Isolation and Identification of an Endophytic Bacteria Bacillus sp. K-9 Exhibiting Biocontrol Activity Against Potato Common Scab

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Abstract

Potato scab is an important soil-borne disease that can significantly reduce the quality and economic value of potatoes. The purpose of this study was to isolate, screen and identify endophytic bacteria that have antagonistic and control effects on potato scab disease, and to determine the control effect and yield traits of the selected strains on potato scab disease in field conditions. A bacterial strain K-9 was isolated from the junction between scab spot and healthy epidermis of potato tuber. The K-9 strain was identified as a new species of *Bacillus* sp. through morphological, physiological and biochemical characterization, and 16S rDNA and gyrB gene sequence analysis. The diameter of the inhibition zone of strain K-9 against *Streptomyces scabies* on the YME plate was 3.82 cm. The K-9 strain could inhibit eight types of crop pathogens, with the highest inhibition rate (70.39%) against another soil-borne potato disease potato black scurf. In the field test, the control effect of K-9 strain against potato scab was not significantly different from that of mixed bacteria or chemical agents, but the disease index and the scab index in the K-9 treatment were significantly lower than in the control. The potato yield in the K-9 treatment was 12.44% higher than the control. In summary, the K-9 strain can prevent not only potato scab, but also increase potato yield. Therefore, the endophytic bacterial K-9 strain may be a potential biological control agent.

Introduction

Potato (*Solanum tuberosum L.*) is rich in carbohydrates and a variety of minerals and nutrients needed by humans, with high nutritional value and strong adaptability to the environment, and can be widely cultivated in diverse climate conditions (Majeed et al. 2017). Early maturing varieties of potato have a short growth period and can be harvested 60 days after emergence, which can quickly alleviate food shortages caused by diseases and other disasters. Potato plays an important role in economy and global food security (Mario et al. 2019).

The potato industry is developing rapidly because of high nutritional value, strong adaptability and short growth period of potatoes. However, with the rapid development of potato industry, the incidence and severity of scab have been increasing year by year due to the continuous planting of potatoes, which negatively influenced the development of potato industry. Potato scab, as a disease spread by soil and seed potato, is widespread in the potato-growing areas and has become an urgent problem in potato growing industry (Lankau et al. 2020). Potato scab is widely distributed in the world, and has been found in Argentina and other South American countries, the United States, Germany, Mexico, Finland, Japan, and South Korea, as well as in Yunnan, Heilongjiang and Shandong provinces of China (Joshi et al. 2010; Cheng et al. 2019; Leiminger et al. 2013; Ciaghi et al. 2018; Hiltunen et al. 2021; Chater 2016; Sousa et al. 2016). More than 20 species of scab pathogens have been reported, the most common of which are *Streptomyces scabies* (*S. scabies*), *S. acidiscabies* and *S. turgidiscabies. S. scabies* is the main pathogen responsible for potato scab. It can invade plants through skin cuts, wounds or young tubers, and can infect also other root crops such as radish, beet, carrot, and parsnip (Flores et al. 2010; Santos-Cervantes et al. 2017).

In recent years, potato scab has become one of the main diseases of potato crops in the world. The control of potato scab depends mainly on the large-scale application of chemical pesticides. Although chemical

agents have certain control effects, environmental pollution and human health problems are becoming increasingly serious. Therefore, researchers began to study biopesticides to control crop diseases. More and more studies have found that endophytic bacteria have potential for biological control of crop diseases (Fu et al. 2018). Endophytic bacteria can resist pathogenic microorganisms or promote plant growth and colonize plants over a long time (Abdallah et al. 2016). The use of endophytic bacteria for the prevention and control of potato scab, potato scab prevention and control effect is stable and lasting. The use of endophytic bacteria to reduce or replace the use of pesticides and fertilizers is of great significance for maintaining the biodiversity of plant microecosystems, maintaining ecological balance and realizing sustainable development.

Materials And Methods

Pathogens and plant samples

S. scabies used in this study was isolated from potato common scab disease by our laboratory and purified and cultured in yeast malt agar medium (YME) (yeast extract 4 g, malt extract 10 g, glucose 4 g, sterile water 1000 mL, pH 7.2). The cultures were store at -80℃. Potato black scurf (*Rhizoctonia solani*), potato early blight (*Alternaria solani*), maize leaf spot (*Bipolaris zeicola*, *Curvularia lunata, Alternaria*), maize root rot (*Fusarium fujikuroi*), wheat take-all (*Gaeumanomyces graminis*), and mung bean anthracnose (*Colletotrichum gloeosporioides*) were isolated and preserved by plant pathology laboratory of Heilongjiang Bayi Agricultural University. Potato tubers were sampled from Keshan, Longzhen and Jianshan farms in Heilongjiang Province, China.

Isolation and screening of antagonistic strains

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). Sterile water (100 µL) of the last rinse was used as control to test whether the surface was thoroughly disinfected (Sun et al. 2003). The isolates were purified and cultured at 28℃ and stored in at -80℃.

Antagonistic strains were screened by the dual culture method (Cui et al. 2019). *Streptomyces scabies* was cultured on YME plates for 7 d and then inoculated in YME liquid medium at 30°C, 180 r/min for 72 h to prepare the *S. scabies* suspension. The antagonistic bacterial isolates were then inoculated on the YME plates (85 mm) containing 200 µL of *S. scabies* suspension. The plates containing only *Streptomyces scabies* were used as the control. The inhibition rate of bacterial strains was determined. The antibacterial activity of antagonistic bacteria was evaluated by the diameter of inhibition zone. The strains with the highest rate of inhibitory of *S. scabies* were selected for further study.

Identification of antagonistic strains

The strain antagonistic to *S. scabies* with the highest bacteriostatic rate were preliminarily identified by morphological characteristics (Jin et al. 2019; Zhang et al. 2017). The antagonistic strains were inoculated on NA medium and cultured at 28℃ for 3 days. The antagonistic strains 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 et al. 1984; Dong et al. 2001).

The antagonistic bacterium was further identified through the analysis of its 16S rDNA and gyrB gene sequences (Lane, 1991; Yu et al. 2010; La et al. 2004). DNA of antagonistic strains was extracted by a Tiangen Bacterial genome kit. The 16S rDNA primers were 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The gyrB amplification was performed with the gyrB-F primer (5 '-GAAGT CATCATGACCGTTCTGCAYGCNGGNGGNAARTTYGA-3') and gyrB-R(5'-AG CAGGGTACGGATGTGCGAGCCRTCNACRTCNGCRTCNGTCAT-3'). The PCR amplification of 16S rDNA 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: pre-denaturation at 95 ℃ for 5 min; 95 ℃ denaturation for 30 s, 62 ℃ annealing for 30 s, 72 ℃ extension for 45 s, 30 cycles; the final elongation was at 72 ℃ for 7 min. The products were sequenced by Sangon Biotech Co. Ltd (Shanghai, China). After BLAST comparison on NCBI, strains with high similarity in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were selected and the neighbor- joining method in MEGA 7 software was used to construct a phylogenetic tree. The nucleotide sequences were deposited in GenBank database, and the accession numbers were obtained.

Inhibition spectrum of *Bacillus* sp .strain K-9

For testing the response of fungal pathogens to *Bacillus* sp. strain K-9, a 6-mm fungal plug of *Rhizoctonia solani*, *Alternaria solani*, *Bipolaris zeicola*, *Curvularia lunata*, *Fusarium fujikuroi, Gaeumanomyces graminis*, and *Colletotrichum gloeosporioides* were transferred to the center of a potato dextrose agar (PDA) plate. Antagonistic strains were inoculated at the top, bottom, left, and right of the bacterial colonies cultured for 24 h, followed by additional culturing at 28 ℃ for 5 days. Petri dishes inoculated with only pathogenic bacteria were used as control, and each treatment was repeated 3 times. The cross-crossing method was used to calculate the bacteriostatic rate.

Effects of K-9 in controlling common scab of potato in field

The plots infected with potato scab (incidence rate = 100%) were used. The total rainfall from April to September was 643 mm, and the average temperature was 15.76 ℃. 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.

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

Soil properties	Alkali-hydrolyzale nitrogen（mg·kg^{-1})	Available phosphate （mg·kg^{-1})	Available potassium （mg·kg^{-1})	pH	Organic matter （g·kg^{-1})
Value	155.25±0.86	28.62±0.52	388.5±4.37	6.12±0.14	31.52±0.55

In this experiment, we used the potato variety 'Eugene' (highly susceptible to disease). Potato scab disease was controlled by antagonistic strain, mixture of bacteria and vernamycin (chemical pesticide). 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 method of hole application, with antagonistic strain fermentation broth (10^8 cfu/mL) applied at 50 mL/hole. The biological agents and chemical pesticides were applied according to the concentration given by producers: eprobiotics (249 g/667 m^2, water consumption 166 L) at 50 mL/hole, vernamycin (830 mL/667 m^2, water consumption 166 L) 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 four treatments and three replicates, with a total of 12 plots (20 m^2 each). A central part (12 m^2) of each potato plot was selected to investigate potato yield and disease incidence, disease index, and the control effect at harvest. The severity of disease (grades 1-6) was judged based on the percentage of tuber area with lesions.

Table 2 Grading criteria for potato common scab

Disease stage	Representative value	Grading criteria
1	0	No spots on the potato
2	1	Scab spots account for 0-5% of the potato
3	2	Scab spots account for 5%-12.5% of the potato
4	3	Scab spots account for 12.5%-25% of the potato
5	4	Scab spots account for 25%-50% of the potato
6	5	Scab spots account for 50% of the potato

The formula used in this section is as follows(Li Cuiping.2021):Yield (kg/hm^2) = measured yield/measured yield area ×10000 m^2; Yield increase effect = (treatment yield - control yield)/control yield ×100%; Disease index = {[$\sum$(number of diseased potatoes at each level × representative value at each level)]/ (number of total potato investigated × highest representative value)} ×100; Control efficiency = [(disease index of control area - disease index of treatment area)/ disease index of control area] ×100%; Scab index =[mean grade of lesion area (MA)× mean grade of lesion depth (MD)]/20×100%.

Statistical analysis

Statistical analysis was performed using SPSS software(IBM SPSS Statistics, Version 22.0, International Business Machines Corporation, New York, in USA). Mean values were compared using Duncan's new

multiple range test at the 1% (P< 0.01) level of significance between treatments.

Results

Isolation and screening of antagonistic bacteria

According to their morphological characteristics, 73 strains of endophytic bacteria were isolated from potato tubers, purified and stored at -80 ℃. The antagonistic activity to *S. scabies* was screened by the double culture method. Twelve endophytic bacteria showed antagonistic activity to *S.scabies*. The diameter of inhibition zone ranged from 1.97 to 3.82 cm (Table 3). The maximum antibacterial diameter of strain K-9 was 3.82 cm, and the antibacterial rate of strain K-9 was 44.90%. When screened in fermentation broth, the strain K-9 had the antibacterial rate of 53.92% (Fig. 1).

Table 3 Bacteriostasis zones of potato isolated bacteria against *Streptomyces scabies*

Strains	The diameters of inhibition zones（cm）
CK	0
1(L)	2.90±0.08cC
2(L)	1.97±0.02aA
3(L)	2.89±0.14cC
4(J)	3.37±0.12cC
5(J)	2.93±0.08deD
6(K)	3.41±0.14bB
7(J)	2.55±0.04bB
8(L)	2.60±0.07dD
9(K)	3.82±0.17fE
10(J)	3.60±0.04eDE
11(K)	2.83±0.12cBC
12(K)	3.40±0.04deD

Data are mean ± standard error. Different letters mean significantly different according to Duncan's multiple range test (P < 0.05 and P < 0.01). CK:*Streptomyces scabies*，*L* Heilongjiang Longzhen, *J* Heilongjiang Jianshan, *K* Heilongjiang Keshan, 9(K):K-9 strain.

Morphological characterization of antagonistic strain

The colony of strain K-9 was round or oval, white, opaque, with uneven surface, regular edge, and no pigment production (Fig. 2-a,b). Under a light microscope, K-9 bacteria were rod-shaped and Gram-positive (Fig. 2-c).

Physiological and biochemical characterization of antagonistic strain

The results of physiological and biochemical identification showed that strain K-9 was Gram-positive and produced hydrogen sulfide gas during metabolism. Strain K-9 decomposed starch and gelatin. Citric acid and phenylalanine deaminase reactions were negative. Nitrate reduction, indole, V-P, and MR reactions were positive. The strain could grow normally on LB medium containing 1-10% w/v NaCl (Table 4). According to the physiological and biochemical tests, and combined with morphological characteristics, according to the handbook of Systematic Identification of Common Bacteria and Berger Handbook of Identification of Bacteria, the strain was preliminarily identified as *Bacteriaceae*, *Firmicutes*, *Bacida*, *Bacillus*, *Bacillus*, *Bacillus* genus.

Table 4 Physiological and biochemical characteristics of strain K-9

Test index	Result	Test index	Result
Gram staining	+	MR	-
pH10	+	5%Nacl	+
V-P	+	7%Nacl	+
Contact Enzyme	+	10%Nacl	+
Starch hydrolysis	+	Phenylalanine amino acid deaminase	-
Nitrate	+	Ammonia production test	+
Indole test	+	Gelatin Liquefaction	+
Citrate	-	Hydrogen sulfide test	+

Note: +: Positive; −: Negative

Genomic identification of antagonistic strain

The length of 16S rDNA of strain K-9 was 1447 bp. The GenBank accession numbers of the 16S rDNA gene sequence and the gyrB sequence of strain K-9 are OL378201 and PRJNA796832, respectively. The 16S rDNA 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. The results of phylogenetic tree showed that K-9 and *Bacillus* sp. SA3 strain branched closely, with 63% similarity rate, but *Bacillus* sp. SA3 strain was only identified at the genus level.

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 K-9 was 99% similar to that of *Bacillus velezensis* strain FJAT-52631. The K-9 was closely related also to *Bacillus velezensis* strain FJAT-52631, but did not cluster together, with similarity rate of 70% (Fig.4).

Inhibition spectrum of antagonistic strain

Strain K-9 had significant inhibitory effect on eight crop pathogens (Fig. 5, Table 5). The strongest inhibitory effect was against *Rhizoctonia solani* (70.39%) and *Colletotrichum gloeosporioides* (70%), and these two inhibition rates were significantly higher than against other pathogens (P<0.01). The inhibition rate of strain K-9 against the pathogenic bacteria (*Bipolaris zeicola*, *Curvularia lunata* and *Alternaria*) in maize leaves was more than 58%. The inhibition rate of strain K-9 against *Gaeumanomyces graminis* was 46.41%. In summary, strain K-9 had a broad spectrum of antimicrobial activity, indicating a great potential in biocontrol.

Table 5 Inhibition rate of strain K-9 against crop pathogens

Pathogens	Disease	Treatment colony diameter (mm)	Inhibition rate(%)
Rhizoctonia solani	Potato black scurf	25.17±0.96aA	70.39±1.13cC
Alternaria solani	Potato early blight	30.17±1.12bB	50.55±3.96aA
Bipolaris zeicola	Maize leaf spot	34.58±1.71cC	59.31±2.01bB
Curvularia lunata	Maize leaf spot	26.33±1.25aA	60.35±1.83bB
Alternaria	Maize leaf spot	26.00±1.27aA	58.40±1.91bB
F.fujikuroi	Maize root rot	24.08±0.24aA	49.83±1.58aA
Gaeumanomyces graminis	Wheat take-all	25.50±0.41aA	46.41±2.55aA
Colletotrichum gloeosporioides	Mung bean anthracnose	24.17±0.59aA	70.04±0.66cC

Data are mean ± standard error. Different letters mean significantly different according to Duncan's multiple range test (P < 0.05 and P < 0.01).

Inhibition rate (%) = [(diameter of the pathogen in control − diameter of the pathogen in treatment) / diameter of the pathogen in control] × 100

Antagonistic efficacy of strain K-9 against potato scab in the field

The complex microbial inoculant, rapamycin and strain K-9 treatments had no significant effect on potato yield, with the percentage of large potatoes 54.05-60.81%, and the percentage of commodity tubers 82.69-88.56% (Table 6). The yield of rapamycin treatment was lower than that of the control, but the percentage of large tubers and commercial tubers was higher than that of the control. The yield of complex microbial inoculant and strain K-9 treatment was 7.03% and 12.44% higher than control, respectively. The percentage of large tubers was 1.4% higher in the treatment with complex microbial inoculant compared with the control, whereas the strain K-9 was associated with a 8.2% increase in the yield compared with the control. The rate of commercial tubers was lower in the treatment with complex microbial inoculant than the control, but in the treatment with strain K-9 it was 5.4% higher than the control. The results showed that the strain K-9 treatment could increase potato yield and the percentages of large and commercial-size tubers, thus resulting in an economic benefit of potato planting under the same conditions.

Table 6 Application in planting hole of strains K-9 on potato yield, large potato and commercial potato percentage

Treatment	yield per plot （kg/12m^2）	Equivalent yield （kg/hm^2）	Increase yield（%）	Large potato percentage （%）	Commercial potato percentage （%）
Control (NA medium166L/667m^2)	38.89±3.16a	32422.65	_	52.61±0.08a	83.15±1.23a
Mixed bacteria （249g/667m^2）	41.83±1.29a	34873.65	7.03	54.05±0.04a	82.69±4.84a
Rapamycin (830ml/667m^2)	37.43±2.50a	31205.4	-3.98	56.97±0.13a	87.59±5.18a
Strain K-9 [16.5L(10^{9}CFU/mL)/667m^2]	43.73±2.33a	36457.65	12.44	60.81±0.14a	88.56±3.22a

Data are means ± standard errors. Different letters indicate significant differences according to the Duncan's multiple range test ($P < 0.05$).

Yield (kg/ha) = measured yield/measured yield area ×10000; Increase in yield =[(yield of treatment area - yield of control area)/yield of control area]×100%

The field efficacy assessments showed that the treatments with mixed bacteria, rapamycin and strain K-9 had a control effect on potato scab. The disease and scab indices of all three treatments were significantly lower than those in the control (Fig. 6, Table 7). The potato treated with rapamycin had the lowest disease incidence and the control effect was 35.27%, which was not significantly different from the other two treatments, but the scab index was higher in the rapamycin compared with the K-9 treatment (i.e., the spots were deeper in the rapamycin than the K-9 treatment). The disease and scab indices in the complex microbial inoculant treatment were higher than in the K-9 treatment (i.e., the area and depth of potato tuber disease spots were larger in the mixed bacteria treatment than in the K-9 treatment). Taken together, the results on yield, disease index, control effect, and scab index indicated the strain K-9 can be used as a biopesticide to control potato scab.

Table 7 Application in planting hole of strains K-9 field efficacy evaluation of antagonistic against potato scab

Treatment	Disease index（%）	Control efficiency（%）	Scab index(MSI)
Control (NA medium166L/667m^2)	68.98±0.007aA	_	25.26±3.52aA
Mixed bacteria （249g/667m^2）	47.36±0.019bB	31.34±1.09aA	19.03±2.22bAB
Rapamycin (830ml/667m^2)	44.65±0.017bB	35.27±0.87aA	16.72±2.06bB
Strain K-9 [16.5L(10^{9}CFU/mL)/667m^2]	46.20±0.009bB	33.01±0.96aA	14.95±1.07bB

Data are mean ± standard error. Different letters mean significantly different according to Duncan's multiple range test ($P < 0.05$ and $P < 0.01$).

Disease index = {[∑(number of diseased potatoes at each level × representative value at each level)]/(number of total potato investigated × highest representative value)} ×100; Control efficiency = [(disease index of control area - disease index of treatment area)/ disease index of control area] ×100%; Scab index = [mean grade of lesion area (MA)× mean grade of lesion depth (MD)]/20×100%.

Discussion

After pathogen scab infects potato tubers, forming flat, raised or sunken brown corkification spots on the tuber surface; they affect the commercial value and storage resistance, resulting in a 20%-40% reduction in price and considerable economic losses. Potato scab has restricted the sustainable development of potato industry (especially the seed potato industry). Under the premise of ensuring environmental safety and balanced development of agro-ecosystems, the best way to control potato scab is by changing soil microenvironment and enriching beneficial bacteria on the tuber surface. With that in mind, we applied beneficial bacteria to potato soil holes in order to increase the abundance of beneficial bacteria, reduce the accumulation of pathogenic bacteria and reduce the incidence of scab.

In this study, 73 endophytic strains of potato were isolated from the junction of diseased spot and healthy epidermis of potato tuber, and 12 strains had antagonistic effect on potato common scab. Among them, strain K-9 had the largest inhibition zone against potato common scab pathogen, with the inhibition zone diameter of 3.82 cm and the inhibition rate of 44.90%. The results showed that endophytic bacteria antagonistic to pathogen could be isolated from the junction of diseased spot and healthy epidermis of potato tuber. This is consistent with some previous similar studies. Iranian researchers isolated endophytic bacteria (*Bacillus atrophaeus* DM6120 strain) from strawberry roots to inhibit anthrax, with an inhibition rate

of 54.92% (Zahra et al. 2021). Researchers in India isolated 14 strains of bacterial endophytes from rice. Among them S3 strain (*Bacillus polymyxis*) showed good antibacterial activity against *Fusarium oxysporum*, *Pythium aphanidermatum*, *Phytophthora infestans*, and other pathogens (Radhakrishnan et al. 2021).

In this study, the 16S rDNA and the gyrB gene of strain K-9 were amplified. The specific taxonomic status of strain K-9 was not determined completely by phylogenetic analysis due to the complexity of the genome, and strain K-9 was defined as a new species of *Bacillus*. The entire genome sequence is currently being analyzed to shed more light on the classification of strain K-9. The double culture experiment showed that strain K-9 had antimicrobial effects not only on *Streptomyces scabies*, but also on other pathogens, such as *Rhizoctonia solani*, *Alternaria solani, Bipolaris zeicola, Curvularia lunata, Fusarium fujikuroi, Gaeumanomyces graminis*, and *Colletotrichum gloeosporioides*. Hence, the strain K-9 has a potential application value in biological control. This study demonstrated the control effect of this antagonistic bacterium on potato scab in the field as well. In this experiment, only the double culture experiment with the eight crop pathogens listed above was carried out in the laboratory, without field control evaluation. Hence, further work on the control effects against these eight crop pathogens in the field is warranted.

- Field control effects of biocontrol agents are affected by environmental conditions, application methods and colonization (Cui et al. 2019). In order to further understand the biocontrol effect of strain K-9 on potato scab, we applied it to the field plots with a long history of scab occurrence. The morbidity in the untreated plots was 100%. In this study, the control effect of strain K-9 on variety ‘Eugene’ (highly susceptible to potato scab) was 33.01%. The control effect of the complex microbial inoculant and rapamycin treatments was 31.34% and 35.27%, respectively. There were no significant differences among the three treatments. In the literature, there are many reports on the biological control of potato scab. For example, the control effect of *Streptomyces* PBSH9 strain in the 1-year field experiment in China using the moderately susceptible potato variety ‘Atlantic ocean’ reached 47.64% and the yield increased by 9.7% (Zhang et al. 2020). The control effect of *Bacillus* sp. *sunhua* strain on potato scab in a pot experiment was 53.33% (Han et al. 2005). In the study presented here, the infection rate in all treatments in the field was 100%, with the plants seriously diseased. Under such environmental conditions, the disease and scab indices of the strain K-9 treatment were significantly lower than those of the control treatment. The yield in the treatment with strain K-9 was 12.44% higher than that of the control, and the percentage of large tubers and commercial tubers was also higher than in the control, indicating that strain K-9 had a good growth-promoting effect. It was confirmed that the screened endophytic bacterium K-9 could be used as a biological agent to control potato scab disease. This study is the first report of a new species of *Bacillus* controlling potato scab disease.

Conclusion

The strain K-9 isolated from the junction of diseased and healthy epidermis of potato has a good control effect against potato scab and also increases yield. The strain K-9 had inhibitory effects on potato common scab pathogen and eight crop pathogenic fungi. In this study, the control effect was shown only in one field experiment with continuous potato cultivation, and there was only one highly susceptible variety of potato tested. Therefore, multi-location and multi-variety trials with strain K-9 should be used to evaluate the control

effect in field. Further study on the inhibition of potato scab by the bacterial strain K-9 and the underlying mechanism is needed before the biological control capacity of the strain can be ascertained.

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conficts of interest.

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Figures

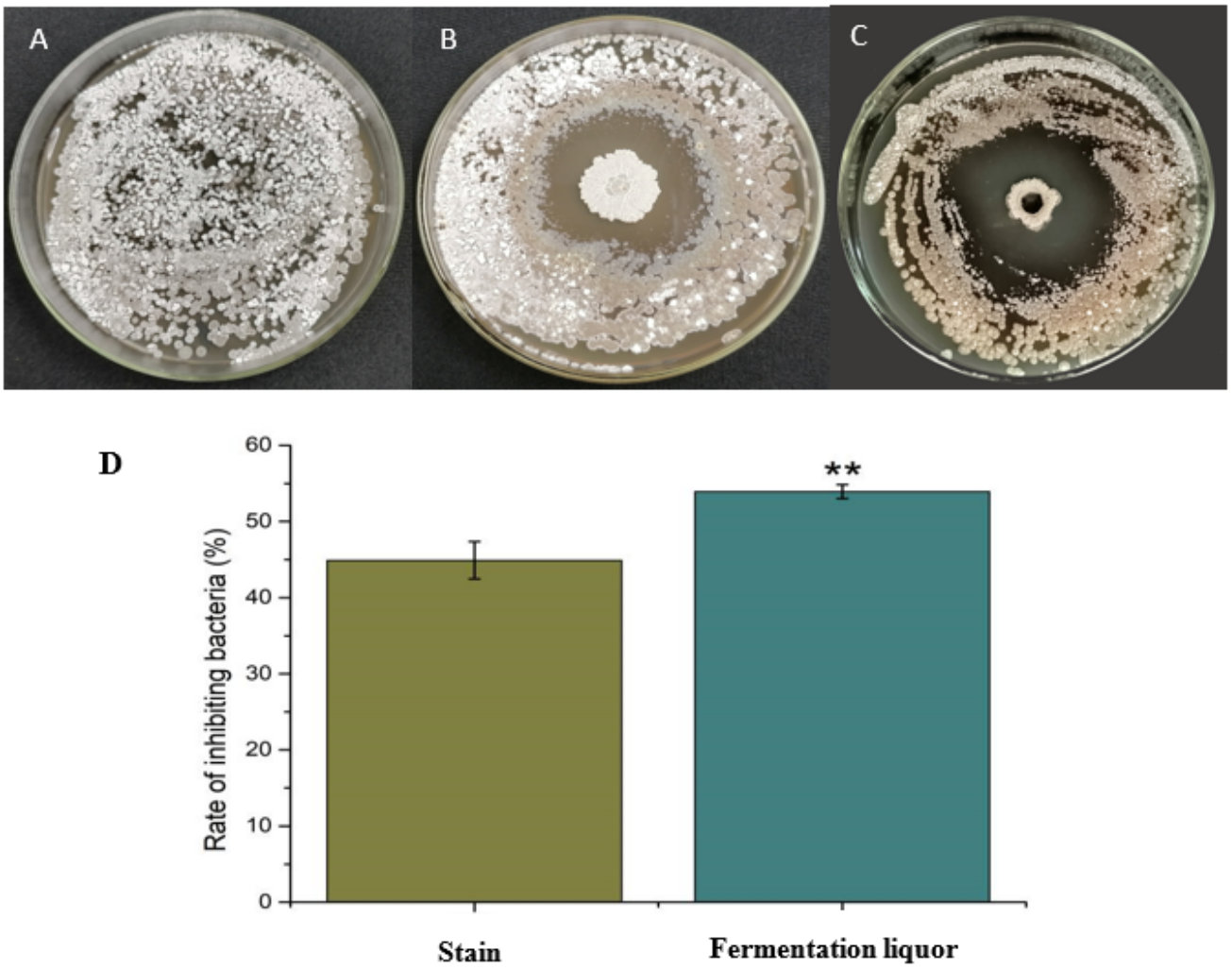

Figure 1

Antimicrobial activity of strain K-9 and fermentation broth against Streptomyces scabies

A, B, C, D: Control of S.scabies, Dual-culture of strain K-9 against S.scabies, Strain K-9 fermentation liquid against S. scabies, Bacteriostatic ratio

**: Different letters mean significantly different according to Duncan's multiple range test ($P < 0.01$).

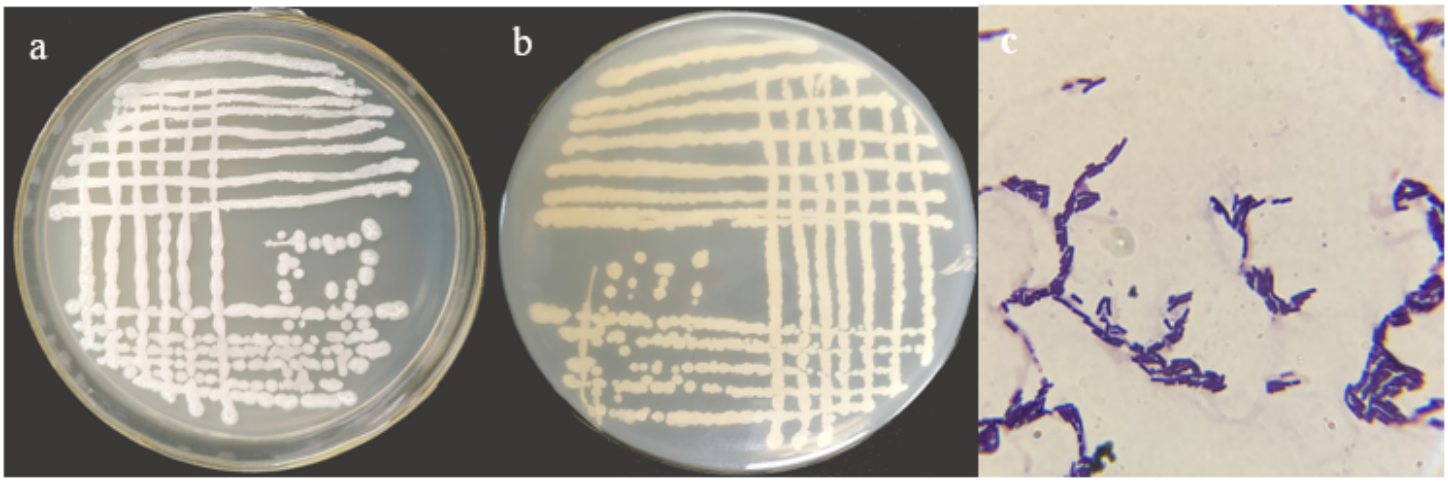

Figure 2

Colony morphology and gram staining of strain K-9

a,b: Colony morphology of strain K-9 on NA plate c: Gram staining of strain K-9(100× magnifica- tion)

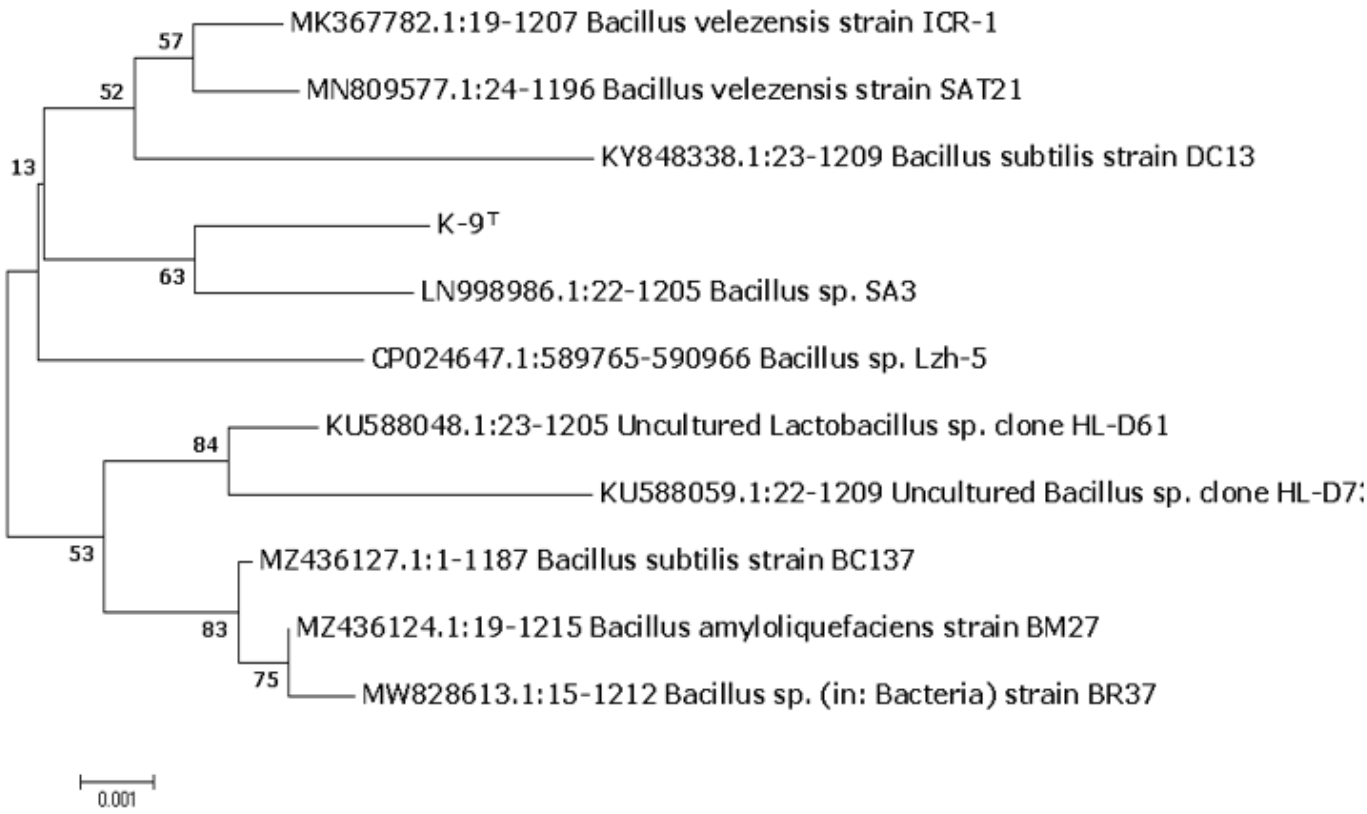

Figure 3

Phylogenetic tree of strain K-9 constructed based on 16S rDNA gene sequence

Bootstrap values (%) presented at the branches were calculated from 1000 replications. The scale bar means 0.1% sequence difference.

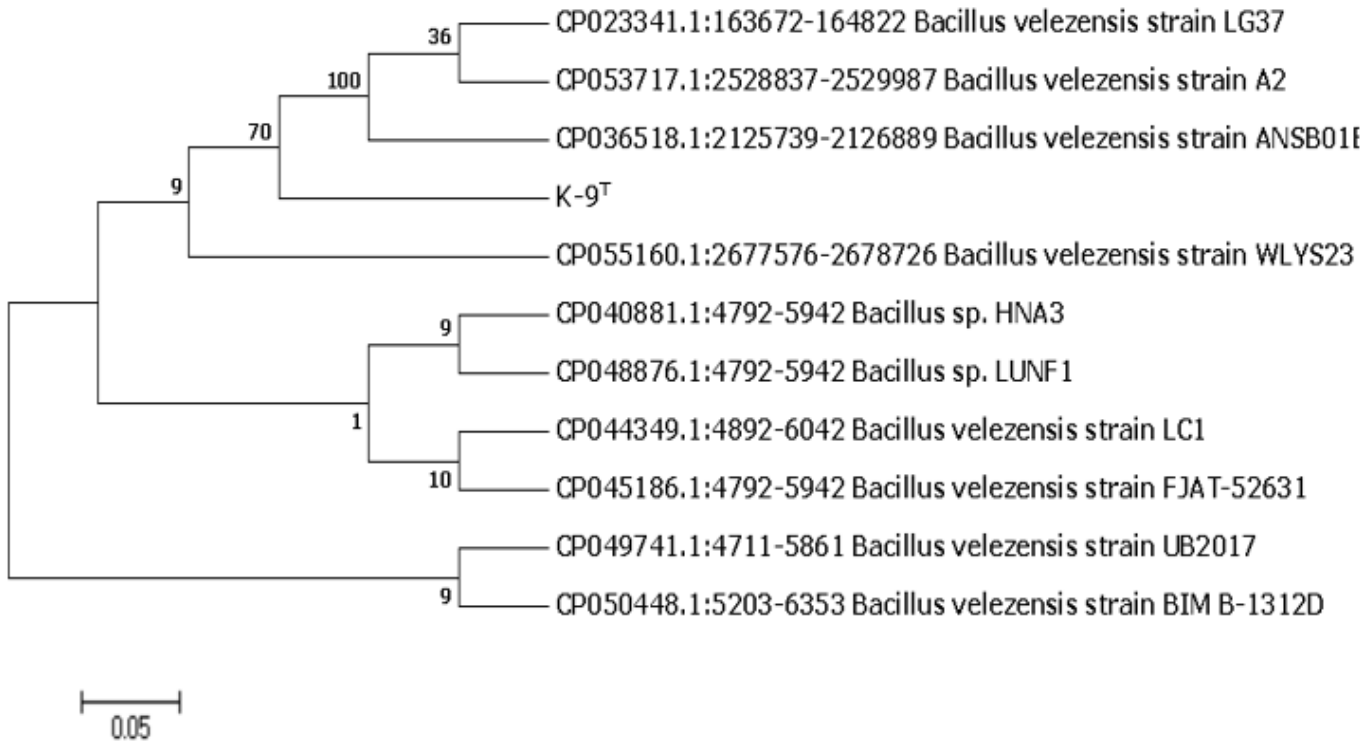

Figure 4

Phylogenetic tree of strain K-9constructed based on gyrB gene sequence

Bootstrap values (%) presented at the branches were calculated from 1000 replications. The scale bar means 5% sequence difference.

Figure 5

Determination of antibacterial spectrum of strain K-9

A:*Rhizoctonia solani* B:Alternaria solani C:*Bipolaris zeicola* D:*Curvularia lunata* E: *Alternaria* F:*F.fujikuroi* G: *Gaeumanomyces graminis* H:*Colletotrichum gloeosporioides*

-1: Control of pathogenic fungus -2:Dual-culture of strain K-9 against pathogenic fungus

Figure 6

Determination of field control effect of strain K-9

A:Control B:Mixed bacteria C:Rapamycin D: Strain K-9