Shuang *et al. Annals of Microbiology* (2022) 72:40
https://doi.org/10.1186/s13213-022-01697-1

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE

Open Access

Identification and growth-promoting effect of endophytic bacteria in potato

Ma Shuang[1,2], Jiying Sun[1] and Wang Teng[2*]

Abstract

In agriculture, *Bacillus*species are efficient and ecologically tool for promote the growth of theplant.

Purpose: This study obtains the plant growth-promoting (PGP) ability ofendophytic bacteria isolated from the potato tubers.

Methods: Using endophyticbacteria to promote potato growth, achieve the purpose of increasing production.In this experiment, the growth- promoting ability of the strain was verified bylaboratory identification and field test validation.

Result: The isolateswere identified as *Bacillus* species based on a 16S rRNA gene sequenceand gyrB gene sequence analysis. DNA hybridization finally identified it as *Bacillusvelezensis*. Among the PGP attributes, the strain K-9 was found to be positivefor indole acetic acid (IAA) production, phosphate solubilization, siderophoreproduction, and nitrogen fixation. The isolate was found negative for potassiumsolubilization. The quantitative estimation of IAA product to 9.09 µg/ml. Theisolate also had the ability to produce lytic enzymes such as amylase andprotease. The quantitative estimation of protease activity is 89.16 µg/ml. The inoculation strain K-9 improved bioaccumulation of rootsand buds and yield in the potato compared to uninoculated control plants.

Conclusion: These findings give an insight into the ways to use PGP bacteria to increasepotato production.

Keywords: *Bacillus*, Potato tubers, Endophytic bacteria, PGPR

Background

The bacteria that live in or near plant roots support the plant growth and are usually referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper et al., 1980). The plant rhizosphere is having multitude of plant-microbe interactions that are influenced by rhizo-secretions and resident microflora (Zahar et al., 2014). In sustainable agriculture, biocontrol and plant growth promotion activities are significant characteristics of commercial agents (Qiao et al., 2017).

The genus *Bacillus* comprises more than 300 species with validly published names (Sun et al., 2021). *Bacillus* species have come to play an increasingly important role in applied microbiology. *Bacillus* species have strong adaptability to the environment (Liu et al., 2019; Cui et al., 2015). *Bacillus* species are gram-positive, rod-shaped, sporulating bacteria that are able to control plant diseases through a variety of mechanisms (antibiosis, defense responses in the host plant, competition) (Zahra et al., 2021). *Bacillus* have been isolated from marine sediments (Xu et al., 2020), soda lake (Menes et al., 2019), salt mine (Roohi et al., 2014), plant rhizosphere (Weihui et al., 2020), plant leaves (Zhang et al., 2017), and plant tubers (Cui et al., 2019). Specific methods and procedures are summarized in Fig. 1. Endophytic bacteria screened from plants are particularly adaptable to host

*Correspondence: wteng1129@126.com

[2] College of Agriculture, Heilongjiang Bayi Agricultural University, Daqing, China
Full list of author information is available at the end of the article

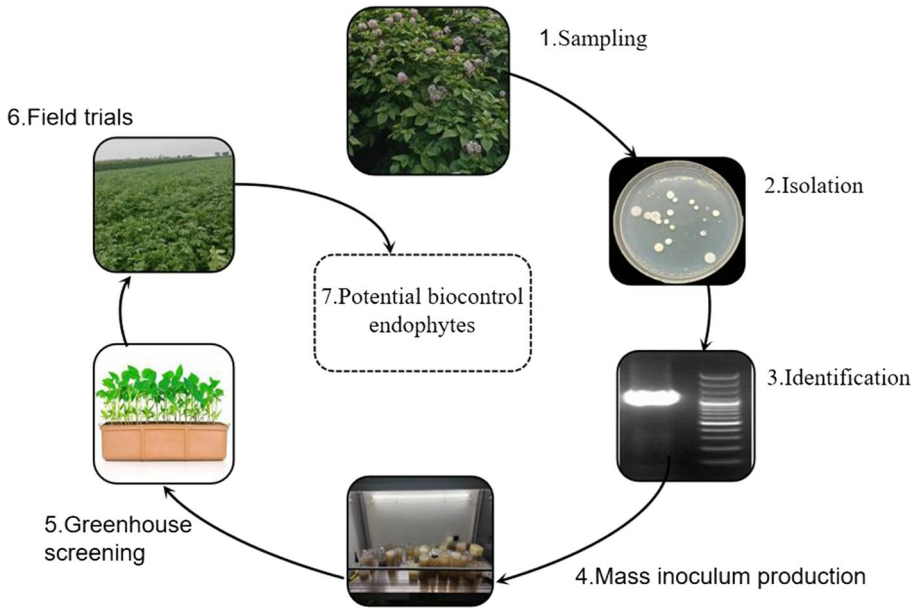

Fig. 1 Isolation and screening of endophytic bacteria for biocontrol. 1, sampling from thriving plants. 2, isolation from surface sterile tissues using artificial media. 3, molecular identification using ribosomal genes. 4, mass production tests. 5, screening test of endophytic bacteria to plants in greenhouse. 6, endophytic bacteria with growth-promoting effect were tested in the field. 7, the potential PGPR can enter the industrial stage

crops (Abedinzadeh et al., 2019). A few endophytic bacteria have the function of promoting crop growth directly and indirectly. They can dissolve phosphorus, potassium, and other minerals in the soil, fix nitrogen in the air for plants to use, generate siderophores, better absorb iron in the soil for plants, synthesize indoleacetic acid, and promote plant growth (Amaresan et al., 2012). In the present study, we proposed a species of the genus *Bacillus*, strain K-9, isolated from the potato tubers. Strain K-9 can increase potato yield and is a potential green pesticide. It provides a basis for further understanding the growth-promoting function of potato endophytic bacteria and its agricultural development and utilization and is of great significance to the sustainable utilization of agricultural ecosystem.

Methods

Isolation and cultivation

Strain K-9 was isolated from the junction of the healthy epidermis and scab spots of the potato tuber collected at Keshan County in the China (122°E, 46°N). The sample was obtained by potato field sampling of September 2020. The potato tubers were surface disinfected with 75% v/v ethanol for 30 s and 5% v/v sodium hypochlorite for 1 min followed by rinsing with sterile water. A sterile scalpel was used to cut the interface between the diseased potato spot and the healthy potato skin and ground it in a sterile mortar. The homogenate was placed in a sterile centrifuge tube, diluted with 10 mL sterile water to 10^{-2}–10^{-6}, and 100 μL of each dilution was spread on a beef extract medium (NA) plate (beef extract 3 g, peptone 5 g, yeast extract 1 g, sucrose 10 g, agar 17 g, sterile water 1000 mL, pH 7.2). The isolates were purified and cultured at 28 °C and stored in at −80 °C (Ma et al., 2022).

Morphological, physiological, and biochemical characteristics

Characteristics of strain K-9 were examined using routine cultivation methods on NA media at 28 °C (except where indicated otherwise). *Escherichia coli* was obtained from the Microbiology Laboratory of Bayi Agricultural University as a reference strain for biological chemical test.

Strain K-9 were cultured in NA plate for 24 h, then gram-stained, observed under a microscope, and photographed. The physiological and biochemical properties of the strain were determined by referring to Bergey's Bacteria Identification Manual and Manual of Identification of Common Bacterial Systems (Buchanan and Gibbons 1984; Dong and Cai 2001). Growth at various NaCl concentrations (0–15%, at intervals of 1.0%) was determined at 28 °C in NA broth media that contained all constituents of NA broth but lacked NaCl, and NaCl was added as a supplement at various concentrations. Catalase activity was determined by bubble formation in a 3% (v/v) H_2O_2 solution. The pH range for growth was determined from pH 3.0 to 11.0 (at intervals of 1.0 pH unit) in NA

broth. Utilization of carbohydrates (sorbitol, saccharose, glucose, xylose, lactose, cellobiose, raffinose, rhamnose, fructose, galactose, mannitol, maltose, inositol, arabinose, sodium alginate, sodium gluconate, glycine) was tested using the basic medium with the corresponding carbohydrates (5 g/L). The basic medium contained (per liter): 2 g $(NH_4)_2SO_4$, 25 g NaCl, 0.5 g $NaH_2PO_4{\cdot}H_2O$, 0.5 g K_2HPO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, and 0.1 g $CaCl_2{\cdot}2H_2O$. Fatty-acid analysis of strain K-9 was undertaken by the analytical services of Microbial Identification Systems (Panomix Biotechnology Company, China), determined by targeted metabolism.

Phylogenetic and genomic analysis

Strain K-9 was further identified through the analysis of its 16S rRNA, gyrB gene sequences (Lane 1991; La et al., 2004) and complete genome sequence. DNA of antagonistic strains was extracted by a Tiangen Bacterial Genome Kit. The 16S rRNA primers were 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The gyrB amplification was performed with the gyrB-F primer (5′-GAAGTCATCATGACCGTTCTGCAYGCNGGNGGNA ARTTYGA-3′) and gyrB-R(5′-AGCAGGGTACGGATGTGCGAGCCRTCNACRTCNGCRTCNGTCAT-3′). The PCR amplification of 16S rRNA was done in a mix containing PCR MIX 25 μL, positive and negative primers 1 μL each, and bacterial DNA 2 μL. The PCR reaction procedure was as follows: pre-denaturation at 95 °C for 5 min; 95 °C denaturation for 30 s, 62 °C annealing for 30 s, 72 °C extension for 45 s, 30 cycles; the final elongation was at 72 °C for 7 min. The products were sequenced by Sangon Biotech Co. Ltd. (Shanghai, China). After BLAST comparison on NCBI and EzTaxone server (http://eztaxon-e.ezbiocloud.net/, (Kim et al., 2012)), strains with high similarity in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and IPSN (https://lpsn.dsmz.de/species/bacillus) were selected, and the neighbor-joining method in MEGA 7 software was used to construct a phylogenetic tree. The minimum evolution (ME) tree was constructed using close-neighbor-interchange heuristic search method and complete deletion option (Sun et al., 2018). The phylogenetic trees were confirmed by bootstrap analysis based on 1000 replications.

In vitro assessment of plant growth promotion (PGP) traits

Qualitative assay

Freshly grown bacterial isolates were streaked on Jensen's medium (HiMedia) (Suárez-Moreno et al., 2019, Mohammed et al., 2020), and plates were incubated at 28 °C for 2 days. Growth on Jensen's medium shows the ability of isolates to fix nitrogen. The strain K-9 were measured for the production of siderophore using the Chrome Azurol S (CAS) agar medium described by Schwyn and Neilands (1987, Reang et al., 2022). The strain K-9 culture was streaked on the CAS medium and incubated at 28 °C for 48 h. Siderophore production was confirmed by the formation of an orange-to-yellow halo around the colonies. Phosphate and potassium solubilization using the agar medium described by Rushabh et al. (2020). ACC deaminase production and cellulolytic activity conducted by Suarez's method was used for qualitative detection (Suárez-Moreno et al., 2019).

Quantification assay

IAA was quantitatively determined by Suárez-Moreno et al. (2019) method. The strain to be tested was inoculated in LB liquid medium containing sterile tryptophan (0.5 g/L), cultured at 180 r/min at 28 °C for 5 days, centrifuged at 6000 r/min for 10 min, and 1 mL supernatant was added to 2 mL Salkowski's colorimeter, and the reaction was performed at 40 °C for 30 min in the dark. The absorbance value was measured at 530 nm, and 1 mL of sterile tryptophan high medium without strain was added to 2 mL of Salkowski's colorimetric solution to adjust to zero. Calculating IAA concentration was established using pure IAA and expressed as microgram per milliliter. The protease activity was determined by folinal method (Ruth et al., 2016; Borah et al., 2019). Calculating casein concentration was established using pure casein and expressed as microgram per milliliter.

Effect of strain K-9 on germination of potato tubers

Potato seeds were treated for 3 min with a 2% (v/v) sodium hypochlorite solution for surface sterilization; 75% alcohol was disinfected for 1 min, washed three times sterile water, and air-dried. The strain K-9 inoculums were prepared in the nutrient broth medium for 24 h at 34 °C and diluted to a final concentration of 10^8 cells per milliliter with sterile distilled water. The seeds (30 g) were sown into sterilized vermiculite, then soaked each potato tuber in 10 mL of fermentation broth, and covered with 7-cm-thick vermiculite. Cultured in a greenhouse at 20 °C. Potted seed traits were investigated on day 25. Medium-treated seeds served as controls.

Effects of strain K-9 on growth promotion of potato in field

The total rainfall from April to September was 643 mm, and the average temperature was 15.76 °C. The soil type was chernozem soil, with uniform fertility and level ground. The properties of the soil in the surface layer of 10–30 cm are shown in Table 1.

In this experiment, we used the potato variety 'Eugene'. The experiment was sown on May 3, 2021, with precision spot seeding. The spacing of each hole was 25 cm, and the row spacing was 80 cm. The seedling preservation rate in the field was 98%. The treatments adopted the

Table 1 Physicochemical properties of the soil (10–30 cm soil layer)

Soil properties	Alkali-hydrolyzable nitrogen ($mg \cdot kg^{-1}$)	Available phosphate ($mg \cdot kg^{-1}$)	Available potassium ($mg \cdot kg^{-1}$)	pH	Organic matter ($g \cdot kg^{-1}$)
Value	155.25 ± 0.86	28.62 ± 0.52	388.50 ± 4.37	6.12 ± 0.14	31.52 ± 0.55

method of hole application, with antagonistic strain fermentation broth (10^8 cfu/mL) applied at 50 mL/hole. The NA medium was applied at 50 mL/hole as the control. The experiment was set up as a completely randomized block design, consisting of three replicates, with a total of 6 plots (20 m^2 each). Plant samples were taken at the formation stage for biomass determination. Each part of potato was killed in an oven at 105 °C for 30 min, and then, the oven was adjusted to 80 °C to dry to constant weight. The dry matter weight of potato stem, leaf, tuber, and root was measured. A central part (12 m^2) of each potato plot was selected to investigate potato yield. The formula used in this section is as follows (Li et al., 2021): yield increase effect = (treatment yield−control yield)/control yield × 100%.

Tubers were collected, weighed, and graded by size according to the standards of local growers (Matteau et al., 2022). The three size-based classes included the following: small tubers (between 0 and 75 g), medium tubers (between 75 and 150 g), and large tubers (over 150 g).

Results and discussion

Morphological, physiological, biochemical, and chemotaxonomic characteristics

Morphological, cultural, physiological, and biochemical characteristics of strain K-9 are given in the species description in Tables 2 and 3. In this study, the major fatty acids of strain K-9 contained C16:0, C18:0, C22:4, C22:5N6, C22:5N3, and C22:6N3 (Table 3). Cells are gram-stain-positive, rod-shaped, pH3.0–10.0 (optimum, 7.0) and with 0–10% (w/v) NaCl. Nitrate is reduced. Indole, ammonia production, and hydrogen sulfide test is positive, citrate and phenylalanine amino acid deaminase are negative. Sorbitol, saccharose, glucose, xylose, lactose, cellobiose, raffinose, rhamnose, fructose, galactose, mannitol, maltose are utilized as carbon and energy sources.

Phylogenetic and genomic analysis

The length of 16S rRNA of strain K-9 was 1220 bp. The 16S rRNA gene sequence of strain K-9 was related to *Bacillus velezensis*, *Bacillus amyloliquefaciens* and *Bacillus subtilis* and other strains, sharing more than 97% of the genetic sequences. Then, strain K-9 was compared with type strains; it was found to be on the same branch as *Bacillus velezensis* NRRL B41580 (Fig. 2). It was difficult to distinguish the types of strain K-9 (Fig. 3). The length of gyrB gene sequence of strain K-9 was 1189 bp. Blast comparison found that the gyrB sequence of strain K-9 was similar to that of *Bacillus amyloliquefaciens* and *Bacillus velezensis* strain (Fig. 3). In order to identify the scientific and reliable results, physiological and biochemical characteristics of the similar strains were distinguished, and DNA hybridization was identified.

Table 4 shows the main features that distinguish *Bacillus velezensis* and *Bacillus amyloliquefaciens* from other phenotypically and phylogenetically related taxa. The strains are characterized by their capacity to produce

Table 2 Physiological and biochemical characteristics of strain K-9^T

Characteristic	1	2
Gram staining	+	-
Phenylalanine amino acid deaminase	-	-
Contact enzyme	+	+
Starch hydrolysis	+	ND
Ammonia production test	+	ND
Gelatin liquefaction	+	-
Hydrogen sulfide test	+	-
MR	-	+
V-P	+	-
10% NaCl	+	ND
pH 10	+	ND
Nitrate	+	+
Indole test	+	-
Citrate	-	-
Sorbitol, saccharose, glucose, xylose, lactose	+	+
Cellobiose, raffinose, rhamnose	+	-
Fructose, galactose, mannitol, maltose	+	+
Inositol	-	-
Arabinose, sodium alginate, sodium gluconate, glycine	-	+

Strains: 1, K-9; 2, *E. coli*. Data are from this study, unless otherwise indicated.
+positive, −negative

ND not determined

Table 3 Cellular fatty acid (μg/g) compositions of strain K-9^T

Fatty acid	K-9	Fatty acid	K-9
C6:0	1.22522	C17:1	4.82156
C8:0	0.85903	C18:0	274.17641
C10:0	—	C18:1N12T	16.45
C11:0	—	C18:1N7	16.9
C12:0	0.65133	C18:2N6T	17.59
C13:0	—	C19:1N12T	1.59901
C14:0	4.56188	C20:0	2.61903
C14:1	—	C20:5N3	27.7
C14:1T	2.43339	C21:0	—
C15:0	3.574	C22:0	0.24846
C15:1T	7.81137	C22:4	29.23
C15:1	3.19354	C22:5N6	29.74
C16:0	346.94394	C22:5N3	30.43
C16:1T	1.13127	C22:6N3	30.93
C16:1	4.04883	C23:0	—
C17:0	1.30701	C24:0	—
C17:1T	3.34883	C24:1	1.90637

C16:0, strain K-9 much higher than the other two strains. The length of the whole genome also varies.

DNA-DNA hybridization was conducted following the methods of Ruiz-Garcia et al. (2005). Strain K-9 was not related to the *Bacillus subtilis* and *Bacillus amyloliquefaciens*, showing less than 70% hybridization with them (Table 5). Strain K-9 was related to the *Bacillus velezensis* NRRL B41580, showing more than 70% hybridization with it. The K-9 strain was identified as *Bacillus velezensis*.

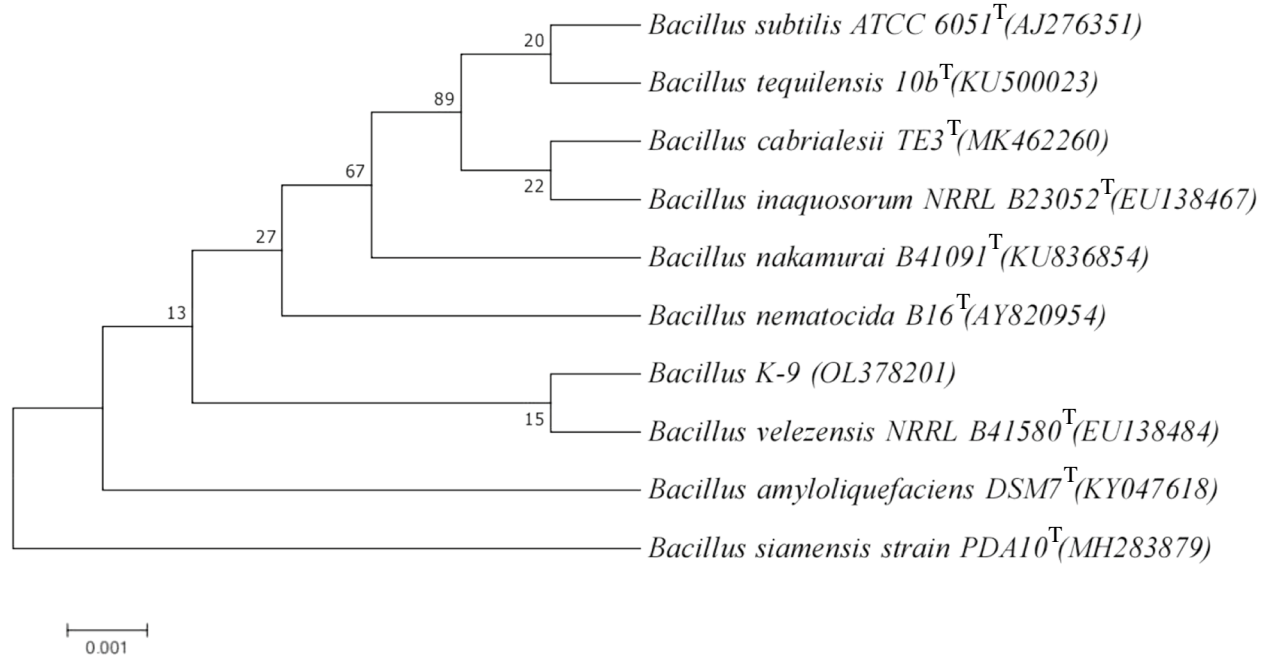

Fig. 2 Phylogenetic tree of strain K-9 constructed based on type strain 16S rRNA gene sequence. Bootstrap values (%) presented at the branches were calculated from 1000 replications. The scale bar means 0.1% sequence difference

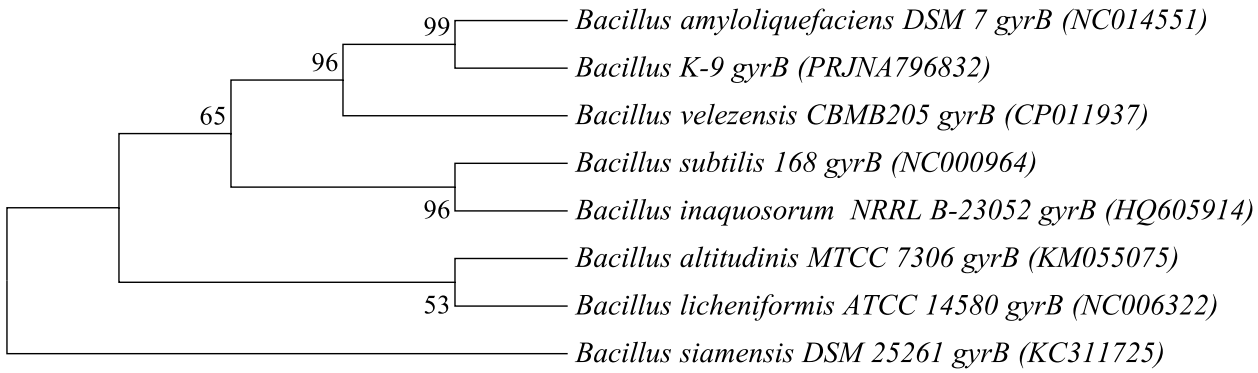

Fig. 3 Phylogenetic tree of strain K-9 constructed based on gyrB gene sequence. Bootstrap values (%) presented at the branches were calculated from 1000 replications. The scale bar means 5% sequence difference

In vitro assessment of plant growth promotion (PGP) traits

In this experiment, in vitro method was assessed to screen the potential of isolated microorganisms to promote plant growth (Table 6, Fig. 4). The assessment of growth-promoting abilities is important. It is considered an effective tool in the investigation of microbes (Rushabh et al., 2020). In this study, strain K-9 had nitrogen-fixing activities, positive activity for IAA, and siderophore production. Strain K-9 was positive able to solubilize organoposphorus. The PGPR containing ACC deaminase can hinder the abiotic stress of induced ethylene production and its associated adverse effect on plants (Reang et al., 2022). In this study, strain K-9 was capable to produce ACC deaminase enzyme. Protease and amylase are indicators of biocontrol characteristics, and strain K-9 can produce these two enzymes, indicating that this strain has certain biocontrol characteristics.

Strain K-9 on potted potato germination in greenhouse

After seed tubers were treated with strain K-9, potato growth was examined. Potato tubers had 100% sprouting. The length of seed bud and fresh bud weight with K-9 treatment was not significantly higher than control (Fig. 5). Root number, fresh and dry weights of potato root, and dry bud weight were significantly higher than control.

Strain K-9 on growth promotion of potato in field

The more dry matter a crop accumulates, the better it grows. Figure 6 shows the plant height, stem diameter, and biomass accumulation of potato in the tuber formation stage. The stem diameter of strain K-9 treatment was higher than the control, but it was not significant. The plant height, biomass fresh weight, and biomass dry weight of strain K-9 treatment were significantly higher than those of

Table 4 Characteristics which distinguish strain K-9^T from other related species of *Bacillus*

Characteristic	*Bacillus* sp. K-9	*Bacillus velezensis* NRRL B41580[b]	*Bacillus amyloliquefaciens* DSM7[b]
Pigmentation	Creamy white	Creamy white	Creamy white
D-Raffinose	+	+	+
Arabinose	—	ND	+
Citrate	—	ND	+
Cellulase	—	ND	+
Indole	+	—	ND
C14:0[a]	0.64	2.96	—
C16:0[a]	48.60	13.41	4.25
Genome size (bp)	3891530	4034335	3980199
Number of protein-coding genes	3915	ND	3754
tRNA	79	80	94
G + C content (%)	46.45	46.1–46.4	46.08

[a] main differences in cellular fatty-acid composition (%) between strain K-9 and type strains of other related species of *Bacillus*. [b] Data from Ruiz-Garcia et al. (2005), Rückert et al. (2011), Zhang (2012), and NCBI

ND not determined

Table 5 Strain K-9^T of DNA hybridization rate with similar type strains

DDH (%)	*Bacillus subtilis* ATCC 6051	*Bacillus velezensis* NRRL B41580	*Bacillus amyloliquefaciens* DSM7
K-9^T	27.63	86.90	69.23

Table 6 Traits related with direct plant growth promotion

Quantitative assay		Qualitative production					
Protease activity (μg/ml)	IAA (μg/ml)	Nitrogen fixation	Organoposphorus	Iron production	Protease	Amylase	ACC deaminase
89.16 ± 5.76	9.09 ± 0.43	+	+	+	+	+	+

the control. The results indicated that strain K-9 had better growth promotion ability.

The yield of strain K-9 treatment was 12.44% higher than control, but had no significant effect on potato yield. This is because the yield difference between the strain K-9 treatment and the control treatment was small. The weight of large tubers treated with strain K-9 was 7.03 kg and significantly higher than the control (Table 7). The weight of medium tubers in strain K-9 treatment was higher than that in control, but the difference between treatments was not significant. The weight of small tubers with the strain K-9 was significantly lower than that of the control. The results showed that the strain K-9 treatment could increase the yield, weight of large tubers and medium tubers, and significantly reduce the weight of small tubers, thus resulting in an economic benefit of potato planting under the same conditions.

Discussion

Compared with chemical fertilizer promoting growth, plant rhizosphere growth-promoting bacteria has attracted more and more attention due to its advantages of environmental protection and high safety to human beings. Studies have confirmed that *Bacillus* (Ben et al., 2018) can play a better role in promoting plant growth. *Bacillus* strains promote plant growth directly or indirectly (Yánez-Mendizábal and Falconí 2018, Ben et al., 2018, Gardener 2008), and this phenomenon has also been confirmed in the indoor pot experiment and field experiment of this study. In the present study, strain K-9 exhibited multiple PGPR properties. PGPR inoculation significantly increased the accumulation of matter in buds and roots of potato under greenhouse condition. To further verify whether the strain can promote plant growth, the yield experiment of strain K-9 was carried

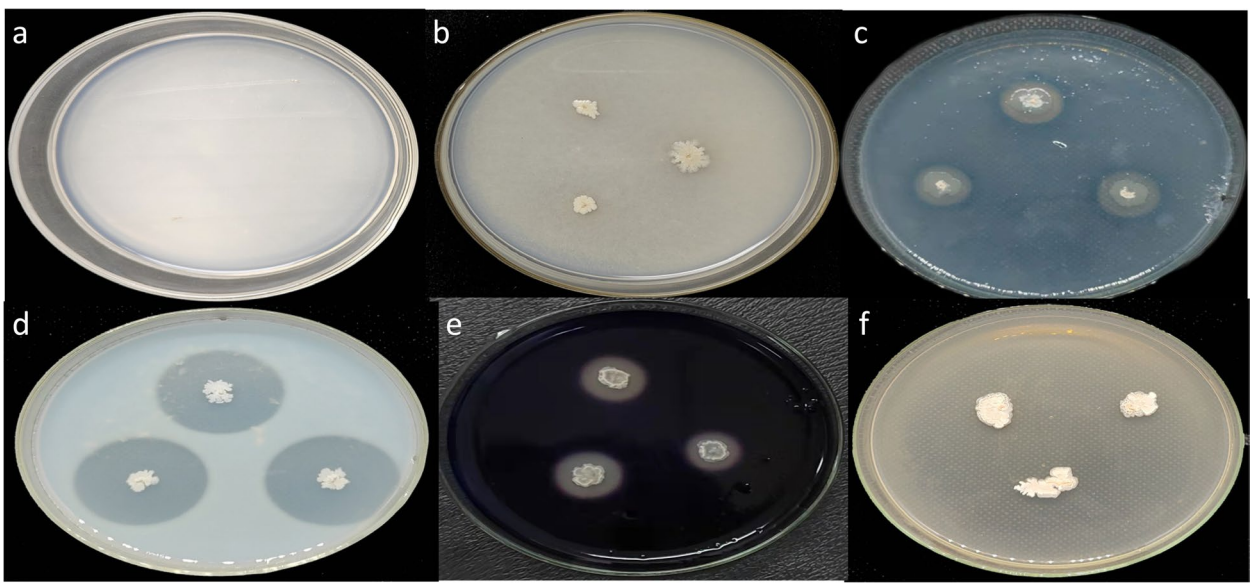

Fig. 4 Evaluation of strain K-9 for key traits related to direct plant growth promotion. **a** Nitrogen fixation. **b** organophosphorus qualitative assay. **c** Iron qualitative assay. **d** Protease qualitative assay. **e** Amylase qualitative activity assay. **f** ACC deaminase activity assay

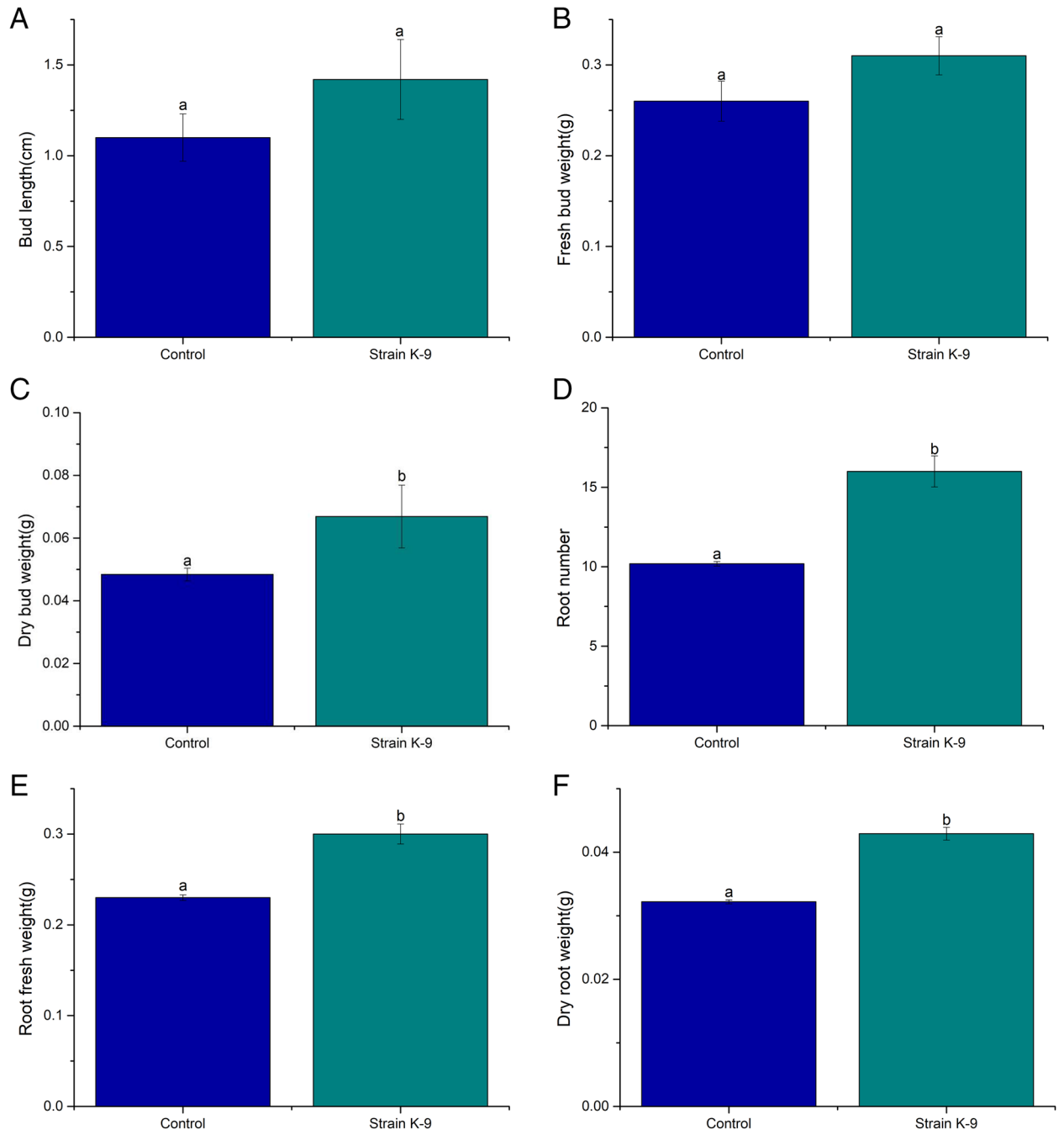

Fig. 5 Effect of strain K-9 on potted potato germination in greenhouse. Each treatment had 3 replications. Different letters indicate significant differences according to the Duncan's multiple range test ($P < 0.05$). **A** Bud length. **B** Fresh bud weigh. **C** Dry bud weight. **D** Root number. **E** Root fresh weight. **F** Dry root weight

out under field conditions. The strain may be exploited as microbial inoculants for potato crop as they enhanced plant growth via diverse mechanisms and offered an attractive strategy to replace synthetic fertilizers.

Conclusion

Strain K-9 was analyzed using 16s rRNA sequences, gyrB gene sequence. They have close evolutionary relationship with *Bacillus velezensis* and *Bacillus*

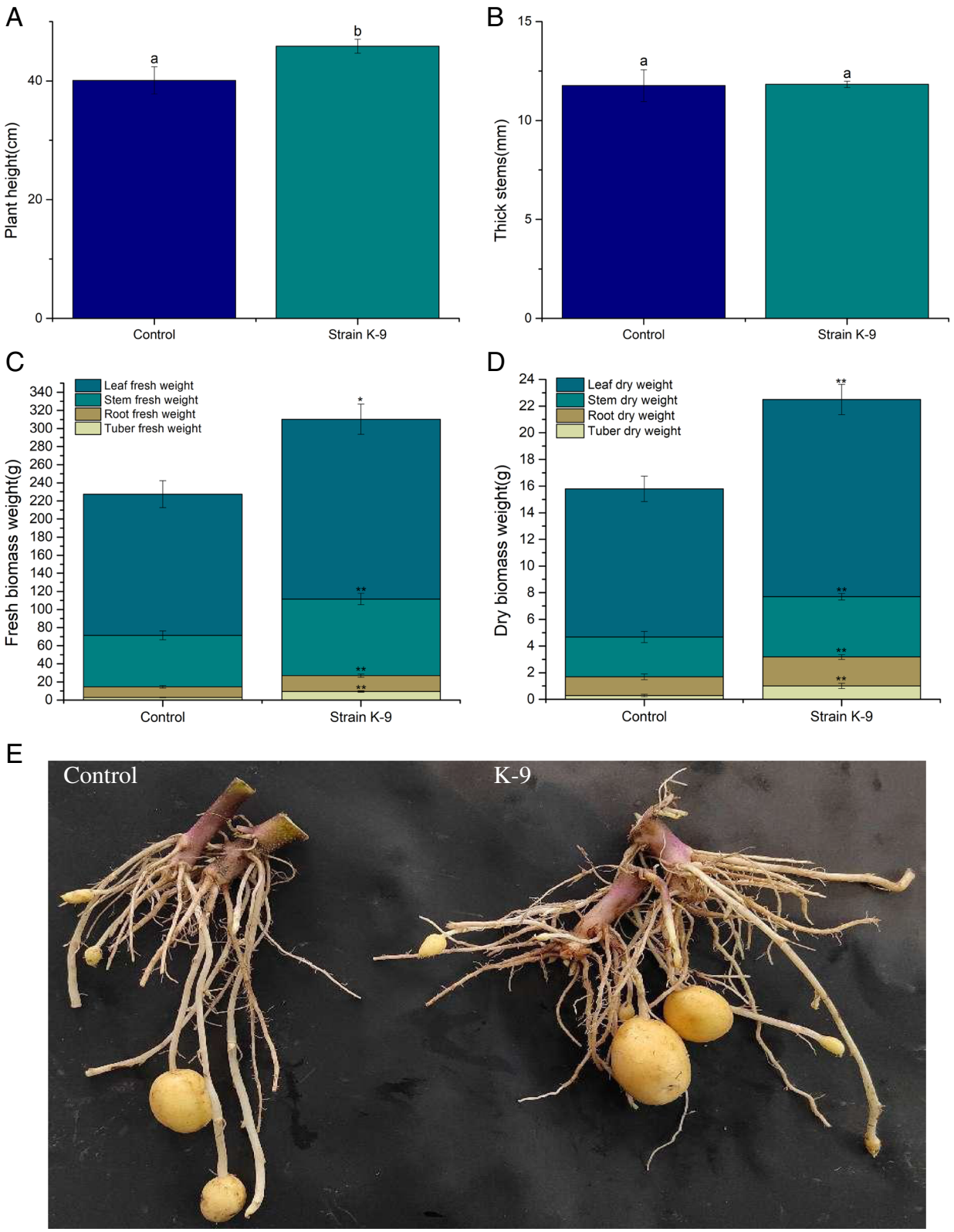

Fig. 6 Effect of strain K-9 on growth promotion of potato tuber during formation. Each treatment had 3 replications. Symbol indicates significant differences according to the Duncan's multiple range test ($P < 0.05$, $P < 0.01$). **A** Plant height. **B** Thick stems. **C** Fresh biomass weight. **D** Dry biomass weight. **E** Root growth phenotype of potato

Table 7 Application in planting hole of strain K-9 on potato yield, large potato, and commercial potato percentage

Treatment	Yield per plot (kg/12 m^2)	Increase yield (%)	Small tubers (kg/2.4 m^2)	Medium tubers (kg/2.4 m^2)	Large tubers (kg/2.4 m^2)
Control (NA medium 166 L/667 m^2)	38.89 ± 3.16a	_	0.31 ± 0.04b	1.09 ± 0.07a	5.15 ± 0.51a
Strain K-9 [16.5 L (109 CFU/mL)/667 m^2]	43.73 ± 2.33a	12.44 ± 4.00	0.21 ± 0.05a	1.11 ± 0.14a	7.03 ± 0.23b

Data are mean ± standard errors. Different letters indicate significant differences according to the Duncan's multiple range test ($P < 0.05$). Increase in yield = [(yield of treatment area — yield of control area)/yield of control area] × 100%; large tubers > 150 g, medium tubers ≥ 75 g, small tubers < 75 g

amyloliquefaciens. DNA hybridization eventually identified it as *Bacillus velezensis*. Strain K-9 can produce IAA, fix nitrogen, and degrade organophosphorus in laboratory tests of growth-promoting properties. The growth-promoting ability of the strain K-9 was verified in the field, and we found that it can increase potato yield by 12.44%.

Acknowledgements
The authors thank teacher Wang Teng, College of Agronomy, Heilongjiang Bayi Agricultural University, for helping with the collection of potato tuber samples.

Authors' contributions
MS and WT wrote the main manuscript text and JS repaired language change. MS and WT were responsible for the design and implementation of the experiment. All authors reviewed the manuscript. The authors read and approved the final manuscript.

Funding
This research was supported by special task of "Agricultural Science and Technology Innovation Spanning Project" of Heilongjiang Academy of Agricultural Sciences — study and integration of soil and water conservation technology for slope farmland in black soil area of northern Songnen Plain (HNK2019CX13).

Availability of data and materials
The GenBank accession number of the 16S rRNA gene sequence of strain K-9 is OL378201. The GenBank accession number of the gyrB sequence of strain K-9 is PRJNA796832. The GenBank accession number of complete genome sequence of strain K-9 is JAKQYO000000000.

Declarations

Ethics approval and consent to participate
This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication
All of the authors have read and approved the manuscript. This work has not been published previously, nor is it being considered by any other peer-reviewed journal.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Keshan Branch, Heilongjiang Academy of Agricultural Sciences, Keshan, China. [2]College of Agriculture, Heilongjiang Bayi Agricultural University, Daqing, China.

Received: 13 May 2022 Accepted: 27 September 2022
Published online: 20 October 2022

References

Abedinzadeh M, Etesami H, Alikhani H A.(2019) Characterization of rhizosphere and endophytic bacteria from roots of maize (Zea mays L.) plant irrigated with wastewater with biotechnological potential in agriculture. Biotechnol Rep 21:e00305. https://doi.org/10.1016/j.btre.2019.e00305

Amaresan N, Jayakumar V, Kumar K, Thajuddin N (2012) Isolation and characterization of plant growth promoting endophytic bacteria and their effect on tomato (Lycopersicon esculentum) and chilli (Capsicum annuum) seedling growth. Ann Microbiol 62(2):805–810. https://doi.org/10.1007/s13213-011-0321-7

Ben F, Cong W, Song XL, Ding XL, Wu LM (2018) Bacillus velezensis FZB42 in 2018: the gram-positive model strain for plant growth promotion and biocontrol. Front Microbiol 9:2491. https://doi.org/10.3389/fmicb.2018.02491

Borah A, Das R, Mazumdar R, Thakur D (2019) Culturable endophytic bacteria of Camellia species endowed with plant growth promoting characteristics. J Appl Microbiol 3. https://doi.org/10.1111/jam.14356

Buchanan RE, Gibbons NE (1984) Bergey's Bacteria Identification Manual, 8th edn. Science Press, Beijing. https://doi.org/10.1007/bergeysoutline200405

Cui LX, Yang CD, Wei LJ, Li TH, Chen X (2019) Isolation and identification of an endophytic bacteria Bacillus velezensis 8-4 exhibiting biocontrol activity against potato scab. Biol Control 141:1–20. https://doi.org/10.1016/j.biocontrol.2019.104156

Cui X, Wang Y, Liu J, Chang M, Zhao Y, Zhou S, Zhuang L (2015) Bacillus dabaoshanensis sp. nov., a Cr(VI)-tolerant bacterium isolated from heavy-metal-contaminated soil. Arch Microbiol 197:513–520. https://doi.org/10.1007/s00203-015-1082-7

Dong XZ, Cai MY (2001) Manual of identification of common bacterial systems. Science Press, Beijing http://www.uzzf.com/soft/185540.html

Gardener MS (2008) Biocontrol of plant pathogens and plant growth promotion by Bacillus. Recent Dev Manag Plant Dis 1:71–79. https://doi.org/10.1007/978-1-4020-8804-9_6

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxone: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721. https://doi.org/10.1099/ijs.0.038075-0

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature. 286:885–886

La DMT, Satomi M, Agata N, Venkateswaran K (2004) gyrB as a phylogenetic discriminator for members of the Bacillus anthracis-Cereus-thuringiensis group. J Microbiol Methods 56:383–394. https://doi.org/10.1016/j.mimet.2003.11.004

Lane DJ (1991) 16S/23S rRNA sequencing. Nucl Acid Tech Bacterial Syst:115–175. https://doi.org/10.4135/9781446279281.n7

Li C, Wencong S, Wu D, Renmao T, Wang B, Lin R, Gao Z (2021) Biocontrol of potato common scab by Brevibacillus laterosporus BL12 is related to the

reduction of pathogen and changes in soil bacterial community. Biol Control 153:104496–104508. https://doi.org/10.1016/j.biocontrol.2020.104496
Liu GH, Narsing Rao MP, Dong ZY, Wang JP, Chen Z, Liu B, Li WJ (2019) Two novel alkaliphiles, Bacillus alkalisoli sp. nov., and Bacillus solitudinis sp. nov., isolated from saline-alkali soil. Extremophiles. 23:759–764. https://doi.org/10.1007/s00792-019-01127-2
Ma S, Yanjie W, Wang T et al (2022) Isolation and identification of an endophytic bacteria *Bacillus* sp. K-9 exhibiting biocontrol activity against potato common scab. Arch Microbiol 204(8):483–493. https://doi.org/10.1007/s00203-022-02989-5
Matteau J-P, Paul C, Guillaume L, Thiago G et al (2022) Effects of irrigation thresholds and temporal distribution on potato yield and water productivity in sandy soil. Agric Water Manag 264:107483. https://doi.org/10.1016/j.agwat.2022.107483
Menes RJ, Machin EV, Iriarte A, Langleib M (2019) Bacillus natronophilus sp. nov., an alkaliphilic bacterium isolated from a soda lake. Int J Syst Evol Microbiol 70:562–568. https://doi.org/10.1099/ijsem.0.003792
Mohammed MA, Tadege CM, Assefa TF (2020) Phenotypic, stress tolerance, and plant growth promoting characteristics of rhizobial isolates of grass pea. Int Microbiol 23:1–12. https://doi.org/10.1007/s10123-020-00131-3
Qiao J, Yu X, Liang X, Liu Y, Borriss R, Liu Y (2017) Additionof plant-growth-promoting Bacillus subtilis PTS-394 on tomato rhizosphere has no durable impact on composition of root microbiome. BMC Microbiol 17:131. https://doi.org/10.1186/s12866-017-1039-x
Reang L, Shraddha B, Singh TR, Kavita J, Shital P, Vyas UM, Kumar KJ (2022) Plant growth promoting characteristics of halophilic and halotolerant bacteria isolated from coastal regions of Saurashtra Gujarat. Sci Rep 12(1):4699. https://doi.org/10.1038/S41598-022-08151-X
Roohi A, Ahmed I, Paek J, Sin Y, Abbas S, Jamil M, Chang YH (2014) Bacillus pakistanensis sp. nov., a halotolerant bacterium isolated from salt mines of the Karak area in Pakistan. Antonie Van Leeuwenhoek 105:1163–1172. https://doi.org/10.1007/s10482-014-0177-5
Rückert C, Blom J, Chen XH et al (2011) Genome sequence of B. amyloliquefaciens type strain DSM7 T reveals differences to plant-associated B. amyloliquefaciens FZB42. J Biotechnol (1). https://doi.org/10.1016/j.jbiotec.2011.01.006
Ruiz-Garcia C, Bejar V, Martinez-Checa F, Llamas I, Quesada E (2005) Bacillus velezensis sp. nov., a surfactant-producing bacterium isolated from the river Velez in Malaga, southern Spain. Int J Syst Evol Microbiol 55:191–195. https://doi.org/10.1099/ijs.0.63310-0
Rushabh S, Amaresan N, Patel P et al (2020) Isolation and characterization of Bacillus spp. endowed with multifarious plant growth-promoting traits and their potential effect on tomato (Lycopersicon esculentum) seedlings. Arab J Sci Eng 45(2):4579–4587. https://doi.org/10.1007/s13369-020-04543-1
Ruth M, Rohmattusolihat R, Nuryati N, Rahmani N, Yopi Y (2016) The selection and identification of potential endophyte bacteria as protease enzyme producer from Halimun Mount National Park. Biopropal Industri 7(2):73–82 https://schlr.cnki.net/zn/Detail/index/GARJ2016/SJDJ8EFA8CAAD7053413C7ADBA2B95DB5401
Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56. https://doi.org/10.1016/0003-2697(87)90612-9
Suárez-Moreno ZR, Marcela V-VD, Isabel V-MD, Nubia M-S (2019) Plant-growth promotion and biocontrol properties of three Streptomyces spp. isolates to control bacterial rice pathogens. Front Microbiol 10:290–307. https://doi.org/10.3389/fmicb.2019.00290
Sun QL, Yu C, Luan ZD, Lian C, Hu YH, Sun L (2018) Description of Bacillus kexueae sp. nov. and Bacillus manusensis sp. nov. isolated from hydrothermal sediments. Int J Syst Evol Microbiol 68:829–834. https://doi.org/10.1099/ijsem.0.002594
Sun YY, HaiZhen Z, QingLei S (2021) Bacillus fonticola sp. nov., isolated from deep sea cold seep sediment. Arch Microbiol 203:4127–4132. https://doi.org/10.1007/s00203-021-02401-8
Weihui X, Wang K, Wang H et al (2020) Evaluation of the biocontrol potential of *Bacillus sp.* WB against Fusarium oxysporum f. sp. niveum. Biol Control 147(C):104288. https://doi.org/10.1016/j.biocontrol.2020.104288
Xu X, Yu L, Xu G, Wang Q, Wei S, Tang X (2020) Bacillus yapensissp. nov., a novel piezotolerant bacterium isolated from deep-sea sediment of the Yap Trench. Pacifc Ocean Antonie Van Leeuwenhoek 113:389–396. https://doi.org/10.1007/s10482-019-01348-7
Yánez-Mendizábal V, Falconí CE (2018) Efficacy of Bacillus spp. to biocontrol of anthracnose and enhance plant growth on Andean lupin seeds by lipopeptide production. Biol Control 122:67–75. https://doi.org/10.1016/j.biocontrol.2018.04.004
Zahar HF, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated interactions belowground. Soil Biol Biochem 77:69–80. https://doi.org/10.1016/j.soilbio.2014.06.017
Zahra A, Amini J, Ashengroph M, Bahramnejad B, Mozafari AA (2021) Biocontrol of strawberry anthracnose disease caused by Colletotrichum nymphaeae using Bacillus atrophaeus strain DM6120 with multiple mechanisms. Trop Plant Pathol. https://doi.org/10.1007/S40858-021-00477-7
Zhang R S. (2012). Genetic diversity and biocontrol agent development of rice bacterial stripe (ph. D. dissertation, Nanjing Agricultural University). https://kns.cnki.net/KCMS/detail/detail.aspx?dbname=CDFD1214&filename=1013282637.nh
Zhang X, Zhou YY, Li Y, Fu XC, Wang Q (2017) Screening and characterization of endophytic Bacillus for biocontrol of grapevine downy mildew. Crop Prot 96:173–179. https://doi.org/10.1016/j.cropro.2017.02.018

Publisher's Note