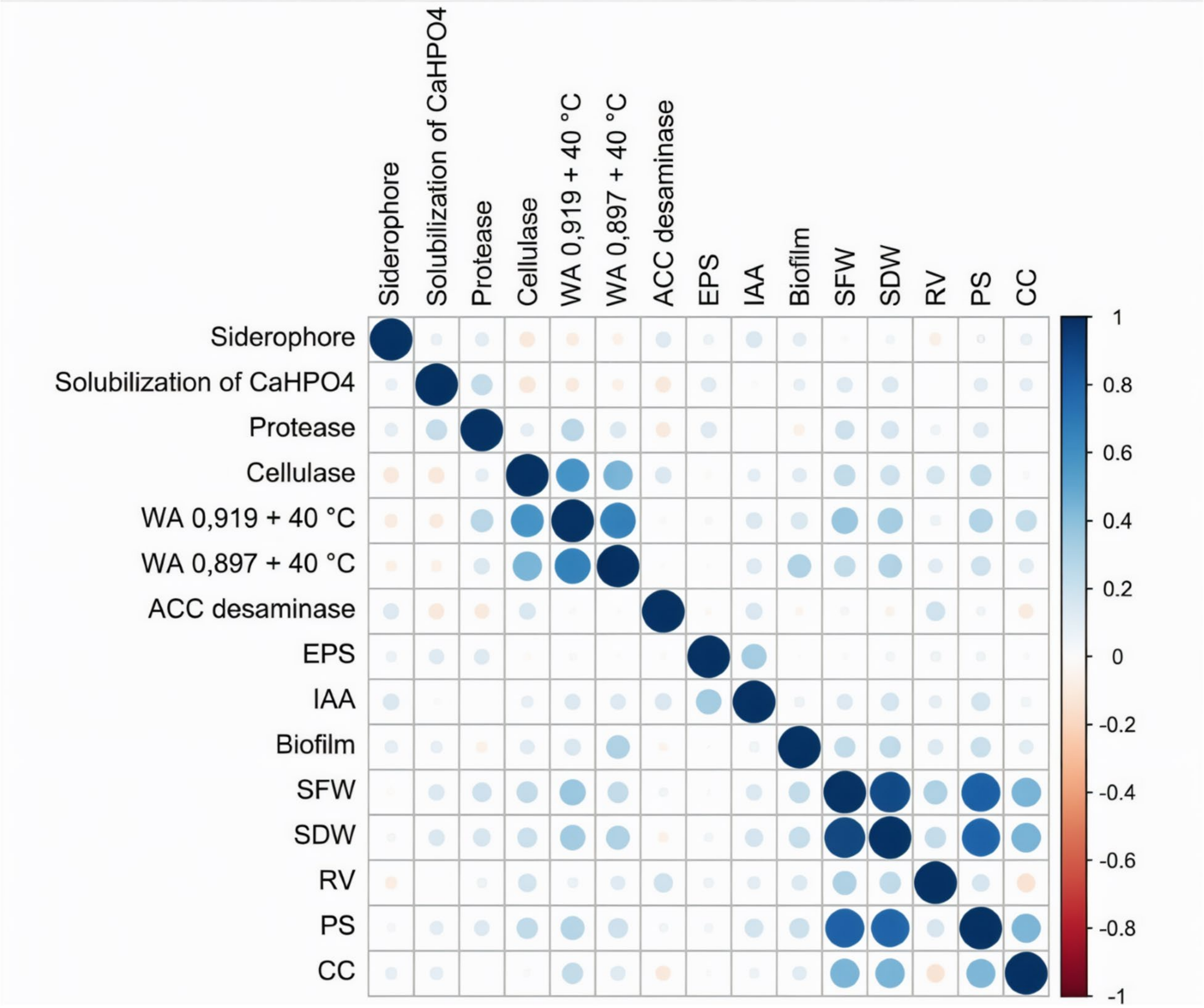

Fig. 4 Pearson correlation analysis among the in vitro *and* in vivo variables of 100 bacterial strains of the Microbial Culture Collection of Embrapa Soja. The color gradient indicates the direction and magnitude of the correlation, ranging from blue for strong positive correlations (+ 1 = 100%) to red for strong negative correlations (−1 = −100%), with white representing no correlation (0 = 0%). The size of the circle reflects the intensity of the correlation: larger circles indicate stronger correlations, while smaller circles indicate weaker correlations. Statistical significance was assessed at $p \leq 0.05$

chlorophyll content, when subjected to stress or physiological disorders, are more effective to convert light energy and accumulate biomass, relieving the negative effects [59].

For selection of the most promising strains in Experiment I, a principal component analysis was applied to check correlations among the variables (Fig. 5A) in a factorial plan (Fig. 5B). Shoot fresh and dry weights, and plant size, were related to each other, as represented by PC1, which explains 56.65% of the total variance. Considering the information gathered by PC1, the previously highlighted variables show a positive correlation; on the other hand, root volume and chlorophyll content were weakly related to the other variables, and were best represented by the PC2.

According to the factorial plan representing the treatments, there was a great variation in the response among different strains. As an example, plants inoculated with *B. pachyrhizi* CNPSo 2259, *A. indigens* CNPSo 2541, and *P. agglomerans* CNPSo 2602 (ID 39, 44, and 45, respectively) responded differently from those inoculated with *Delftia* sp. CNPSo 3288, *Agrobacterium pusenese* CNPSo 3315, and *Agrobacterium* sp. CNPSo 4045 (ID 83, 85 and 98, respectively).

Fifteen bacterial strains that resulted in more vigorous plants grown under water limitation and that were positive in most in vitroassessments were selected for a second greenhouse trial (Table 3).

Assessment of the most promising strains in non-sterile soil

In the second experiment conducted with 15 selected strains, plant photosynthetic rates varied over time and among the different strains. A general reduction in CO_2 assimilation rate was observed on the fifth day of stress, followed by partial or complete recovery by the ninth day. Control I, with plenty water supply, showed the highest values at all assessing times, while Control II, under drought stress, exhibited a marked reduction in CO_2 assimilation, especially on the fifth and ninth days. Among the strains, *B. velezensis* CNPSo 2384 stood out for maintaining a high maize photosynthetic rate throughout the experimental period (Table 3).

Among the plants under water restriction, some strains showed potential to mitigate the stressful condition, maintaining a high photosynthetic rate, such as *C. violaceum* CNPSo 1954 and *B. velezensis* CNPSo 2384, which presented, respectively, photosynthetic rates 26 And 51% higher than the non-inoculated plants (Control I). Plants under water restriction and inoculated with *B. velezensis* CNPSo 2384 showed a reduction of only 5% in photosynthetic rate in the last evaluation compared with those irrigated throughout the whole period (Control II). Similar trends were observed for stomatal conductance and transpiration rates (Table 3), which are closely linked to photosynthetic activity, indicating a consistent physiological response among these parameters.

Shoot fresh weight ranged from 122.5 to 203.3 g per plant. The lowest value was observed in non-inoculated plants under water restriction, while the highest was recorded in plants that received plenty of water supply throughout the experiment. Inoculated plants showed superior performance compared with non-inoculated ones. For shoot dry weight, the highest average was found for well-watered plants (26.14 g), while plants under water restriction but inoculated with the *Bacillus* sp. CNPSo 2658 had the highest average (19.97 g). For plant size, the average of well-watered plants also stood out from the other treatments, reaching 1.35 m. Among the treatments under water restriction, plants inoculated with *C. violaceum* CNPSo 1954 and *G. azotocaptans* CNPSo 2783 reached 1.27 m (Table 4).

The root volume of plants inoculated with the strains of *C. violaceum* CNPSo 1954, *Pseudomonas soli* CNPSo 1987, *Pantoea agglomerans* CNPSo 2602, *Bacillus subtilis* CNPSo 2605, and *B. subtilis* CNPSo 2606 were similar to those of well-watered plants, while no differences were found for root dry weight (Table 4). The average root length ranged from 261.4 to 491.8 m per plant, with the highest value in plants inoculated with *C. violaceum* CNPSo 1954. In addition, the length of roots of plants inoculated with *Pseudomonas soli* CNPSo 1987 and *Pantoea agglomerans* CNPSo 2602 were also significantly increased (Table 4). It is worth mentioning the importance of the contact between the root system and the soil, enhancing the uptake of water and nutrients [49, 96]. We found that plants inoculated with three strains, *C. violaceum* CNPSo 1954, *Pseudomonas soli* CNPSo 1987, and *Pantoea agglomerans* CNPSo 2602 stood out in volume, mass, and length of roots. Under field conditions, this ability could have a significant impact on drought tolerance.

Water constraints in a warmer climate concern humanity and agriculture [97]. Furthermore, global patterns of increasing crop yield [98] also enhance the demand for water. In addition, higher temperatures with more frequent hotter days, stronger radiation, and land cover/land use changes [98] may intensify the impacts of water stress [99]. The survival capacity of plants will depend on their adaptation ability but also on the severity and duration of the restrictive period [100]. In this scenario, inoculation with PGPB should be investigated, as it can help mitigate plant damages. Indeed, it has been recently shown that strains used in our study were able to mitigate the negative impacts of a 2 °C warming on the photosynthesis, growth, and nutritional value of a tropical C4 grassland under field conditions [101].

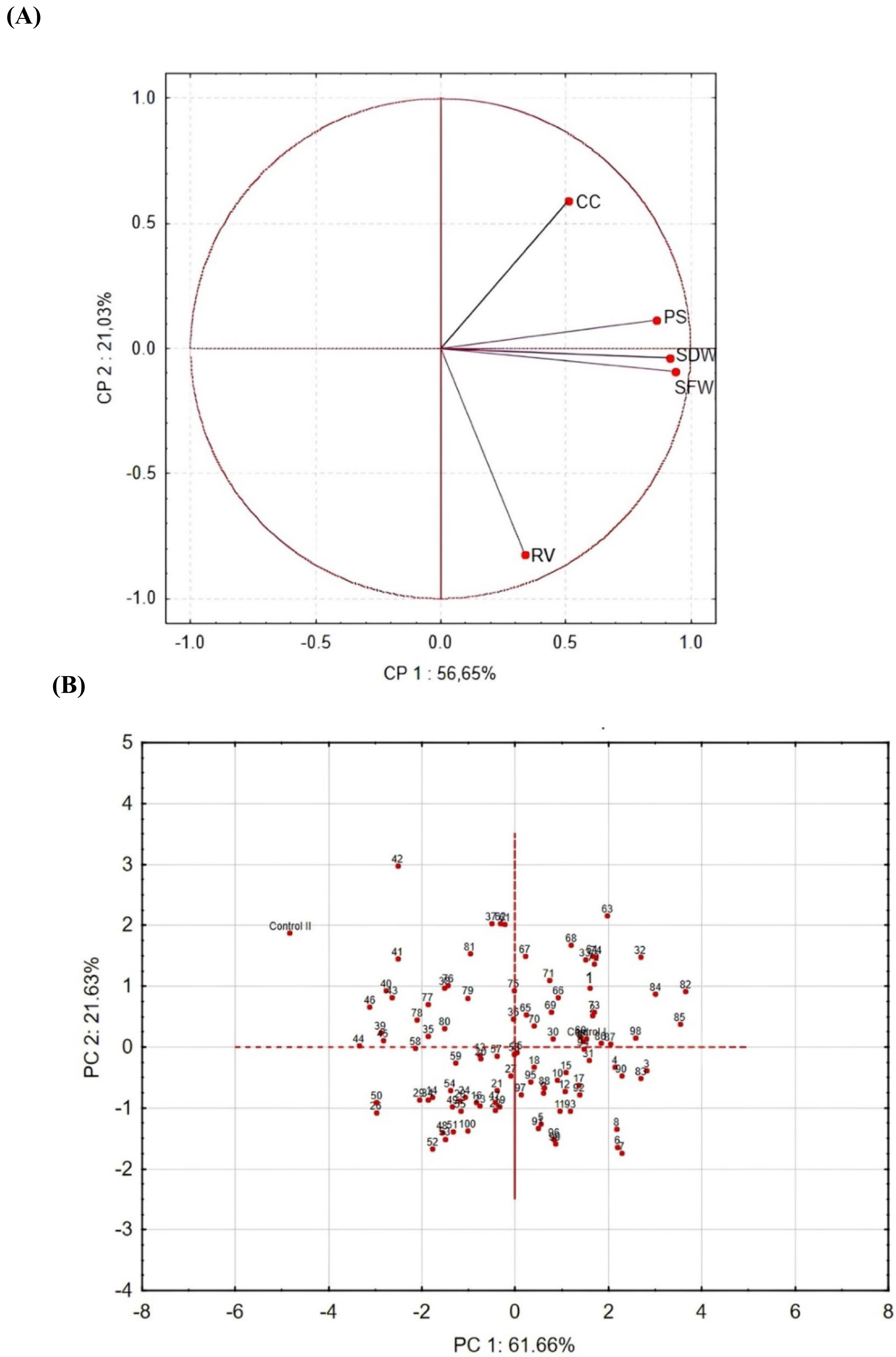

Fig. 5 PCA graphics of variables and treatments. **A** Correlation circle among the variables: shoot fresh weight (SFW), shoot dry weight (SDW), root volume (RV), plant size (PS) and chlorophyll content (CC) of maize plants inoculated with 100 bacterial strains of the Microbial Culture Collection of Embrapa Soja and grown in sterile substrate under water restriction. The proximity and direction of the variables indicate their correlation and contribution to the components. **B** Factorial plan under water restriction conditions. The numbers corresponding to each strain are shown in Table S1. The percentage of variance explained by each principal component is shown in the axis's labels

Table 3 Photosynthetic rate, stomatal conductance and transpiration of maize plants inoculated with the 15 selected bacterial strains grown in non-sterile soil for 3, 5, and 9 days under water restriction

Treatment	**Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})**			**Stomatal conductance (mol H_2O m^{-2} s^{-1})**			**Transpiration rate (mol H_2O m^{-2} s^{-1})**		
	3 days	**5 days**	**9 days**	**3 days**	**5 days**	**9 days**	**3 days**	**5 days**	**9 days**
Control I (non-inoculated under water restriction)	35.38a[a]	9.37d	22.79b	0.17a	0.05c	0.08c	3.52a	0.99c	1.79d
Control II (non-inoculated without water restriction)	33.38a	41.35a	32.37a	0.21a	0.25a	0.23a	4.01a	4.92a	4.64a
Bacillus aryabhatai CNPSo 2603	29.61a	16.27c	23.74b	0.14b	0.10b	0.10c	3.06b	1.59c	2.26d
Bacillus subtilis CNPSo 2605	29.93a	19.83c	25.08b	0.14a	0.06b	0.10c	2.94b	1.81c	2.21d
Bacillus subtilis CNPSo 2606	24.15b	19.80c	24.96b	0.11b	0.05c	0.09c	2.56b	1.47c	2.32d
Bacillus subtilis CNPSo 2620	31.60a	22.76c	34.24a	0.16a	0.13b	0.14c	3.83a	3.25b	1.92d
Bacillus velezensis CNPSo 2384	32.15a	31.96b	34.60a	0.17a	0.05c	0.16b	3.49a	0.72c	3.71b
Bacillus velezensis CNPSo 2657	30.80a	25.69c	27.37b	0.12b	0.05c	0.11c	3.11b	1.38c	2.61c
Bacillus sp. CNPSo 2658	32.32a	22.49c	30.05a	0.10b	0.07c	0.12c	2.24b	1.89c	2.73c
Bacillus sp. CNPSo 2723	22.86b	19.46c	32.38a	0.11b	0.06c	0.14c	2.47b	1.34c	2.61c
Bacillus sp. CNPSo 2725	29.22a	21.77c	33.53a	0.15a	0.07c	0.18b	2.86b	2.07c	2.94c
Chromobacterium violaceum CNPSo 1954	31.68a	30.43b	28.83b	0.16a	0.10b	0.12c	3.56a	2.86b	2.93c
Gluconacetobacter azotocaptans CNPSo 2783	20.28b	19.36c	25.69b	0.10b	0.06c	0.10c	2.34b	1.56c	2.51c
Pantoea agglomerans CNPSo 2602	34.37a	13.73d	26.97b	0.18a	0.06c	0.13c	3.81a	1.11c	2.29d
Pantoea sp. CNPSo 3282	31.99a	9.05d	29.17b	0.15a	0.05c	0.12c	3.26a	0.91c	2.66c
Paraburkholderia franconis CNPSo 3157	32.59a	19.20c	32.23a	0.17a	0.06c	0.16b	3.73a	1.61c	2.58c
Pseudomonas soli CNPSo 1987	27.84a	19.01c	30.04a	0.12b	0.06c	0.12c	2.55b	1.39c	2.51c

[a] Values represent the mean of five replicates and when followed by the same letter are not statistically different according to the Scott-Knott test ($p \leq 0.05$)

Table 4 Growth parameters[a] of maize hybrid ATL100 inoculated with 15 selected bacterial strains and grown in non-sterile soil under water restriction

Treatment	**SFW (g)**	**SDW (g)**	**PS (m)**	**RV (mL)**	**RDW (g)**	**RL (m)**
Control I (non-inoculated under water restriction)	122.5c[b]	18.10c	1.22b	65.66a	6.32a	353.2b
Control II (non-inoculated without water restriction)	203.3a	26.14a	1.35a	72.05a	7.30a	356.2b
Bacillus aryabhatai CNPSo 2603	145.2b	18.06c	1.23b	55.86b	5.98a	320.9b
Bacillus subtilis CNPSo 2605	132.6c	17.12c	1.22b	66.58a	6.10a	347.7b
Bacillus subtilis CNPSo 2606	146.8b	19.54b	1.26b	63.43a	6.96a	376.6b
Bacillus subtilis CNPSo 2620	145.1b	18.32c	1.24b	52.58b	6.22a	322.7b
Bacillus velezensis CNPSo 2384	142.2b	19.28b	1.25b	59.17b	5.93a	350.4b
Bacillus velezensis CNPSo 2657	137.3c	17.13c	1.20b	56.79b	6.47a	325.9b
Bacillus sp. CNPSo 2658	144.9b	19.97b	1.24b	61.13b	6.50a	344.3b
Bacillus sp. CNPSo 2723	145.3b	18.60b	1.25b	61.36b	6.38a	291.7b
Bacillus sp. CNPSo 2725	138.1c	17.83c	1.21b	55.28b	6.50a	261.4b
Chromobacterium violaceum CNPSo 1954	132.4c	19.01b	1.27b	67.05a	6.78a	491.8a
Gluconacetobacter azotocaptans CNPSo 2783	148.4b	18.87b	1.27b	60.50b	7.23a	384.7b
Pantoea agglomerans CNPSo 2602	142.6b	18.26c	1.26b	69.87a	6.55a	485.1a
Pantoea sp. CNPSo 3282	139.3b	19.42b	1.25b	56.75b	6.39a	310.5b
Paraburkholderia franconis CNPSo 3157	133.3c	16.52c	1.22b	62.08b	6.82a	349.8b
Pseudomonas soli CNPSo 1987	143.4b	19.22b	1.26b	69.41a	6.52a	427.3a

[a] Shoot Fresh Weight (SFW); Shoot Dry Weight (SDW); Plant Size (PS); Root Volume (RV); Root Dry Weight (RDW); and Root Length (RL) of maize plants inoculated with PGPB strains under water restriction

[b] Values represent the mean of five replicates and when followed by the same letter are not statistically different according to the Scott-Knott test ($p \leq 0.05$)

Considering all the data analyzed, we highlight the outstanding performance of three strains, *B. velezensis* CNPSo 2384, *B. subtilis* CNPSo 2606, and *Bacillus* sp. CNPSo 2723, with high potential to be used as new bio-inputs (Supplementary Figure S5). In addition, the multifunctional traits of these three selected strains confirm that *Bacillus* may have multiple uses [30, 102–104], which highly benefits agricultural sustainability.

According to Radhakrishnan et al. [92], during water restriction and the consequent increase in the concentration of toxic salts and metals in the soil solution, *Bacillus* spp. can stimulate the production of EPS and siderophores. This ability can also control other microbial species in the soil, including pathogens. Regarding the imbalance resulting from water deficiency, the synthesis of IAA and ACC-deaminase regulates intracellular phytohormone metabolism, leading to increased stress tolerance in plants. Sivasakthi et al. [105] pointed out that *Pseudomonas* and *Bacillus* have predominant abilities among other PGPB to survive in a wide range of stressful environments – whether through phosphate solubilization, the production of siderophores, or the biocontrol of plant pathogens. In this study, the production of siderophores and EPS in vitro was mostly found in *Pseudomonas* spp. strains. Besides the production of metabolites, the ability of *Bacillus* to form endospores may increase the cell viability under adverse conditions, even in formulations of commercial products [106, 107]. Despite the promising results obtained under controlled conditions, further studies are needed to evaluate the behavior of selected strains under field conditions, where multiple biotic and abiotic factors influence microbial dynamics. Adaptation to the local ecosystem is essential for introduced microorganisms to establish themselves, interact with native microbiota, and promote tangible benefits to plants. According to Li et al. [108], land use plays a decisive role in shaping soil microbial communities, influencing their genomic and functional traits. These environmental factors can directly influence the success of inoculation and the performance of selected strains. Consequently, field trials are crucial for evaluating microbial inoculants under real agricultural conditions. The next step will involve verifying the agronomic potential of these strains and their consistency across diverse environments.

Conclusions

- ➢ In vitro evaluations may accelerate the bioprospection of elite strains for bio-inputs; for example, we found a high correlation between growth in vitro in culture medium with reduced water activity and the capacity to increase maize tolerance to drought;
- ➢ Searching for elite strains of agronomic interest in microbial culture collections may be facilitated by starting from specific genera, such as *Bacillus* for drought tolerance and *Pseudomonas* for phosphate solubilization.
- ➢ Microbial culture collections represent a valuable reservoir of biotechnological solutions for agricultural sustainability. Among them, plant growth-promoting bacteria stand out, offering a sustainable alternative to mitigate the impacts of abiotic and biotic stresses on plants. Many strains exhibit multiple traits of agronomic interest, and their use in agriculture should be encouraged.

Abbreviations

ACC	1-Aminocyclopropane-1-carboxylate deaminase
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BCCM	Belgian Coordinated Collections of Microorganisms
BRC	Biological Resource Centre
CAS	Chromazurol
CBS	Central Bureau of Fungal Cultures
CC	Chlorophyll content
CFBP	Collection Francaise de Bacteries Phytopathogenes
Ci intercelular	CO_2 concentration
CMC	Carboxymethyl cellulose
DAE	Day after emergence
DSMZ	German Collection of Microorganisms and Cell cultures GmbH
EPS	Exopolysaccharides
IAA	Indole-3-acetic acid
ICMP	International Collection of Microorganisms from Plants
ISR	Induced systemic resistance
JCM	Japan Collection of Microorganisms
MCC	Microbial culture collections
PGPB	Plant growth-promoting bacteria
PS	Plant size
RDW	Root dry weight
RL	Root length
RV	Root volume
SDW	Shoot dry weight
SFW	Shoot fresh weight
WA	Water activity
WDCM	World Data Centre for Microorganisms
WFCC	World Federation for Culture Collections

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-04376-8.

Supplementary Material 1.

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Authors' contributions

Conceptualization: M.H., M.A.N.; Methodology: N.C.V., P.C., M.A.N., M.H.; Conduction of analysis: N.C.V, P.C.; Data analysis: N.C.V., P.C., A.R.T., M.A.N., M.H.; Resources: M.H., M.A.N.; Writing, review and editing: N.C.V., P.C., A.R.T., M.A.N., M.H. All the authors have read and approved the final manuscript.

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Data availability

The data presented in this study are available in the article. Other data (crude data from the greenhouse experiments) will be given upon request to the corresponding author: mariangela.hungria@embrapa.br.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Soil Biotechnology Laboratory, Embrapa Soja, C.P. 4006, CEP 86.085-981, Londrina, Paraná, Brazil
[2]Department of Microbiology, Universidade Estadual de Londrina, C.P. 10.011, CEP 86.057-970, Londrina, Paraná, Brazil
[3]National Council for Scientific and Technological Development (CNPq), Federal District, Brasília, Brazil

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