Research Article

Idriss Djoko Kouam*, Joseline Mabah, Pierre Germain Ntsoli, Laverdure Tchamani, Aoudou Yaouba*, Bridget Katte, Dieudonné Bitom

Growth promotion potential of *Bacillus* spp. isolates on two tomato (*Solanum lycopersicum* L.) varieties in the West region of Cameroon

https://doi.org/10.1515/opag-2022-0154
received May 25, 2022; accepted November 15, 2022

Abstract: Inoculation of plants with plant growth-promoting bacteria (PGPB) is an ecological approach to improve plants growth and yield. The objective of this study was to screen native PGPBs in tomato root rhizosphere and evaluate their performance in germination and growth of seedlings in the greenhouse in the western region of Cameroon. Isolates were selected based on their ability to solubilize phosphate, produce indole-3-acetic acid, and improve seed germination and seedling growth under laboratory conditions. Following this screening, eight bacteria were selected (S1, S4, S13, S14, S16, B9, B11, and B15) to assess their performance on seedling growth in greenhouse. Seeds of two tomato varieties (one improved, Raja, and the local one, Foolewouh) were used in this study. The experiment was performed in sterilized and non-sterilized soils. Phylogenetic analysis of the 16S rRNA sequence showed that these bacteria belong to the genus *Bacillus*, including *B. thuringiensis*, *B. cereus*, *B. pacificus*, and *B. aerius*. The bacterial performance depended on the variety and conditions of soil treatment. Isolates improved seed germination (up to 31.93%) and seeding strength (up to 78.59%) *in vitro*. In greenhouse, inoculation with PGPB significantly increased stem height, root length, and weight of fresh and dry stems and roots. Raja variety was more sensitive to bacteria and performed better than the Foolewouh variety. Bacterial isolates on sterilized soil allowed to better seedling growth compared to non-sterilized soil. The study showed that *B. thuringiensis* (strain B9), *B. pacificus* (strain B11), and *Bacillus* sp. (strain B15) can be used as inoculant formulations to improve seedling growth of tomato plant.

Keywords: PGPBs, *Bacillus* spp., tomato, germination, seedling growth, inoculation

*** Corresponding author: Idriss Djoko Kouam,** Department of Agriculture, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. BOX 222, Cameroon, e-mail: kouamidriss@gmail.com
*** Corresponding author: Aoudou Yaouba,** Department of Agriculture, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. BOX 222, Cameroon, e-mail: yaoubaaoudou@yahoo.fr
Joseline Mabah: Department of Agriculture, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. BOX 222, Cameroon, e-mail: joselinemabah@yahoo.com
Pierre Germain Ntsoli: Department of Agriculture, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. BOX 222, Cameroon, e-mail: pierrentsoli@yahoo.fr
Laverdure Tchamani: Department of Biochemistry, Faculty of Sciences, University of Dschang, P.O. BOX 67, Cameroon, e-mail: laverduretchamani@yahoo.com
Bridget Katte: Department of Animal Production, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. BOX 122, Cameroon, e-mail: brikatte@hotmail.com
Dieudonné Bitom: Department of Soil Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. BOX 222, Cameroon, e-mail: bitomoyono@yahoo.fr

1 Introduction

Tomato (*Solanum lycopersicum* L.), a plant of the Solanaceae family, is one of the most widely grown and consumed vegetable crops in the world [1]. In 2020, its world production was estimated at 186.8 million tons for a surface area of about 5.05 million hectares, with an average yield of 37 t ha^{-1} [2]. Its cultivation presents socio-economic challenges for the population. Socially, it contributes to the balance of the population's diet through its nutritional composition, while economically, it is an important source of income for many populations [3,4]. In 2020, its production in Cameroon was estimated at 1.24 million tons [2] for a total land area of 101,459 ha. Although it is grown in all five agro-ecological zones of Cameroon, 92% of its production is mainly in the West (65.70%), Centre (18.10%), and Adamawa (7.97%) regions [5].

In Cameroon, despite the annual increase in production, its productivity remains low due to the intensive use of fertilizers. This is due to demographic pressure which leads to intensive land use [6], thus leading to a decline in soil fertility. Moreover, like most tropical soils, the soils are predominantly acidic due to the abundance of iron and aluminum oxides in the soil [7]. This acidity directly affects the fertility of the soil because several mineral elements needed for the proper development of the plant are not available at low pH. This is the case for phosphorus, whose available form (HPO_4-phosphate ions) for the plant declines as the soil pH decreases. Phosphorus reacts with cations of iron, aluminum, and calcium precipitates, and becomes insoluble, and hence unavailable for plants [8]. Thus, only approximately 4% of the total phosphorus found in the soil for plants is in the form of orthophosphate [8]. Consequently, 75–90% of the P injected into the soil in the form of chemical fertilizer is transformed into an insoluble form ref. [9]. As a result, tomato production to ensure food security and a significant income for producers remains dependent on the intensive use of mineral fertilizers and pesticides [10]. Second, the intensive use of fertilizers, in particular nitrogen and phosphate, means that fertilizer elements that are not absorbed by plants or retained by the soil can be transported through groundwater, by leaching, or into waterways through soil erosion, causing eutrophication and deteriorating water quality [11]. Moreover, these chemical fertilizers are often responsible for soil acidification or alkalinization and increased plant susceptibility to diseases and pests from high nitrogen fertilization [12].

Face of this situation, it is thus imperative to rethink all agricultural production systems. There is a clear need for new agronomic practices that improve the efficiency and sustainability of agroecosystems. In response to these challenges, one of the proposed solutions is the use of biofertilizers. To this end, several soil microorganisms particularly plant growth-promoting bacteria (PGPB) have been used to replace or reduce the number of chemical fertilizers and pesticides [13]. These PGPBs produce numerous secondary metabolites that improve plant growth and development through direct and indirect mechanisms, the most well-known of which are solubilization of inorganic phosphate and mineralization of organic phosphate or other nutrients, enhancement of non-symbiotic nitrogen fixation, ability to produce plant growth regulators or phytohormones such as indole-3-acetic acid (IAA), cytokinin, and gibberellin. In addition, the antagonistic effect against phytopathogenic microorganisms through the production of siderophores, synthesis of antibiotics, enzymes and/or fungicidal compounds has been reported [14,15]. Aware of their importance, the rhizospheres of many crops have been studied in several countries. Thus, many bacteria species have been identified and recognized as beneficial. This is the case of the genera *Bacillus*, *Pseudomonas*, *Pantoea* in Spain [16]; *Serratia*, *Enterobacter* in Pakistan [17]; and *Bradyrhizobium* in China [18], which have been isolated in the rhizosphere of tomatoes. Their effects on yield increase and fruit quality have been shown. Many formulations based on these bacteria exist and are marketed in many Western countries for sustainable organic agriculture. However, these bacteria remain largely under-exploited in Africa. In Cameroon in particular, little information is available on the use of PGPBs in general; and to the best of our knowledge, no research has yet been done on the use of indigenous PGPBs in tomatoes in particular.

The objective of the present study was to isolate and characterize native ectophytic bacteria from the rhizosphere of tomatoes in West Cameroon, to evaluate their growth-promoting properties, as well as their tolerance to various environmental stresses, and finally to evaluate the *in vitro* and *in vivo* effect of PGPBs on seedling growth.

2 Materials and methods

2.1 Study site

Soil samples were collected from agricultural fields in two localities in Menoua Division, West region of Cameroon: Santchou (5°16′55″ North and 9°58′27″ East), altitude 700 m, mean annual temperature 24.6°C, rainfall 1,759 mm per year, with ferralitic, humic, and hydromorphic soils [19]; and Baleveng (5°29′41″ North and 10°09′13″ East), altitude 1,400 m, mean annual rainfall 1,911 mm, mean annual temperature 20.1°C, with mostly tropical ferralitic soils [20]. These localities were chosen based on their different pedoclimatic characteristics and representatives of the main production areas of the Division.

2.2 Soil samples collection from rhizosphere

Rhizosphere soil samples from ten plants were collected from three plots in each locality, using the simple random sampling technique [21]. Soil particles finely attached to the roots were collected from ten healthy plants. Rhizosphere samples from each plot were homogenized to form a composite sample and transported to the Plant

Pathology Laboratory of the University of Dschang, in sterile plastic bags labeled with the sample references. Once at the laboratory, the soil samples were oven-dried at 30°C for 24 h, ground in a porcelain mortar, and sieved to remove large particles and plant fragments. The fine particles obtained were stored at 4°C in a refrigerator in labeled bags for further analysis.

Similarly, soil samples were collected at a depth of 0–20 cm using an auger for physico-chemical analyses. The physico-chemical analyses were performed according to the standard methods recommended by Pauwels et al. [22]. The characteristics of the studied soils are presented in Table 1.

2.3 Isolation and purification of the bacteria

Isolation of ectophytes was done on a nutrient agar (NA) medium (Flumedia, Hi Flown Global Resources Ltd, UK) containing 5 g peptic digest of animal tissue, 5 g NaCl, 1.5 g beef extract, 1.5 g yeast extract, 15 g agar, pH 7.4 at 25°C. Decimal order dilution methods were used. Ten grams of composite rhizosphere sample from each locality (indicating 3.33 g of soil per plot in each locality) were introduced into a 250 mL conical flask containing 90 mL of sterile distilled water. The mixture was vortexed and a series of dilutions were made up to 10^{-7} dilution. Then 100 µL of aliquots of the dilutions 10^{-5} and 10^{-7} were plated on NA with three replicates. Petri dishes were incubated at 37°C for 24–48 h. Isolates were selected based on differences in colony morphology and pigment production by visual examination [23], and sub-cultured separately for pure strains. Isolates were stored at 4°C until use.

Table 1: Physico-chemical characteristics of soils samples in the studied localities

Locality	Variables					
	% clay	% silt	% sand	OM (%)	total N (%)	Av P (ppm)
Santchou	13	19	68	1.24	0.29	42.6
Baleveng	31	28	41	1.91	0.39	36.2
	Variables					
	K	Na	Ca	Mg	CEC	pH H_2O
Santchou	0.58	0.47	1.16	0.84	17.92	5.4
Baleveng	1.03	0.69	2.68	0.24	15.98	5.8

K, Na, Ca, Mg, CEC are in meq./100 g. CEC – cation exchange capacity; OM – organic matter.

2.4 Biochemical characterization of bacterial isolates

Gram staining was performed as described by Vincent and Humphrey [24]. Biochemical tests were done following the criteria described in Bergey's Manual of Systematic Bacteriology [25]. The catalase test was done as described by Majeed et al. [26]. The citrate test was done following the protocol described by Simmons [27] using Simmons citrate agar medium. The ability of isolates to hydrolyze urea to produce ammonia is carried out on a urea broth medium with phenol red as a color indicator which will turn pink if ammonia is released. The motility test is performed using the drop-on-slide technique as described by Benson [28].

2.5 Molecular identification of bacterial isolates

Molecular identification was carried out in the Molecular Parasitology and Entomology Research Unit of the University of Dschang. Bacterial isolates selected for their plant growth-promoting properties were identified by 16S rRNA gene sequencing. The 16S rRNA gene fragments were amplified using a universal primer pair 27F 5′-AGAGTTTGATCCTGG CTCAG-3′ and 1492R 5′-CTACGGCTACCTTGTTACGA-3′. The PCR reaction was performed in a 50 µL reaction volume containing 25 µL of 2× Master Mix (Tsingke, China), 2 µL of each primer, 21 µL of distilled deionized water (ddH_2O), and one bacterial colony of pure isolate. PCR reactions were performed in a thermal cycler under the following conditions: pre-denaturation at 95°C for 10 min, followed by a PCR reaction consisting of a 30-cycle denaturation program consisting of three steps (denaturation at 94°C for 30 s, hybridization at 55°C for 30 s, and elongation at 72°C for 2 min) and final elongation at 72°C for 10 min. The amplification product was verified using 1.5% (w/v) agarose gel electrophoresis with Tris-acetate-EDTA buffer (TAE 0.5×, pH 8.5). Subsequently, the amplified products were purified and sequenced by Macrogen, Amsterdam using the Sanger method. The resulting sequences were introduced into the National Center for Biotechnology Information GenBank database (https://blast.ncbi.nlm.nih.gov/) and compared with the standard sequences available in the

database. The previous sequences were deposited in the same database and the accession numbers of each strain were obtained. The phylogenetic tree was constructed using MEGA 11 software.

2.6 Evaluation of growth-promoting activities

The growth-promoting properties of the bacterial isolates were assessed using the inorganic phosphate solubilization test, the IAA production test, the hydrogen cyanide (HCN) production test, and the ammonia production test.

2.6.1 Phosphate solubilization

The estimation of the amount of solubilized phosphate by the bacterial isolates was done on liquid Pikovskaya medium containing 1 per litre, 10 g *D*-Glucose, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.2 g KCl, 0.5 g yeast extract, 0.0001 g $MnSO_4{\cdot}5H_2O$, 0.0001 g $FeSO_4{\cdot}7H_2O$ and 5 g insoluble calcium phosphate $(Ca_3PO_4)_2$ as the only source of phosphate [29]. The inoculated medium with each of the isolates was incubated at 28°C for 5 days in constant agitation. After incubation, the medium was centrifuged at 4,000 rpm for 20 min, the supernatant was used to determine the solubilized phosphate content by using the colorimetric method of Murphy and Riley [30] at 882 nm. The soluble phosphate concentration is calculated using a standard calibration curve of a KH_2PO_4 solution.

2.6.2 IAA production

The ability of bacterial isolates to produce IAA was done following a similar protocol to that described by Cavite et al. [31]. Bacterial isolates were inoculated onto a nutrient broth (NB) supplemented with 0.5 g L^{-1} L-tryptophan under constant agitation and incubated for 3 days at 28°C. After incubation, the cultures were centrifuged at 4,000 rpm for 20 min. To 1 mL of the supernatant, 2 mL of Salkowski's reagent (1.5 mL 0.5 M $FeCl_3{\cdot}6H_2O$, 50 mL distilled H_2O, and 30 mL concentrated H_2SO_4) was added and incubated in the dark in a room laboratory temperature for 20 min [32]. IAA production is observed by the change in color to pink or red depending on the concentration. The absorbance was measured at 530 nm using a 752P ultraviolet-visible spectrophotometer (Techmel & Techmel, USA). The concentration of IAA produced by the isolates is determined using a standard IAA calibration curve following dilution series.

2.6.3 Ammonia production

Ammonia production by the bacterial isolates was evaluated in a peptone water medium. Test tubes containing 10 mL of culture medium were inoculated with 100 µL of the bacterial suspension of each isolate. After incubation at 28°C for 72 h, 500 µL of Nessler reagent was added to each tube. Observation of yellow or orange color indicates NH_3 production [33].

2.6.4 HCN production

The ability of the isolates to produce HCN was evaluated according to the method of Lorck [34] on an NA medium supplemented with glycine (4.4 g L^{-1}). A No. 1 Whatman paper disc (90 mm in diameter), was impregnated with a solution of sodium picrate (5% picric acid and 2% sodium carbonate) and placed inside the lid of the Petri dish. The dish was sealed with parafilm and incubated at 28°C for 4 days. The color change of the Whatman paper from yellow to orange or red indicated the production of HCN.

2.7 Tolerance of bacterial isolates to environmental stresses

2.7.1 Tolerance to salinity

The ability of bacterial isolates to grow at different salt concentrations was evaluated in an NB supplemented with different salt concentrations (2, 4, 6, and 8% w/v) [21]. Control treatments are maintained at 0.5% NaCl (w/v). Each treatment was inoculated with 100 µL of one of the bacterial suspensions (OD = 0.1 at 600 nm) and incubated at 28°C for 48 h under constant agitation. The non-inoculated medium was used as a negative control. Growth was assessed by determining the absorbance at 600 nm using a 752P ultraviolet-visible spectrophotometer (Techmel & Techmel, USA). The experiment was performed in three replicates for each isolate.

2.7.2 pH tolerance

The effect of pH was evaluated on the NB medium to assess the ability of bacterial isolates to grow at various pH. The culture medium was adjusted to pH 4, 5.5, 7, 8.5, and 10 using NaOH and HCl solutions. The bacterial growth was assessed after 48 h of incubation by measuring

the optical density at 600 nm. The experiment was repeated three times.

2.8 Effect of PGPBs on germination and growth of tomato seedlings

2.8.1 Preparation of bacterial inocula

In constant agitation, bacterial isolates were grown on an NB medium for 24 h at 37°C. Cultures were centrifuged at 4,000 rpm for 5 min and the pellet was suspended in physiological water until turbidity comparable to that of the 0.5 points of the McFarland nephelometer scale was obtained. The cell density of each culture was determined at 600 nm using a spectrophotometer and adjusted if necessary until an absorbance of 0.1 corresponding to a cell density of 10^8 CFU mL^{-1} was obtained [35].

2.8.2 Effect of PGPRs on seed germination and seedling growth

Eighteen [18] bacterial isolates were tested for their ability to stimulate seed germination and growth of tomato seedlings by the blotting paper method [36]. Tomato seeds (*S. lycopersicum* L., cv Raja) were obtained from a seed distributor in Dschang, while the local variety (*S. lycopersicum* L., cv Foolewouh) were obtained from the NGO "Semence d'avenir." The seeds were calibrated according to their form to homogenize their germination response.

A modified protocol of Laradj Zazou [37] was used for seed treatment. Seeds of the different varieties were disinfected in a 2% NaClO solution for 2–3 min and rinsed successively three times with distilled water for 5 min. Disinfected seeds were immersed in each of the bacterial suspension or physiological water solution (control treatment) for 30 min. Twenty-five [25] seeds of each variety inoculated with each isolate were sown in Petri dishes (14 cm × 2 cm) lined with three layers of hydrophilic paper moistened with sterilized distilled water. The experiment was performed in three independent replicates in a completely randomized design. The Petri dishes were incubated at 28°C for 5 days. The number of germinated seeds was counted and the lengths of the roots and hypocotyl were measured. The germination percentage and vigor index were calculated from the followed formulae according to Islam et al. [38] (Equations 1–2). The seed is considered to have germinated when the radicle has pierced the seed coat.

$$\text{Percentage of germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sowed}} \times 100, \quad (1)$$

$$\text{Vigor index} = (\text{Average root length} + \text{Average stem length}) \times \%\text{germination}. \quad (2)$$

2.9 Evaluation of the effect of bacterial isolates on seedling growth

The experiment was carried out at the Research and Application Farm (RAF) (5°26′41.88588″N, 10°4′8.83812″E, altitude 1,390 m) of the Faculty of Agronomy and Agricultural Sciences (FAAS) of the University of Dschang, West Region of Cameroon. Eight isolates that significantly stimulated germination and seedling vigor index were used to evaluate their potential for stimulating tomato seedling growth.

It was carried out in plastics pots containing a substrate consisting of a sterile or non-sterile soil–sand (2:1) mixture. The soil samples were collected at the RAF and analyzed to determine their physical and chemical properties. This analysis showed that this soil is a sandy clay loam with the following characteristics: clay 32%, silt 28% and 40%, organic carbon 1.04%, organic matter 1.8%, total N 40%, assimilable phosphorus 19.5 ppm, K 2.48 meq./100 g, Na 0.93 meq./100 g, Ca 1.36 meq./100 g, Mg 0.56 meq./100 g, CEC 16.96 meq./100 g, and pH 6.1.

The treatments were a combination of eight bacterial isolates (S1, S4, S13, S14, S16, B9, B11, and B15), one control (sterilized distilled water), two soil types (sterilized soil and unsterilized soil), and two tomato varieties (Raja and Foolewouh) in a completely randomized factorial design with four replications, resulting in 144 experimental units.

The substrate was sterilized in an autoclave twice at 121°C for 20 min at 24 h intervals to eliminate any microorganisms present in the soil. Plastic pots (19.0 cm × 14.5 cm) were filled with substrates (sterilized and non-sterilized) and arranged in the greenhouse. The seeds of each variety were disinfected and treated with the different bacterial inocula as described above. Three inoculated seeds were sown in each pot and the whole was set up in the greenhouse. After the seedlings emerged, one seedling was left per pot to avoid competition between the seedlings. Three weeks (21 days) after sowing, 1 mL of each of the bacterial suspensions were applied to the base of each seedling [39]. The seedlings were watered daily to maintain sufficient moisture and no fertilization nor chemical treatments were applied. Six weeks (42 days) after sowing, the height of the plants was measured. At this stage, destructive sampling of plants was carried out for growth evaluation. Above and belowground biomass were separated, root, and stem lengths measured, fresh weight of plant parts were weighed and other growth variables according to Saleemi et al. [39]. The plant parts were oven-dried at 75°C for 3 days to obtain the dry mass. The growth-pro-

moting effect of the bacterial isolates was calculated according to the formula:

$$GPE = [(GT - GC)/GC] \times 100,$$

where GPE is the growth promotion efficiency, GT is the growth in treated (inoculated) seedlings, and GC is the growth in non-inoculated seedlings (control).

2.10 Data analysis

Collected data were entered and organized in Microsoft excel before analysis of variance. Separation of homogeneous groups by the LSD test was performed in R software version 4.1. 95% confidence level was used for all analyses and principal component analysis (PCA) and clustering analysis were performed using the mean of all variables measured in each treatment in R Studio.

3 Results

3.1 Biochemical and molecular characteristics of bacterial isolates

A total of 40 bacterial isolates were obtained from the analyzed samples with 24 from Santchou and 16 from Baleveng. They showed white to creamy yellow pigments on the NA culture medium. Of these isolates, 18 were initially selected based on their ability to solubilize insoluble phosphate and produce IAA in the preliminary screening.

Germination and seedling vigor test resulted in the selection of eight isolates for evaluation of their ability to promote seedling growth. All eight isolates were positive for the Gram stain and catalase test. However, they were negative for urease and citrate tests except for isolate S4, which was positive for the citrate test (Table 2, Figure 1).

3.2 Growth promoting properties of bacterial isolates

The results obtained showed that solubilized phosphorus varied from 277.44 to 1929.29 μg mL^{-1} for isolates B15 and B11, respectively (Table 3).

One of the important characteristics of PGPBs is the production of IAA. Results showed that variable amounts of IAA were obtained from the different isolates, ranging from 1.9 to 49.39 μg mL^{-1} with a maximum production for isolate S4 (Table 3).

All isolates tested were positive for ammonia production. However, they were all negative for hydrocyanic acid production, showing that they are not good antagonists against plant pathogens.

3.3 *In vitro* response of bacteria to different environmental stresses

3.3.1 *In vitro* tolerance of bacteria to salinity stress

The results of the evaluation of the ability of bacterial isolates to grow at different concentrations of NaCl (0.5–8%) in NB showed that the growth of the bacteria varied with the salt concentration. In general, it decreased with an increasing salt concentration in the medium. The isolates showed good tolerance to NaCl concentrations ranging from 0.5 to 4%. Optimum growth was achieved at the 2% concentration with ODs ranging from 0.054 for isolate

Table 2: Biochemical characteristics and molecular correspondence of bacterial isolates

Strain	Biochemical analysis					16S rRNA sequencing identification	Accession number
	Gram staining	Catalase test	Citrate test	Urease test	Motility		
S1	+	+	–	–	–	*Bacillus aerius*	OM978377
S4	+	+	+	–	–	*Bacillus* sp.	
S13	+	+	–	–	–	*Bacillus* sp.	
S14	+	+	–	–	–	*Bacillus cereus*	OM978378
S16	+	+	–	–	–	*Bacillus cereus*	OM978379
B9	+	+	–	–	–	*Bacillus thuringiensis*	OM978375
B11	+	+	–	–	–	*Bacillus pacificus*	OM978380
B15	+	+	–	–	–	*Bacillus* sp.	

'–' indicates a negative response; '+' indicates a positive response.

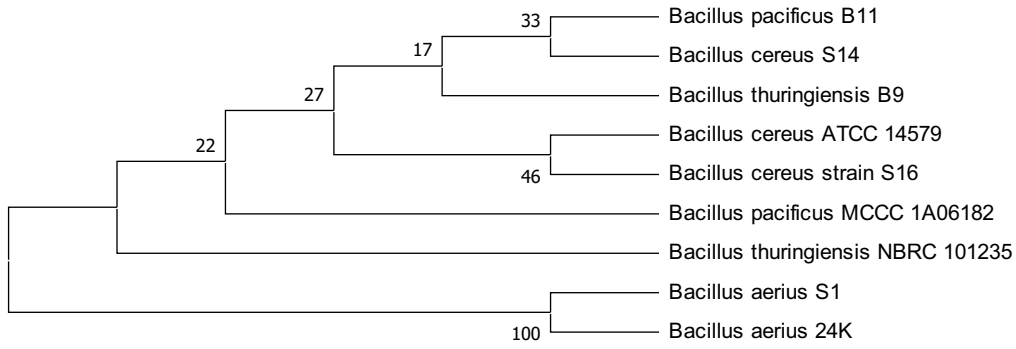

Figure 1: Phylogenetic tree of identified bacteria.

Table 3: Amount of phosphate and IAA produced according to bacterial isolates

Strains	Amount of IAA ($\mu g\ mL^{-1}$)	Amount of phosphate ($\mu g\ mL^{-1}$)	NH_3 production	HCN production
S1	26.09 ab*	1340.07 ab*	+ve	–ve
S4	49.39 a	1494.28 ab	+ve	–ve
S13	1.9 bc	1638.38 ab	+ve	–ve
S14	10.36 bc	1268.69 ab	+ve	–ve
S16	3.2 bc	1652.52 ab	+ve	–ve
B9	3.88 bc	898.99 bc	+ve	–ve
B11	3.76 bc	1929.29 a	+ve	–ve
B15	2.73 bc	277.44 c	+ve	–ve

'–ve' indicates a negative response; '+ve' indicates a positive response. *Different letters indicate significant differences between means ($P < 0.05$).

S13 to 0.682 for isolate S14. No growth was observed at the 8% concentration for all isolates (Figure 2a).

3.3.2 Growth of bacterial isolates as a function of pH

The growth of bacteria on a NB adjusted to different pH was evaluated by measuring their absorbance. The results obtained show that the bacteria tested can tolerate a pH range of 4–10. The optimum pH varied from 5.5 to 8.5 depending on the isolates. Twenty-five percent of the isolates had a pH optimum of 5.5, 25% at pH 7, and 50% at pH 8.5. Maximum growth was obtained with isolate S13 (OD = 1.777) at pH 8.5 and minimal growth (OD = 0.0033) with isolate B9 at pH 4 (Figure 2b).

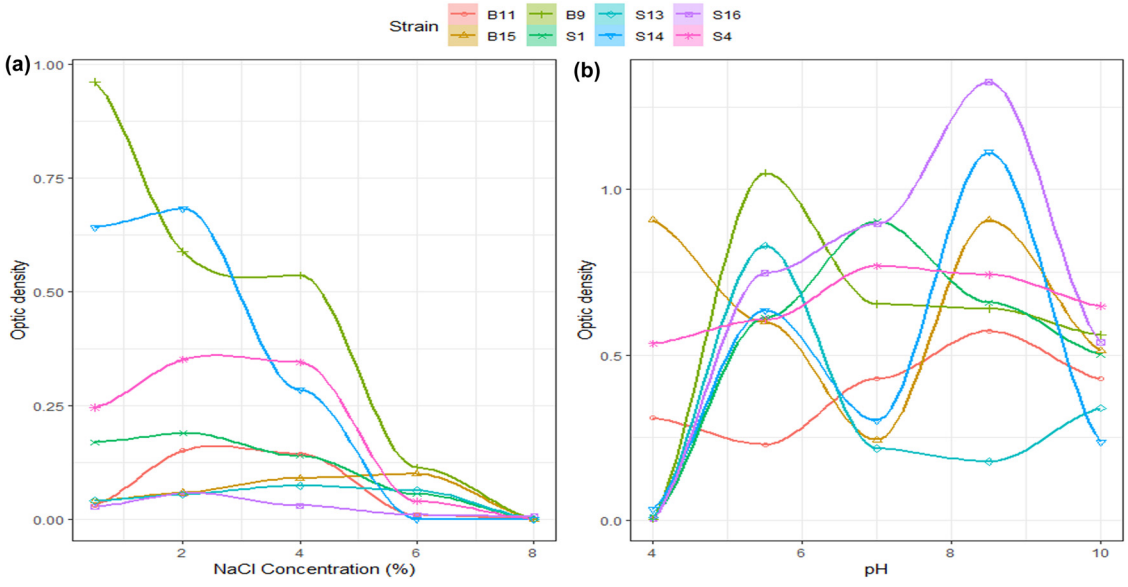

Figure 2: Growth of bacterial isolates as a function of NaCl concentration (a) and pH (b).

3.4 Seed germination and seedling vigor index according to bacterial isolates and tomato varieties

The results show that the bacterial treatments significantly ($P < 0.05$) increased the germination percentage and seedling vigor index compared to the control (Figure 3a and b). For the Raja variety (Figure 3b), the increase rate ranged from 4.4 to 28.93% for seed germination and from 5.34 to 55.91% for vigor index. In Foolewouh, the isolates improved the percentage of seed germination and seedling vigor index by 1.12–31.93 and 15.75–78.59%, respectively, compared to the control (Figure 3a). Isolate B9 increased the germination percentage and vigor index by 31.93 and 78.59% on the Foolewouh variety, followed by isolate S14 which increased by 28.93 and 56.01% on Raja variety.

3.5 Growth of tomato seedlings as a function of bacterial isolates

Eight bacterial isolates were used to evaluate their ability to promote seedling growth in the greenhouse. The results obtained showed that these isolates had a variable influence (positive or negative) on the different growth variables evaluated in the two tomato varieties (Figures 4 and 5). However, growth parameters varied depending on bacterial isolate, tomato variety, and soil treatment.

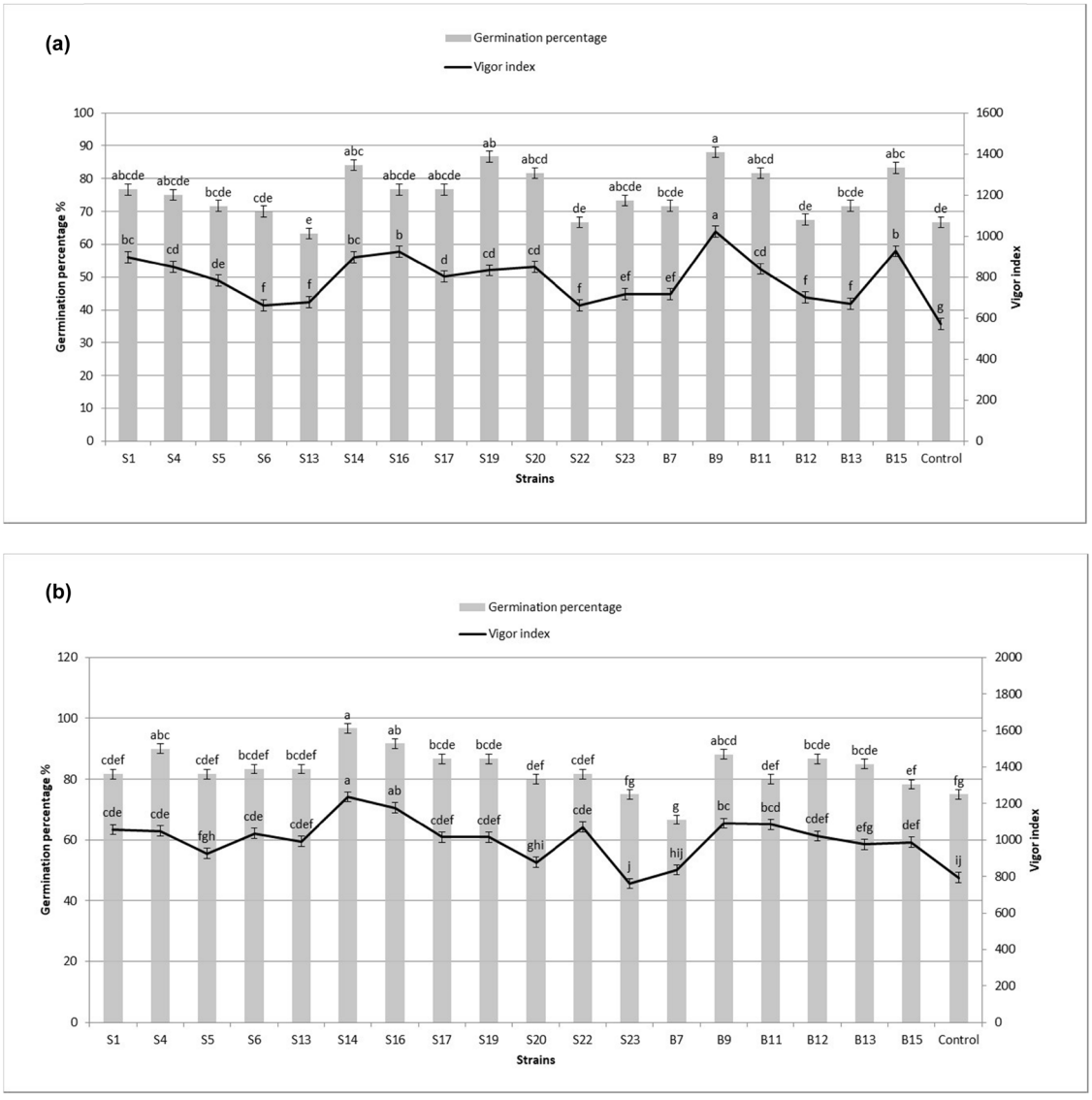

Figure 3: Germination percentage and vigor index of bacterial treatments according to varieties (a) Foolewouh and (b) Raja.

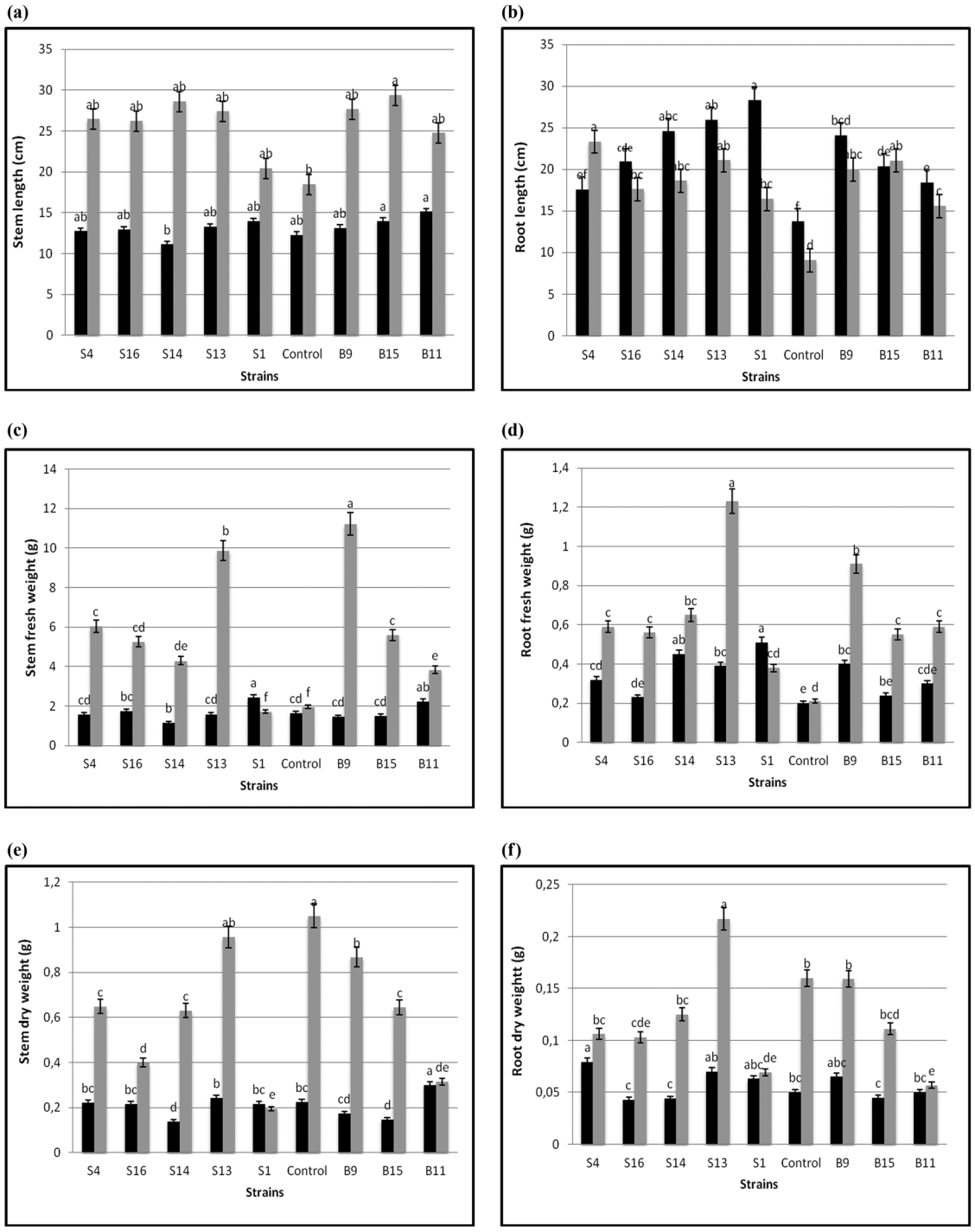

Figure 4: Growth parameters as a function of bacterial inocula of the Raja variety. Different letters indicate significant differences between categories ($P < 0.05$). Error bars indicate ±SD. (a) stem length, (b) root length, (c) stem fresh weight, (d) root fresh weight, (e) stem dry weight and (f) root dry weight.

Stem length was significantly influenced by bacterial treatments, soil treatment, and tomato variety. In general, all isolates significantly increased the stem length compared to the control ($P < 0.05$). Isolate B15 performed best on variety Raja with increases in length of 58.98% on sterilized soil but on unsterilized soil, B11 showed the best results with an increase of 23.21% compared to the control. However, there were no significant differences between the strains on the unsterilized soil and the control. While, in Foolewouh, the best performance was

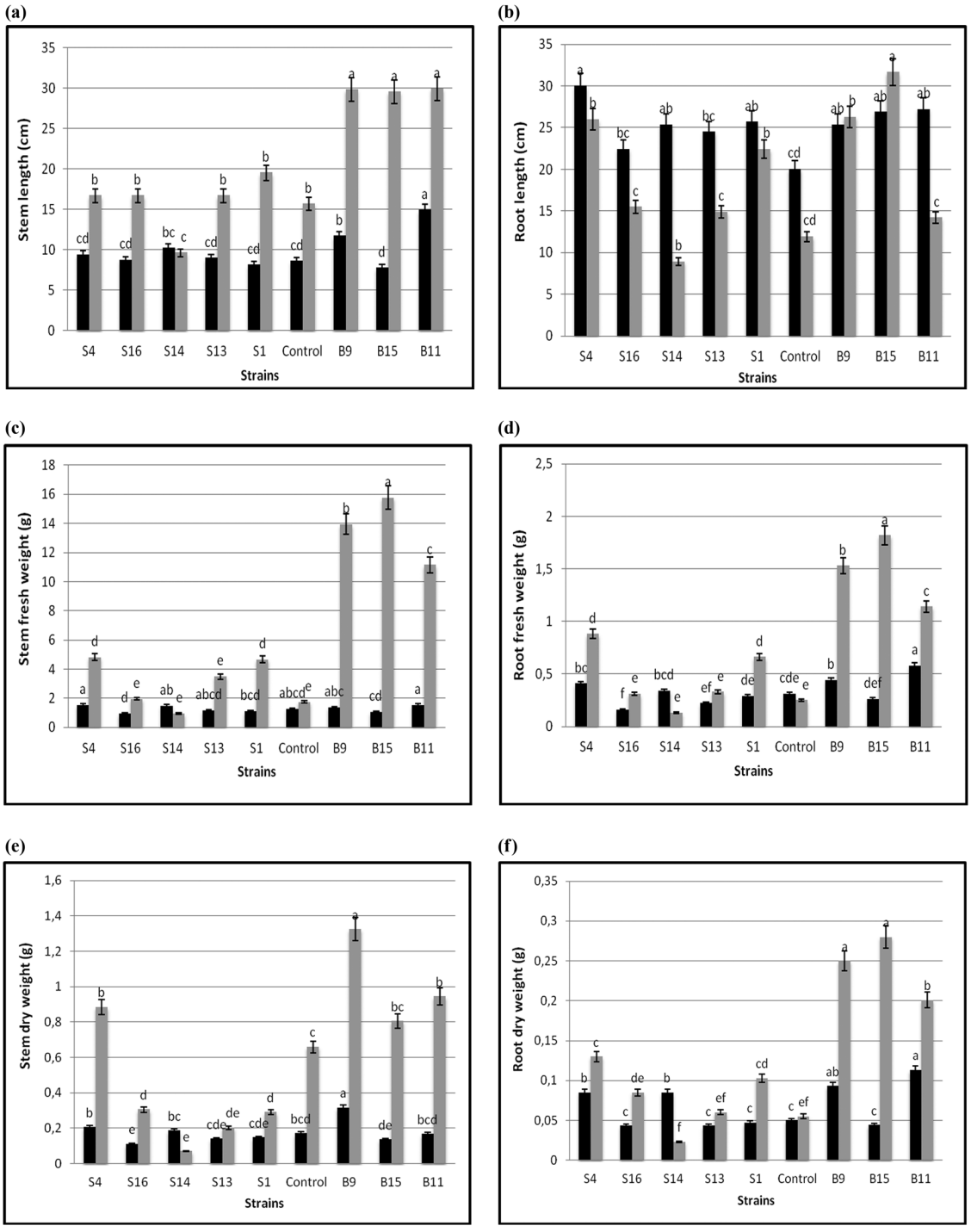

Figure 5: Growth parameters as a function of bacterial inocula of the Foolewouh variety. Different letters indicate significant differences between categories ($P < 0.05$). Error bars indicate ±SD. (a) stem length, (b) root length, (c) stem fresh weight, (d) root fresh weight, (e) stem dry weight and (f) root dry weight.

obtained with isolate B11 with increases of 90.69 and 73.43% on sterilized and unsterilized soil, respectively. Stem length was significantly higher on sterilized soil than on unsterilized soil (Figures 4a and 5a).

All isolates significantly improved root length of both varieties, except isolate S14 which had negative effects on sterilized soil. The maximum rate of improvement was observed with isolate B15 on Foolewouh with an increase

of 165.23% on sterilized soil, while on unsterilized soil isolate S4 showed the best result with an increase of 49.63%, although not significantly different from B15. Similarly, on the Raja variety, isolates S4 and S1 increased root length by 156.94 and 106.18% on sterilized and non-sterilized soil, respectively. A significant increase in root length was observed on unsterilized soil compared to sterilized soil (Figures 4b and 5b).

Fresh stem and root weights varied significantly with bacterial treatment, soil treatment, and tomato variety. In Foolewouoh, the maximum improvement rates in stem and root fresh weight were obtained with isolates S4 and B11 on unsterilized soil with increases of 20.31 and 87.09%, respectively. While on sterilized soil, the best performance was obtained with isolate B15 on the different fresh weight. In the Raja variety, isolate S1 gave the best fresh stem and root weight on unsterilized soil with increase of 47.88 and 155%, respectively. However, on sterilized soil, isolates B9 and S13 obtained the best stem and root fresh weight, respectively.

The isolates showed variable effects on stem and root dry weight. In the majority of cases, these effects were negative. However, some isolates showed positive effects compared to the control. B9 isolate showed the best improvement in stem dry weight on sterilized and unsterilized soil with increased values of 85.29 and 100.76%, respectively. In the same variety, the best performance in root dry weights was obtained with isolates B11 and B15 on sterilized and unsterilized soil, respectively. With Raja, the maximum improvement rates of 33.33 and 58% in stem and root dry weights were obtained with isolates B11 and S4, respectively, on sterilized soil. All isolates showed negative effects on unsterilized soil.

3.6 Relationship between bacterial treatments and growth parameters

To study the relationship between bacterial treatments and growth parameters of tomato seedlings, and to group individuals according to their performance, a PCA and a hierarchical clustering of individuals were performed, respectively. They were performed according to varieties and different soil conditions.

At the sterilized soil level, the PCA of the Raja variety shows that the principal component (PC1) and the principal component (PC2) represent 56.3 and 24.4% of the total variability, respectively (Figure 6a). On the Foolewouh variety, the PC1 and PC2 components represent 63.3

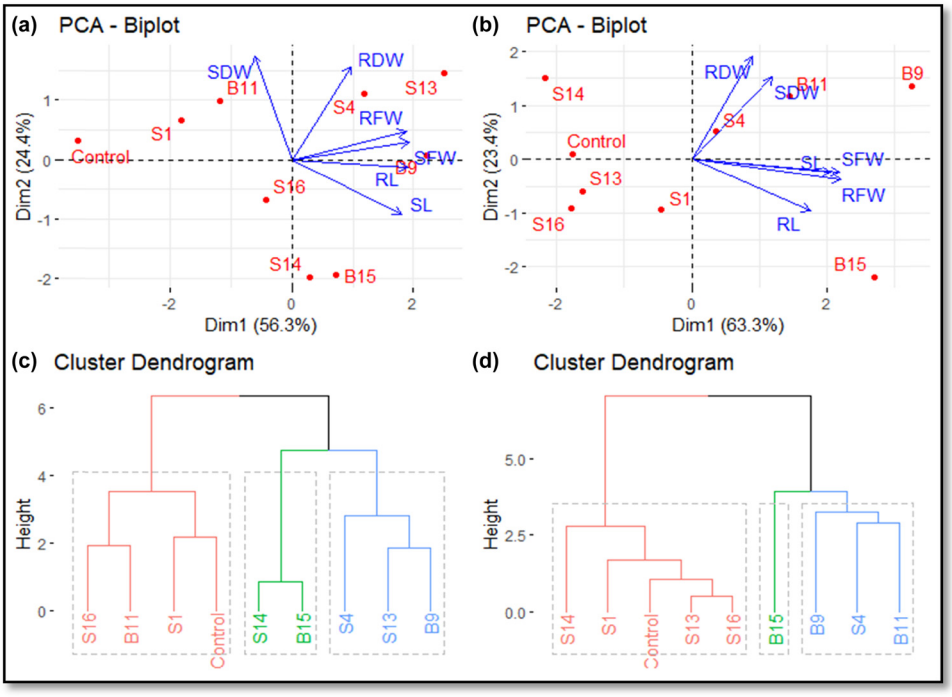

Figure 6: Effect of bacterial treatments on growth parameters of tomato seedlings on sterilized soil. (a and b) PCA of Raja (a) and Foolewouh (b) varieties. (c and d) HAC of individuals for Raja (c) and Foolewouh (d) varieties. SL – stem length; RL – root length; RFW – root fresh weight; RDW – root dry weight; SFW – stem fresh weight; RDW – root dry weight.

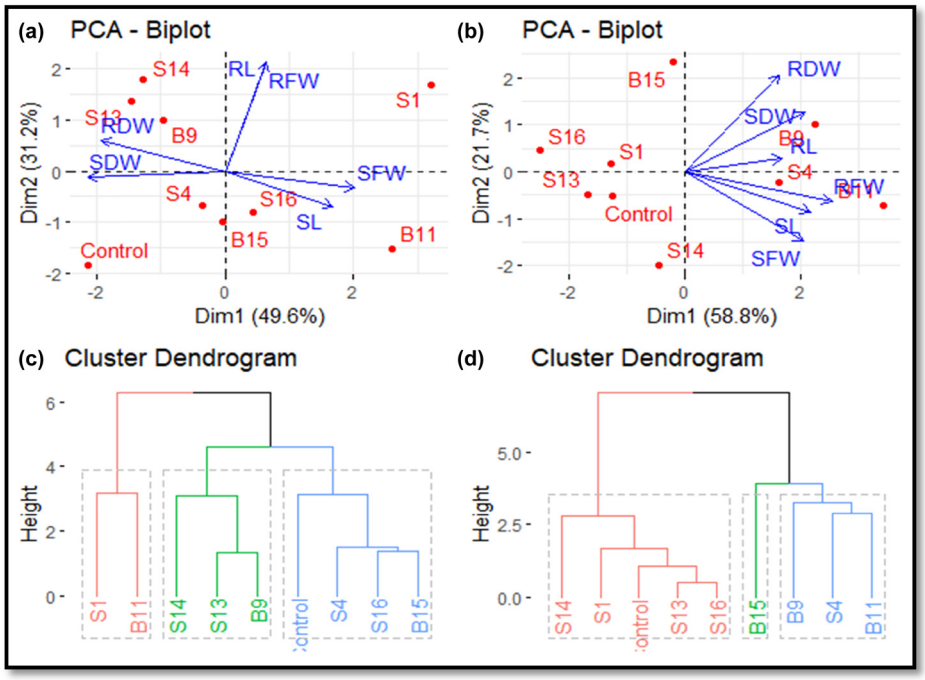

Figure 7: Effect of bacterial treatments on growth parameters of tomato seedlings on non-sterilized soil. (a and b) PCA of Raja (a) and Foolewouh (b) varieties. (c and d) HAC of individuals for Raja (c) and Foolewouh (d) varieties. SL – stem length; RL – root length; RFW – root fresh weight; RDW – root dry weight; SFW – stem fresh weight; RDW – root dry weight.

and 23.4% of the total variability observed, respectively (Figure 6b). Hierarchical ascending classification (HAC) of individuals revealed that treatments with isolates S4 and B9 showed better growth promotion on Raja and Foolewouh varieties (Figure 6c and d).

At the unsterilized soil level, the PCA of the Raja variety revealed that PC1 and PC2 accounted for 49.6 and 31.2% of the total variance, respectively (Figure 7a). PCA of the variety Foolewouh shows that PC1 and PC2 contribute 58.8 and 21.7% of the total variance, respectively (Figure 7b). The HAC of individuals on the Foolewouh variety shows that isolates B9, S4, and B11 have a strong correlation with the different growth parameters evaluated and have similar effects on the growth of this variety (Figure 7d). Furthermore, isolates S1 and B11 showed effects on increasing fresh stem mass while isolates B9, S13, and S14 showed effects on dry stem and root masses (Figure 7a and c).

4 Discussion

From the rhizosphere of tomato, 40 bacterial isolates were obtained, 24 from Santchou and 16 from Baleveng. This variability in microbial diversity could be explained by the variability of soil conditions (especially soil physical and chemical properties, salinity, pH), climatic conditions (temperature, rainfall), agronomic practices, and the land-use system of the different areas [40].

In vitro screening of the main characteristics of the growth-promoting properties of rhizobacteria was done. Phosphorus (P) is one of the essential elements for plant nutrition and is a limiting factor for plant growth [41]. All isolates solubilized P at amounts ranging from 277.44 to 1929.29 µg mL^{-1}. The amount of P released in this study is higher than that observed in other work. The work of Fashi et al. [42] showed a production of 36–278.5 mg L^{-1} by PGPB isolated from the rhizosphere of jujube (*Ziziphus lotus*) plants in Morocco. These rhizobacteria mobilize inorganic phosphate from the soil thereby increasing their availability to plants [8]. Many phosphate solubilization mechanisms are used by PGPBs, the main one being the production of mineral dissolving compounds such as organic acids, protons, hydroxyl ions, and CO_2 [43].

Another important feature of PGPBs is the production of auxin IAA. It is an important phytohormone produced by several rhizobacteria. Quantitative analysis of IAA production in our study showed a concentration ranging from 1.9 to 49.39 µg mL^{-1}. Alemneh et al. [44] obtained a similar trend in IAA production with concentrations ranging from 4.1 to 67.2 µg mL^{-1} with bacteria isolated from

chickpea in Australia. The S4 isolate obtained the highest production. This variation in production could be explained by the fact that PGPBs use different pathways for IAA synthesis [45]. IAA biosynthesis by rhizobacteria is mainly due to tryptophan, an amino acid commonly found in plant root exudates [46].

Regarding ammonia, all isolates were shown to be capable of producing ammonia. This production of ammonia has many advantages for plants, including the improvement of nitrogen uptake by plants [42] and the inhibition of phytopathogens [47].

Inoculation of tomato seeds with PGPB isolates improved the germination percentage and seedling's vigor index compared to the control treated with distilled water. Similar increases by rhizobacteria have been reported in tomato [48] in India, pepper [49] in Argentina, and maize [21]. This improvement in germination could be explained by increased production of phytohormones, particularly gibberellin, which would have triggered enzyme activities thereby favoring early germination such as amylases [50,51]. These amylases increase the uptake of starches by hydrolyzing them into metabolizable sugars, which provide energy for root and stem growth during the germination process [52].

Several environmental factors such as soil pH, salinity, rainfall, and temperature can influence microorganisms' growth, performance, and population density in the rhizosphere [40,53,54]. In this study, the ability of bacterial isolates to grow at different pH and salt (NaCl) concentrations were evaluated. The isolates tolerated NaCl concentrations up to 4%. Beyond that, growth was significantly affected until it was canceled at 8%. Similar results were obtained by Ngo Nkot et al. [55]. This ability of isolates to tolerate NaCl concentrations could be explained by their capacity to synthesize organic osmolytes such as proline and glycine betaine, or carbohydrates [56–58].

One of the most important environmental factors affecting the microbial population is the soil pH [59]. The different isolates grew with optimum growth at pH ranging from 5.5 to 8.5. Some work [60] has shown that soil organisms prefer slightly acidic to slightly basic soils. Thus, variations in pH can influence the soil bacterial community. Indeed, under acidic pH conditions, the N_2 fixation process is reduced due to a low diversity of diazotrophic communities in the soil [61].

In the pot experiment, the PGPB used significantly improved the growth parameters (stem and root lengths, fresh and dry masses of stems and roots) of seedlings of different tomato cultivars in the greenhouse. These results are in accord with those obtained by Cervantes-Vázquez et al. [62] on tomato plantlets in Mexico. Li et al. [63] in China showed that microbial inocula had a growth-promoting effect on *Avena sativa* (oats), *Medicago sativa* (alfalfa), and *Cucumis sativus* (cucumber) by increasing soil enzyme activity and available nutrients. This improvement in plant growth can be attributed to mechanisms or molecules produced by PGPBs such as phosphate solubilization, production of phytohormones (mainly auxin and IAA), and biological nitrogen fixation [21,64,65]. Auxin controls cell elongation and division, and tissue differentiation and assists in apical dominance [45].

The IAA released by rhizobacteria mainly affects root development by increasing their length, secondary root number, size, and mass, which increases their contact surfaces with the soil and thus the enhancement of nutrients needed for plant growth [66,67]. In addition, the bioavailability of phosphate increases plants metabolic processes such as photosynthesis, respiration, and energy transfer [68]. These metabolic processes will stimulate growth, length, and fresh and dry mass of stems and roots [69].

In general, seedlings on sterilized soils showed higher growth rates than those observed on non-sterilized soils. Similar results were observed by Cavite et al. [31] on rice. Indeed, soil sterilization eliminated all indigenous microorganisms that could be pathogenic or antagonistic to the PGPB inocula. As a result, the applied bacterial isolates expressed their full plant growth-promoting potential [70]. In addition, soil sterilization would probably have caused the elimination of denitrifying microorganisms leading to higher availability of nitrogen to plants [71].

The cultivars reacted differently to inoculation with PGPBs. The improved variety performed better than the local variety. These results are different from those obtained on wheat by Valente [72], who showed that plant breeding negatively impacted the relationship between plants and PGPBs due to genetic variation between cultivars or plant species [73]. Indeed, different types of root exudates are produced depending on the variety, species, and crop [74].

5 Conclusions

In the present study, 40 strains were isolated from tomato plants and eight of them belonging to the genus *Bacillus*, including *B. thuringiensis*, *B. cereus*, *B. pacificus*, *B. aerius* exhibited plant growth promoting characteristics and enhanced *in vitro* and *in vivo* growth of tomato seedlings. This result shows the ability of these bacterial strains

to improve the growth of seedlings of different tomato varieties (local and improved) and under different soil treatments (sterile and non-sterile). The tested bacterial strains can be used as biofertilizers for sustainable and ecological production. However, further studies should be conducted to evaluate the effect of their application in the field.

Acknowledgments: The authors thank the members of the Molecular Parasitology and Entomology Research Unit of the University of Dschang for facilitating the sequencing of the bacterial isolates. They thank the members of the Research Unit of Phytopathology and Agricultural Zoology of the University of Dschang for their collaboration.

Funding information: The authors state no funding involved.

Author contributions: Conceptualization, I.D.K. and A.Y.; methodology, I.D.K. and A.Y.; validation, B.K., A.Y., and D.B.; investigation, I.D.K., B.K., J.M., and L.T.; data analysis, I.D.K. and P.G.N.II.; writing – original draft preparation, I.D.K. and P.G.N.II.; writing – review and editing, I.D.K.; supervision, A.Y. and D.B. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Ruf S, Bock R. Plastid transformation in tomato: a vegetable crop and model species. In: Maliga P, editor. Chloroplast biotechnology: mthods and protocols. 2nd ed. UK: Springer; 2021. p. 217–28.

[2] FAOSTAT. Food and Agriculture Organization Corporate Statistical Database. Available Online: https://www.fao.org/faostat/fr/#data/QCL. (Accessed on 9 february 2022).

[3] Kumar M, Giri VP, Pandey S, Gupta A, Patel MK, Bajpai AB, et al. Plant-growth-promoting rhizobacteria emerging as an effective bioinoculant to improve the growth, production, and stress tolerance of vegetable crops. Int J Mol Sci. 2021;22(22):12245. doi: 10.3390/ijms222212245.

[4] Mekonnen H, Kibret M. The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia. Chem Biol Technol Agric. 2021;8(1):15. doi: 10.1186/s40538-021-00213-y.

[5] INS. Annuaire statistique du Cameroun: Recueil des séries d'informations statistiques sur les activités économiques, sociales, politiques et culturelles du pays. Cameroun: INS, Agriculture; 2017.

[6] Djoukeng HG, Dogot T, Tankou CM, Degré A. Contraintes Socio-économiques de Répartition des Terres et Impacts sur la Conservation des Sols dans les Hauts Plateaux de l'Ouest du Cameroun. Tropicultura. 2016;34:231–41.

[7] Hinsinger P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil. 2001;237(2):173–95. doi: 10.1023/A:1013351617532.

[8] Alori ET, Glick BR, Babalola OO. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol. 2017;8:8. doi: 10.3389/fmicb.2017.00971.

[9] Turan M, Ataoğlu N, Şahın F. Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. J Sustain Agric. 2006;28(3):99–108.

[10] Agbodjato A, Noumavo A, Amogou O, Adoko M, Dagbenonbakin G, Falcon Rodriguez A. Impact de l'utilisation des Rhizobactéries Promotrices de la Croissance des Plantes (PGPR) en combinaison avec le chitosane sur la croissance et le rendement du maïs (Zea mays L.) sur sol ferralitique au Sud-Bénin. Symposium International sur la Science et la Technologie. Ouagadougou: Revue burkinabè de la recherche; 2018.

[11] FAO. Code de conduite international sur l'utilisation et la gestion durables des engrais. Rome: 2019. p. 56.

[12] Chen JH, Jeng-Tzung W, Chiu-Chung Y. The combined use of chemical, organic fertilizers and/or biofertilizer for crop growth and soil fertility. In: International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use. Thailand: Land Development Department; 2006. p. 1–11. doi: 10.30058/SE.200706.0001.

[13] Ahmed B, Zaidi A, Khan M, Rizvi A, Saif S, Shahid M. Perspectives of plant growth promoting rhizobacteria in growth enhancement and sustainable production of tomato. In: Zaidi A, Khan MS, editors. Microbial strategies for vegetable production. UK: Springer; 2017. p. 125–49.

[14] Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, Patra JK. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol Res. 2018;206:131–40. doi: 10.1016/j.micres.2017.08.016.

[15] Thakkar A, Sharma S. Study of biocontrol activity of PGPR againt phytophathogen. UK: LAP LAMBERT Academic Publishing; 2021.

[16] de la Osa C, Rodríguez-Carvajal MÁ, Gandullo J, Aranda C, Megías M, Ollero FJ, et al. Plant growth-promoting rhizobacteria modulate the concentration of bioactive compounds in tomato fruits. Separations. 2021;8:223. doi: 10.3390/separations8110223.

[17] Zia MA, Riaz R, Batool A, Yasmin H, Nosheen A, Naz R, et al. Glucanolytic rhizobacteria associated with wheat-maize cropping system suppress the Fusarium wilt of tomato (*Lycopersicum esculentum* L). Sci Horticulturae. 2021;287:110275. doi: 10.1016/j.scienta.2021.110275.

[18] Wang Y, Li W, Du B, Li H. Effect of biochar applied with plant growth-promoting rhizobacteria (PGPR) on soil microbial community composition and nitrogen utilization in tomato. Pedosphere. 2021;31:872–81. doi: 10.1016/S1002-0160(21)60030-9.
[19] Mfewou A, Dorothy F, Tchofo P. Geography of the production of Ipomoeabatatas (Sweet Potato): an analysis in the space of Santchou (West-Cameroon). Int J Humanities Soc Sci. 2019;6:67–72. doi: 10.14445/23942703/IJHSS-V6I2P111.
[20] Fonkou T, Nguetsop VF, Pinta JY, Dekoum VM, Lekeufack M, Amougou A. Macrophyte diversity in polluted and non-polluted wetlands in Cameroon. Cameroon. J Exp Biol. 2005;1(1):26–33.
[21] Tchuisseu Tchakounté GV, Berger B, Patz S, Fankem H, Ruppel S. Community structure and plant growth-promoting potential of cultivable bacteria isolated from Cameroon soil. Microbiol Res. 2018;214:47–59. doi: 10.1016/j.micres.2018.05.008.
[22] Pauwels JM, Van Ranst E, Verloo M, Mvondo Ze A Soil Science Laboratory Manual. Methods of Soil and Plant Analysis, Equipment, Management of Glassware and Chemicals. Bruxellles1992. Report No.: 28.
[23] Shafi J, Mingshan J, Zhi-qiu Q, Xiuwei L, Zumin G, Xinghai L, et al. Optimization of *Bacillus aerius* strain JS-786 cell dry mass and its antifungal activity against *Botrytis cinerea* using response surface methodology. Arch Biol Sci. 2017;69:469–80. doi: 10.2298/ABS160421122S.
[24] Vincent J, Humphrey B. Taxonomically significant group antigens in Rhizobium. J Gen Microbiol. 1970;63:379–82. doi: 10.1099/00221287-63-3-379.
[25] Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, et al. Bergey's manual of systematic bacteriology. Volume 3: The Firmicutes. New York, United States: Springer; 2011.
[26] Majeed A, Abbasi K, Hameed S, Imran A, Rahim N. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Front Microbiol. 2015;6:198. doi: 10.3389/fmicb.2015.00198.
[27] Simmons JS. A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi: with colored plate. J Infect Dis. 1926;39(3):209–14. doi: 10.1093/infdis/39.3.209.
[28] Benson HJ. Microbiological applications: laboratory manual in general microbiology. 8th ed. New york: McGraw Hill; 2002.
[29] Pikovskaya R. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya. 1948;17:362–70.
[30] Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Analytica Chim Acta. 1962;27:31–6. doi: 10.1016/S0003-2670(00)88444-5.
[31] Cavite HJM, Mactal AG, Evangelista EV, Cruz JA. Growth and yield response of upland rice to application of plant growth-promoting rhizobacteria. J Plant Growth Regul. 2021;40(2):494–508. doi: 10.1007/s00344-020-10114-3.
[32] Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. Plant Physiol. 1951;26(1):192–5. doi: 10.1104/pp.26.1.192.
[33] Cappuccino J, Sherman N. Biochemical activities of microorganisms. Microbiology, a laboratory manual. California, USA: The Benjamin/Cummings Publishing Co.; 1992. p. 188–247.
[34] Lorck H. Production of hydrocyanic acid by bacteria. Physiol Plant. 1948;1(2):142–6.
[35] Azeem M, Haider MZ, Javed S, Saleem MH, Alatawi A. Drought stress amelioration in maize (*Zea mays* L.) by inoculation of *Bacillus* spp. strains under sterile soil conditions. Agriculture. 2022;12(1):50. doi: 10.3390/agriculture12010050.
[36] ISTA. The international rules for seed testing. Proc Int Seed Test Ass. 1999;4:3–49.
[37] Laradj Zazou K. Isolement et caractérisation des rhizobactéries promotrices de la croissance des plantes capables de lutter contre le Fusarium. Doctorat de 3ième cycle. Algérie: Université de Djillali Liabes de Sidi Bel Abbes; 2017.
[38] Islam S, Akanda AM, Prova A, Islam MT, Hossain MM. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Front Microbiol. 2016;6:1360. doi: 10.3389/fmicb.2015.01360.
[39] Saleemi M, Kiani MZ, Sultan T, Khalid A, Mahmood S. Integrated effect of plant growth-promoting rhizobacteria and phosphate-solubilizing microorganisms on growth of wheat (*Triticum aestivum* L.) under rainfed condition. Agric Food Security. 2017;6(1):1–8. doi: 10.1186/s40066-017-0123-7.
[40] Simonsen AK. Environmental stress leads to genome streamlining in a widely distributed species of soil bacteria. ISME J. 2022;16(2):423–34. doi: 10.1038/s41396-021-01082-x.
[41] Gupta G, Parihar S, Ahirwar N, Snehi DSK, Singh V. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. Microb Biochem Technol. 2015;7:096–102. doi: 10.4172/1948-5948.1000188.
[42] Fahsi N, Mahdi I, Mesfioui A, Biskri L, Allaoui A. Plant growth-promoting rhizobacteria isolated from the jujube (*Ziziphus lotus*) plant enhance wheat growth, Zn uptake, and heavy metal tolerance. Agriculture. 2021;11(4):316.
[43] Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus. 2013;2(1):587. doi: 10.1186/2193-1801-2-587.
[44] Alemneh AA, Cawthray GR, Zhou Y, Ryder MH, Denton MD. Ability to produce indole acetic acid is associated with improved phosphate solubilising activity of rhizobacteria. Arch Microbiol. 2021;203(7):3825–37. doi: 10.1007/s00203-021-02364-w.
[45] Goswami D, Thakker JN, Dhandhukia PC. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food Agric. 2016;2(1):1127500. doi: 10.1080/23311932.2015.1127500.
[46] Etesami H, Alikhani HA, Akbari AA. Evaluation of plant growth hormones production (IAA) ability by Iranian soils rhizobial strains and effects of superior strains application on wheat growth indexes. World Appl Sci J. 2009;6(11):1576–84.
[47] Mahdi I, Fahsi N, Hafidi M, Allaoui A, Biskri L. Plant growth enhancement using rhizospheric halotolerant phosphate solubilizing bacterium *Bacillus licheniformis* QA1 and *Enterobacter asburiae* QF11 isolated from *Chenopodium quinoa* willd. Microorganisms. 2020;8(6):948. doi: 10.3390/microorganisms8060948.
[48] Konappa N, Krishnamurthy S, Arakere UC, Chowdappa S, Ramachandrappa NS. Efficacy of indigenous plant growth-

promoting rhizobacteria and Trichoderma strains in eliciting resistance against bacterial wilt in a tomato. Egypt J Biol Pest Control. 2020;30(1):1–13. doi: 10.1186/s41938-020-00303-3.
[49] Ureche MAL, Pérez-Rodriguez MM, Ortiz R, Monasterio RP, Cohen AC. Rhizobacteria improve the germination and modify the phenolic compound profile of pepper (*Capsicum annum* L.). Rhizosphere. 2021;18:100334. doi: 10.1016/j.rhisph.2021.100334.
[50] Gholami A, Shahsavani S, Nezarat S. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Int J Biol Life Sci. 2009;1(1):35–40.
[51] Li Q, Yang A, Zhang W-H. Higher endogenous bioactive gibberellins and α-amylase activity confer greater tolerance of rice seed germination to saline-alkaline stress. Environ Exp Botany. 2019;162:357–63. doi: 10.1016/j.envexpbot.2019.03.015.
[52] Akazawa T, Hara-Nishimura I. Topographic aspects of biosynthesis, extracellular secretion, and intracellular storage of proteins in plant cells. Annu Rev Plant Physiol. 1985;36(1):441–72.
[53] Alexandre A, Oliveira S. Response to temperature stress in rhizobia. Crit Rev Microbiol. 2013;39(3):219–28. doi: 10.3109/1040841x.2012.702097.
[54] Abdul Rahman NSN, Abdul Hamid NW, Nadarajah K. Effects of abiotic stress on soil microbiome. Int J Mol Sci. 2021;22(16):9036. doi: 10.3390/ijms22169036.
[55] Ngo Nkot L, Ngo Bisseck M, Fankem H, Adamou S, Kamguia K, Albert N, et al. Isolation and screening of indigenous bambara groundnut (*Vigna subterranea*) nodulating bacteria for their tolerance to some environmental stresses. Am J Microbiol Res. 2015;3:65–75. doi: 10.12691/ajmr-3-2-5.
[56] Csonka LN. Physiological and genetic responses of bacteria to osmotic stress. Microbiol Rev. 1989;53(1):121–47.
[57] Vriezen JA, De Bruijn FJ, Nusslein K. Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. Appl Environ Microbiol. 2007;73(11):3451–9. Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature.
[58] Yan N, Marschner P, Cao W, Zuo C, Qin W. Influence of salinity and water content on soil microorganisms. Int Soil Water Conserv Res. 2015;3(4):316–23. doi: 10.1016/j.iswcr.2015.11.003.
[59] Banda JF, Zhang Q, Ma L, Pei L, Du Z, Hao C, et al. Both pH and salinity shape the microbial communities of the lakes in Badain Jaran Desert, NW China. Sci Total Environ. 2021;791:148108. doi: 10.1016/j.scitotenv.2021.148108.
[60] Sullivan TS, Barth VP, Lewis RW. Soil acidity impacts beneficial soil microorganisms. Washington: Washington State University; 2016. Report No.: 247E.
[61] Smercina DN, Evans SE, Friesen ML, Tiemann LK. To fix or not to fix: controls on free-living nitrogen fixation in the rhizosphere. Appl Environ Microbiol. 2019;85(6):e02546–18. doi: 10.1128/AEM.02546-18.
[62] Cervantes-Vázquez TJÁ, Valenzuela-García AA, Cervantes-Vázquez MG, Guzmán-Silos TL, Fortiz EL, Rangel PP, et al. Morphophysiological, enzymatic, and elemental activity in greenhouse tomato saladette seedlings from the effect of plant growth-promoting rhizobacteria. Agronomy. 2021;11(5):1008. doi: 10.3390/agronomy11051008.
[63] Li H, Qiu Y, Yao T, Ma Y, Zhang H, Yang X. Effects of PGPR microbial inoculants on the growth and soil properties of *Avena sativa*, *Medicago sativa*, and *Cucumis sativus* seedlings. Soil Tillage Res. 2020;199:104577. doi: 10.1016/j.still.2020.104577.
[64] Cherchali A, Boukhelata N, Yahia K, Ouzna A-B, Djebbar R. Isolation and identification of a phosphate-solubilizing *Paenibacillus polymyxa* strain GOL 0202 from durum wheat (*Triticum durum* Desf.) rhizosphere and its effect on some seedlings morphophysiological parameters. Biocatalysis Agric Biotechnol. 2019;19:101087. doi: 10.1016/j.bcab.2019.101087.
[65] Oleńska E, Małek W, Wójcik M, Swiecicka I, Thijs S, Vangronsveld J. Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: a methodical review. Sci Total Environ. 2020;743:140682. doi: 10.1016/j.scitotenv.2020.140682.
[66] Solano BR, Maicas JB, Mañero FG. Physiological and molecular mechanisms of plant growth promoting rhizobacteria (PGPR). In: Ahmad I, Pitchel J, Hayat S, editors. Plant–bacteria interactions – strategies and techniques to promote plant growth. New Jersey: Weinheim-Wiley VCH; 2008. p. 41–54.
[67] Aeron A, Kumar S, Pandey P, Maheshwari D. Emerging role of plant growth promoting rhizobacteria in agrobiology. Bacteria in agrobiology: crop ecosystems; Springer 2011. p. 1–36.
[68] Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA. Plant growth promotion by phosphate solubilizing fungi – current perspective. Arch Agron Soil Sci. 2010;56(1):73–98. doi: 10.1080/03650340902806469.
[69] Rodríguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv. 1999;17(4-5):319–39. doi: 10.1016/S0734-9750(99)00014-2.
[70] Gamliel A, Van Bruggen A. Maintaining soil health for crop production in organic greenhouses. Sci Horticulturae. 2016;208:120–30. doi: 10.1016/j.scienta.2015.12.030.
[71] Rosenblueth M, Ormeño-Orrillo E, López-López A, Rogel MA, Reyes-Hernández BJ, Martínez-Romero JC, et al. Nitrogen fixation in cereals. Front Microbiol. 2018;9:1794. doi: 10.3389/fmicb.2018.01794.
[72] Valente J. Impact du génotype de blé dans les interactions avec les rhizobactéries: quelle influence de la sélection variétale sur les relations entre plantes et bactéries phytostimulatrices? Thèse de Doctorat. Lyon: Université Claude Bernard Lyon1; 2018.
[73] Khalid A, Arshad M, Zahir Z. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol. 2004;96(3):473–80. doi: 10.1046/j.1365-2672.2003.02161.x.
[74] Dazzo F, Yanni Y, Rizk R, de Bruijn FJ, Rademaker J, Squartini A, et al. Progress in multinational collaborative studies on the beneficial association between *Rhizobium leguminosarum* bv. trifolii and rice. In: Ladha JK, Reddy PM, editors. The quest for nitrogen fixation in rice. Makati City (Philippines): International Rice Research Institute; 2000;167–89.