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Research article

An investigation of *Panax ginseng* Meyer growth promotion and the biocontrol potential of antagonistic bacteria against ginseng black spotZhuo Sun¹, Limin Yang^{2,*}, Lianxue Zhang², Mei Han²¹ Cultivation Base of State Key Laboratory for Ecological Restoration and Ecosystem Management of JL Province and MOST, College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun, China² College of Chinese Medicinal Materials, Jilin Agriculture University, Changchun, China

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ABSTRACT

Background: Ginseng black spot disease resulting from *Alternaria panax* Whuetz is a common soil-borne disease, with an annual incidence rate higher than 20–30%. In this study, the bacterial strains with good antagonistic effect against *A. panax* are screened.

Methods: A total of 285 bacterial strains isolated from ginseng rhizosphere soils were screened using the Kirby–Bauer disk diffusion method and the Oxford cup plate assay. We analyzed the antifungal spectrum of SZ-22 by confronting incubation. To evaluate the efficacy of biocontrol against ginseng black spot and for growth promotion by SZ-22, we performed pot experiments in a plastic greenhouse. Taxonomic position of SZ-22 was identified using morphology, physiological, and biochemical characteristics, 16S ribosomal DNA, and *gyrB* sequences.

Results: SZ-22 (which was identified as *Brevundimonas terrae*) showed the strongest inhibition rate against *A. panax*, which showed 83.70% inhibition, and it also provided broad-spectrum antifungal effects. The inhibition efficacies of the SZ-22 bacterial suspension against ginseng black spot reached 82.47% inhibition, which is significantly higher than that of the 25% suspension concentrate azoxystrobin fungicide treatment ($p < 0.05$). Moreover, the SZ-22 bacterial suspension also caused ginseng plant growth promotion as well as root enhancement.

Conclusion: Although the results of the outdoor pot-culture method were influenced by the pathogen inoculum density, the cropping history of the field site, and the weather conditions, *B. terrae* SZ-22 controlled ginseng black spot and promoted ginseng growth successfully. This study provides resource for the biocontrol of ginseng black spot.

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1. Introduction

Panax ginseng Meyer (Araliaceae), is one of the most well-known Chinese herbal medicines, and was formerly a wild plant that is now grown in the northeastern region of China. Generally, *P. ginseng* is primarily cultivated artificially in China; in addition, it is also cultivated in Korea and Japan [1]. Ginseng black spot resulting from *Alternaria panax* infection has become a common soil-borne disease. This disease occurs in the Changbai Mountains of China and other ginseng producing areas, with an annual incidence rate excess of 20–30% [2]. This pathogenic fungus is present in the soil and formed mycelia on the seed surfaces of diseased plants. The overwintering spores invade the leaves through the

host stoma or epidermis. After its onset, the disease initially results in the loss of leaves, withering of plants, reduction in the production of seeds, and decreased root development. At high temperatures and under rainy conditions, the disease induces complete plant metabolic failure and significant damage [3]. Currently, the method of controlling this disease primarily depends on chemical protection and a cure involving azoxystrobin as well as polyxins to limit its spread [4]. However, the prolonged use of chemical pesticides results in disease resistance, soil pesticide residues, soil microecological imbalance, and other problems [5].

Biocontrol can reduce or inhibit the occurrence of plant diseases and have benefits of being environmentally friendly, safety, and high efficiency [6,7]. More than 14 microbial fungicides are

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currently registered as biocontrol agents in Korea [8]. *Paenibacillus polymyxa* GBR-1 was reported to be effective for control of root rot caused by *Cylindrocarpon destructans* (Zins.) Scholt [9]. Joy and Parke [10] reported the biocontrol potential of *Burkholderia cepacia* AMMD against *Alternaria* leaf blight. Thus, biocontrol of ginseng pathogens is an alternative means of reducing the incidence and severity of diseases with no or few negative impacts on the environment compared to chemical controls with fungicides. Screening of highly efficient antagonistic strains is a prerequisite for studies on plant disease protection [11]. Accordingly, it is important to identify microorganisms with the potential for biocontrol.

Previous studies have shown that the rhizosphere acts as an important bridge between plants and soil for the exchange of substances through plant roots and for establishing mutual relationships [12]. The bacteria in rhizospheric soil are responsible for many important ecological functions, such as pest control, and induce disease resistance [13,14]. Using rhizospheric soil bacteria for plant disease control and treatment has been good strategy toward the efficient biocontrol of plant diseases [15–17]. In the present study, we isolated strain SZ-22 from ginseng rhizosphere soil, which exhibited strong antifungal effects, and we examined its ability to prevent diseases and promote ginseng growth. In addition, we determined the taxonomic status of this strain based on morphological, physiological, and biochemical characteristics.

2. Materials and methods

2.1. Microorganisms

Ginseng rhizosphere soil was collected from the Ginseng Standardized Cultivation Base in Fusong county, Jilin Province, China (Y:42°32'N,127°08'E,537 m). A total of 285 bacteria isolates were isolated from the soil samples and stored at -80°C until further use. The bacteria were cultured using nutrient agar medium and beef extract–peptone–yeast extract fermentation liquor [18,19]. All the pathogens (*A. panax*, *Phytophthora capsici*, *Botrytis cinerea*, *Bipolaris maydis*, *Fusarium oxysporum*, *F. bulbigenum*, *F. graminearum*, *Dothiorella gregaria*, *Alternaria brassicae*, *Magnaporthe grisea*, *Rhizoctonia cerealis*, *B. sorokiniana*, *Sclerotinia sclerotiorum*, and *R. solani*) used in the present study were maintained on potato dextrose agar medium [18,19].

2.2. Bacterial suspension preparation

Bacterial suspension preparation was performed as previously described with several modifications [19]. Briefly, the bacteria were inoculated into sterilized beef extract–peptone–yeast extract fermentation liquor and shaken at 190 rev/min for 24 h at 32°C . The resulting fermentation liquor was subsequently subjected to centrifugation (5,000 rev/min, 4°C) for 30 min. The supernatant was discarded, and the remainder bacteria were washed three times with sterilized deionized water; then, the bacteria were adjusted to 10^8 CFU/mL for subsequent use. Microscopic counting was used to determine the content of the bacterial suspension using a blood count board (25 medium-sized lattice \times 16 pint-sized lattice) [20].

2.3. Screening for bacterial antagonistic activity

Preliminary and secondary screening was performed as previously described [19], and *A. panax* was used as the indicator. The Kirby–Bauer disk diffusion method [21] was used for preliminary screening. Colonies with the best fungistatic effect were selected for secondary screening. The Oxford cup plate assay [18] was used for secondary screening. Then the bacteriostasis rate of the

antagonistic strains was calculated. All the pathogens were used for secondary screening using confronting incubation [22].

2.4. Biocontrol and growth promotion by antifungal strains in the plastic greenhouse

Biocontrol and growth promotion were performed as previously described, with several modifications [19]. According to Hessemüller and Zeller [23], forest soil (from the location in Fusong, China) that infested the pathogenic fungus *A. panax* was used to evaluate the biocontrol potential of the antifungal strains. The soil matrix was a blending matrix with the ratio of V (infested forest soil)/V (vermiculite) = 2:1. Polypropylene pots [24 cm (diameter) \times 16 cm (height)] were filled with 2,500 g of soil matrix. The 2-yr-old ginseng seedlings with developed roots and similar growth were selected and cleaned, and their roots were disinfected (sodium hypochlorite was used to clean the surface, and the plants were dipped into the water at 50°C for 5 min). Prior to planting, the ginseng roots were dipped in bacterial suspensions (10^8 CFU/mL) for 25–30 min, and the fungicide control or nontreated control ginseng roots were dipped in tap water. A total of three ginseng seedlings were planted into per pot, respectively. Ten replicates of each treatment were performed in a completely randomized block design. All treatment combinations were repeated three times.

To evaluate the biocontrol potential of the antifungal strains, the root-cut inoculation was used in the pathogenicity assays [19], 150 mL of *A. panax* spore suspension (5×10^4 spores/mL) was poured into the soil, and 50 mL of antifungal bacterial suspension, 25% suspension concentrate azoxystrobin, and water were also simultaneously poured into each pot, respectively. Treatments with antifungal bacterial suspensions were conducted for five groups, as follows: 100% [volume/volume (v/v)] concentration (10^8 CFU/mL), 80% (v/v) concentration, 60% (v/v) concentration, 50% (v/v) concentration, and 40% (v/v) concentration. For the drug control, 0.25 mg/L of 25% suspension concentrate azoxystrobin was used, and for the nontreated control, water was used. The experiments were conducted under plastic greenhouse conditions (approximately 16 h of sunlight at 14 – 29°C on average, over a 10-wk period). Ten weeks after inoculation, the disease index and control effect were calculated [24]. The morbidity degree of ginseng black spot can be divided into nine levels according to Wang et al. [4], where 0 = no disease, 1 = disease spot area is less than 5% of the total leaves, 3 = disease spot area is 6–10% of the total leaves, 5 = disease spot area is 11–20% of the total leaves, 7 = disease spot area is 21–50% of the total leaves, 9 = disease spot area is more than 50% of the total area.

To evaluate the influence of the treatment on yield, nine ginseng seedlings were randomly selected after 10 wk; then, the ginseng plant height, whole plant fresh weight, whole plant dry weight, root length, root fresh weight, and root dry mass were measured and recorded.

2.5. Characterization of bacterial strains

Morphological identification, as well as physiological and biochemical characteristics determination [25] were performed in accordance with *Bergey's Manual of Determinative Bacteriology*.

The 16S ribosomal DNA (rDNA) sequence was amplified using polymerase chain reaction (PCR) [26,27]. The bacterial PCR amplification universal primers [28] 16 S1F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 16S1R: 5'-TACGGCTACCTGTACG-ACTT-3' were used. The PCR amplification reaction system and PCR amplification conditions were performed as previously described [19]. The PCR product was purified, sequenced, and submitted to GenBank.

GyrB gene amplification primer sequences and PCR amplification conditions were used according to Tayeb et al. [29]. The *gyrB* gene amplification primers used were *gyr1F*: 5'-GACAACGGCCGCGGSATTC-3' and *gyr1R*: 5'-CACGCCGTTGTTCAG-GAASG-3'. The *gyrB* gene amplification product was purified, sequenced, and submitted to Genbank. Clustal X [30] was used for multiple comparisons, and MEGA5.2 software (www.mega-software.net/home) was used to construct a phylogenetic tree using the neighbor-joining (NJ) method. Bootstrap analyses were performed with 1,000 repetitions.

2.6. Statistical analysis

The original data were processed using DPS version 9.50 (sinyosoft, China), with Duncan's multiple range test to analyze the difference.

3. Results

3.1. Effect of bacterial treatment on inhibition of *A. panax*

Among the 285 bacterial strains evaluated here, 25 strains showed adequate antifungal activity, and 13 strains showed inhibition rates of up to 40%. Three bacterial strains SZ-2, SZ-8, and SZ-22 were highly effective against *A. panax*, with inhibition rates higher than 70%. Secondary screening revealed that the antagonistic bacteria fermentation filtrate has a different degree of inhibition for *A. panax*, for which the inhibition zones of SZ-22 and SZ-2 were 14.30 mm and 10.06 mm (Table 1), respectively. This inhibition effect was significant and stable according to several experimental verifications. Thus, strains SZ-2 and SZ-22 were used for further study.

3.2. Bacteriostatic spectrum of strains SZ-2 and SZ-22

The bacterial strain SZ-2 had antifungal activity toward 10 fungi. Strain SZ-2 reduced the radial growth of *A. panax*, *F. graminearum*, *B. maydis*, *F. oxysporum*, *B. sorokiniana*, *B. cinema*, *F. bulbigenum*, *R. cerealis*, *M. grisea*, and *D. gregaria* from 29.77% to 78.02%, and was not inhibitory toward *R. solani*, *A. brassicae*, *S. sclerotiorum*, and *P. capsici*. Bacterial strain SZ-22 had antifungal activity toward 12 fungi and showed better broad-spectrum antifungal activity. This strain also exerts less than 50% inhibition of *R. solani*, *A. brassicae*,

Table 1
Antifungal activity of Selected bacterial Strains against *Alternaria panax*

Strain	Inhibition rate (%) ¹⁾	Inhibition zone (mm) ²⁾
SZ-2	72.73 ± 1.81 b	10.06 ± 0.37 b
SZ-8	71.33 ± 1.11 b	7.37 ± 0.21 c
SZ-14	46.17 ± 1.76 g	4.43 ± 0.15 f
SZ-17	50.20 ± 1.06 f	4.37 ± 0.12 f
SZ-20	66.93 ± 0.32 c	5.03 ± 0.32 e
SZ-22	83.70 ± 0.92 a	14.30 ± 0.36 a
SZ-23	57.65 ± 2.27 d	3.83 ± 0.06 g
SZ-39	43.03 ± 2.00 h	2.10 ± 0.10 h
SZ-51	41.17 ± 0.76 i	1.73 ± 0.15 i
SZ-56	65.40 ± 0.70 c	6.13 ± 0.21 d
SZ-72	40.33 ± 1.65 i	1.53 ± 0.06 i
SZ-76	53.33 ± 0.91 e	5.33 ± 0.06 e
SZ-81	65.70 ± 0.60 c	7.63 ± 0.15 c

Data in the table are presented as the means ± standard deviation. Different letters in the same column indicate the results of Duncan's new multiple range test ($p < 0.05$)

¹⁾ The mean values for the inhibition rate represent preliminary screening measured using the Kirby–Bauer disk diffusion method

²⁾ The mean values for the inhibition zone represent secondary screening measured using the Oxford cup plate assay

Table 2
Inhibition effect of SZ-2 and SZ-22 against fungal pathogens

Plant pathogen	Inhibition rate (%)	
	SZ-2	SZ-22
<i>Alternaria panax</i>	72.73 ± 1.81 b	83.70 ± 0.92 a
<i>Fusarium graminearum</i>	54.10 ± 1.55 d	74.30 ± 0.89 b
<i>Bipolaris maydis</i>	29.77 ± 2.44 h	66.47 ± 0.83 c
<i>Fusarium oxysporum</i>	78.37 ± 1.31 a	59.30 ± 1.08 d
<i>Bipolaris sorokiniana</i>	33.10 ± 0.46 g	53.97 ± 0.78 e
<i>Botrytis cinema</i>	66.60 ± 0.96 c	50.90 ± 0.60 f
<i>Rhizoctonia solani</i>	—	46.00 ± 0.66 g
<i>Alternaria brassicae</i>	—	43.43 ± 0.25 h
<i>Sclerotinia sclerotiorum</i>	—	43.33 ± 0.61 h
<i>Fusarium bulbigenum</i>	33.70 ± 1.05 g	38.23 ± 0.25 i
<i>Rhizoctonia cerealis</i>	49.73 ± 0.59 e	33.20 ± 0.20 j
<i>Phytophthora capsici</i>	—	30.10 ± 1.30 k
<i>Magnaporthe grisea</i>	41.47 ± 0.60 f	—
<i>Dothiorella gregaria</i>	53.80 ± 0.92 d	—

Different letters in the same column by Duncan's new multiple range test ($p < 0.05$). The experiment was repeated at least once with three replications/treatment producing similar results

and a total of six types of pathogenic fungi, with 83.70% inhibition for *A. panax*, and less than 50.90–74.30% inhibition for *F. graminearum*, *B. maydis*, and a total of five types of pathogenic fungi (Table 2).

3.3. Control efficiency of strains SZ-2 and SZ-22 against ginseng black spot

In soil that was artificially inoculated with *A. panax*, treating with antifungal strains SZ-2 and SZ-22 resulted in a reduced number of wilted and necrotized plants (Fig. 1). Ten weeks after treatment, at the highest concentration tested, i.e., 100% (v/v, 10⁸ CFU/mL), the bacterial suspensions of strains SZ-2 and SZ-22 significantly reduced ginseng black spot caused by *A. panax*, and the average reduction of ginseng black spot were 69.64% and 82.47% compared with the nontreated control, respectively. The bacterial suspension of SZ-22 at a 60% (v/v) concentration had adequate biocontrol activity ($p < 0.05$) compared with 25% suspension concentrate azoxystrobin. In addition, the bacterial suspensions of SZ-2 and SZ-22 could weakly control ginseng black spot at 40% or 50% (v/v) concentrations (Figs. 2, 3).

3.4. Influence of strain on ginseng seedling growth

In the pot experiments, strains SZ-2 and SZ-22 all showed growth-promoting effects (Table 3). Among these strains, the growth promotion effect of SZ-22 was the most significant, and the ginseng whole plant fresh weight, whole plant dry weight, root fresh weight, and root dry weight increased by 144.03%, 382.94%, 240.28%, and 144.03%, respectively. The strain SZ-22 promoted the increase in ginseng plant height and root length by 83.05% and 83.05%, respectively, compared with the water control ($p < 0.05$).

3.5. Morphological, physical, and chemical properties of strains SZ-22

According to the morphological observation results, the SZ-22 colony was irregular and yellow and showed a central uplift, with a moist, transparent surface (Fig. 4A). Under the electron microscope, the cells of strain SZ-22 were ascertained to be rod shaped. The cell size of strain SZ-22 was found to be 0.6–0.7 μm by 3.2–4.1 μm. Strain SZ-22 was identified as a nonspore-forming, gram-negative aerobic bacterium (Fig. 4B). Strain SZ-22 grew optimally at pH 5.0–9.0 but was inhibited at pH less than 5.0. Strain SZ-22 also grew at 4–48°C but not at temperatures above 50°C. As shown in

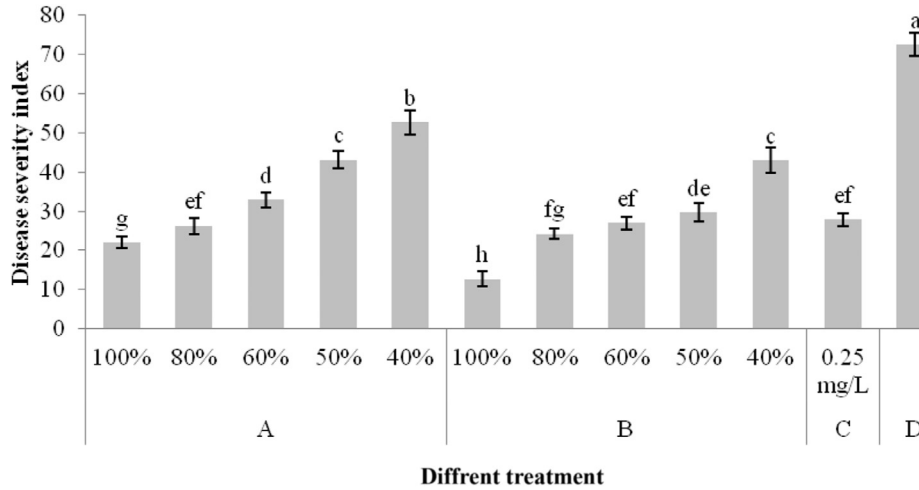


Fig. 1. Effects of the bacterial suspensions (volume/volume) of two selected antifungal strains, SZ-2 and SZ-22, on ginseng black spot caused by *Alternaria panax*. A, SZ-2; B, SZ-22; C, 25% suspension concentrate azoxystrobin; D, nontreated control (water). Each percentage means different concentration of bacterial suspension. At the tested concentrations, i.e., low to 40% (volume/volume) concentrations, the disease severity index of ginseng measured in A or B, was similar to that in D. Under Duncan's new multiple range test, different lowercase letters in the histogram by significant differences ($p < 0.05$) were determined.

Table 4, the strain SZ-22 exhibited catalase, oxidase activity, but did not exhibit lipase activity. Nitrate reduction to nitrite was found, but oxidative response was not detected in the oxidative/fermentative test. The Voges–Proskauer was negative. The strain SZ-22 did not utilize citrate, D-xyllose, casein, and tyrosine. Amylum, gluten were hydrolyzed by strain SZ-22. The strain SZ-22 utilized L-arabinose, α-D-glucose, and D-mannitol. Strain SZ-22 tolerated well up to 5–7% NaCl in the beef extract peptone medium. According to its morphological, physiological, and biochemical characteristics, strain SZ-22 is similar to *Brevundimonas* spp.

3.6. Molecular identification

The genomic DNA from SZ-22 was used to amplify 16S rDNA fragments with a length of 1,274 bp, and the products were submitted to GenBank (registration number: KC511111) and BLAST for comparison. Strain SZ-22 showed the highest homology with *Brevundimonas* sp. X60 (registration number: KF017644), with 99% sequence homology.

The SZ-22 genomic DNA was used to amplify the *gyrB* gene fragment, and the resulting PCR band of 1,124 bp was consistent with the *gyrB* gene of *Brevundimonas* spp. The sequencing results of SZ-22 and known sequences in GenBank were compared, identifying *Brevundimonas* sp. (registration number: JQ653053.1) with a *gyrB* gene sequence homology of 99%. The phylogenetic tree is shown in Fig. 5, and it was obtained using the NJ method. Strain SZ-22 belonged to the same branch as *Brevundimonas terrae* (registration number: DQ335215.1).

Based on the analysis of morphological, physiological, and biochemical indices, 16S rDNA and *gyrB* gene sequence alignment results, and the phylogenetic tree analysis, strain SZ-22 is related to *B. terrae*.

4. Discussion

The bacterial source for plant biocontrol has been isolated from plants and cultivated soil. The rhizosphere structure establishes a closer relationship with the plant root system [6,12,31]. The

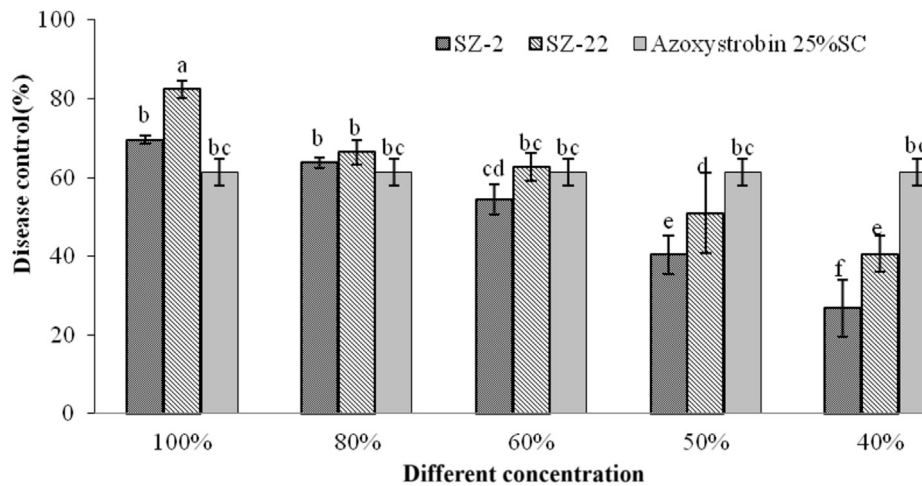


Fig. 2. Individual effects of SZ-2 and SZ-22 bacterial suspensions (volume/volume), on ginseng black spot caused by *Alternaria panax*. In all treatments, the concentration of azoxystrobin 25% suspension concentrate was 0.25 mg/L. The control effect of 25% suspension concentrate azoxystrobin was 61.45%. Under Duncan's new multiple range test, different lowercase letters in the histogram by significant differences ($p < 0.05$) were determined.

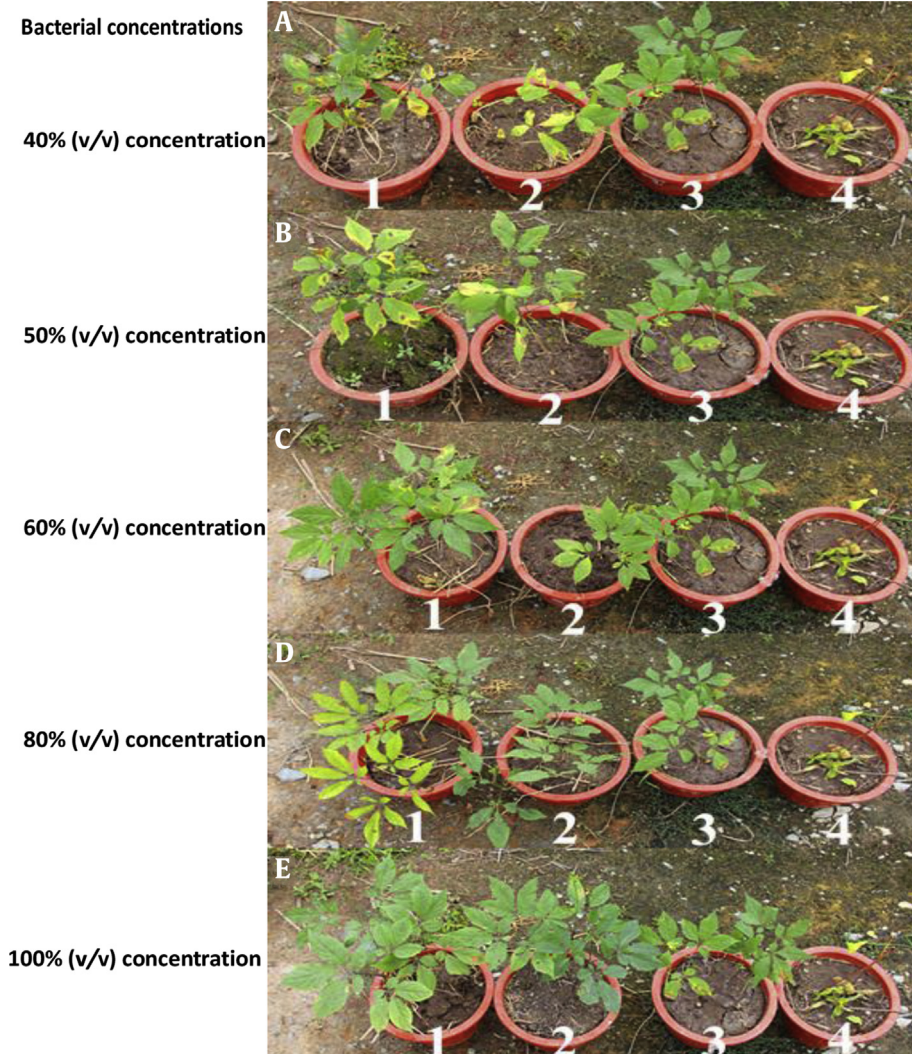


Fig. 3. Symptoms of common ginseng black spot on the leaves of ginseng seedlings that developed 10 wk after root irrigation with *Alternaria panax* (5×10^4 spores/mL). A comparison between ginseng plants obtained from nonbacterized (3, 4) and bacterized ginseng (1, 2). In A, B, C, D, and E, ginseng plants (1, 2) that were treated with bacterial suspensions were attributable to SZ-22 and SZ-2 at a 40% [volume/volume (v/v)] concentration, a 50% (v/v) concentration, a 60% (v/v) concentration, an 80% (v/v) concentration, and a 100% (v/v) concentration, respectively. A, B, C, D, and E, plants (3) treated with a 0.25 mg/L concentration of Azoxystrobin at 25% SC and plants (4) treated with water.

Table 3

Growth-promoting effect of strain SZ-2, SZ-22 on *Panax ginseng* Meyer

Treatment	Height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)
SZ-2	34.67 ± 0.32b	9.5 ± 0.37b	3.54 ± 0.35b	3.57 ± 0.34b	1.97 ± 0.02b	18.4 ± 0.2b
SZ-22	38.8 ± 0.75a	17.12 ± 0.8a	6.49 ± 0.47a	9.41 ± 0.25a	3.58 ± 0.05a	26.5 ± 0.17a
CK (water)	21.2 ± 0.56c	7.04 ± 0.09c	1.34 ± 0.02c	2.73 ± 0.04c	0.69 ± 0.02c	11.3 ± 0.2c

Different letters in the same column by Duncan's new multiple range test ($p < 0.05$)

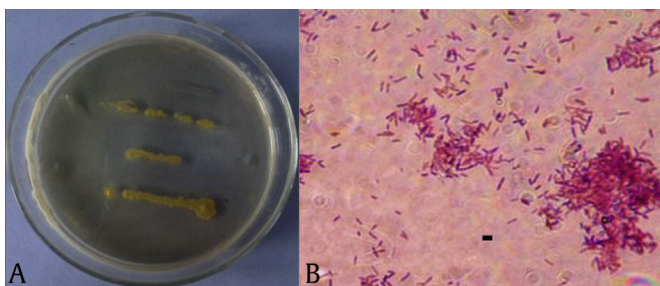


Fig. 4. Cultural and morphological characteristics of strain SZ-22, showing (A) yellow irregular-central uplift and (B) rod-shaped bacterial cells of 0.6–0.7 μm (diameter) \times ca. 3.2–4.1 μm (length). Bar = 2.0 μm .

bacteria in rhizosphere soil exhibit stronger competitiveness and survival abilities when competing for favorable ecological sites. It has been reported that the bacteria in rhizosphere soil can prevent disease and promote production, meeting the screening criteria of biocontrol strains and can be used as biocontrol [32,33]. In the present study, using a systematically flat confrontation method, we screened multiple strains of bacteria from the rhizosphere soil of ginseng plants. Our results showed that SZ-22 has a significant inhibitory effect on *A. panax*, which caused ginseng black spot.

During the biocontrol of plant diseases, the screening of antagonistic strain and various diseases is still in the research stage. Zhang et al. [34] isolated *Bacillus subtilis* from NJ-18, and it exhibited broad-spectrum antifungal activity against *Blumeria graminis* f. sp. *tritici* and eight other plant pathogenic fungi [34].

Table 4
Biochemical and physiological characteristics that differentiate strain

Item	Reaction	Item	Reaction
Motile	+	Gram stain	–
Aerobic	+	Glutin hydrolysis	+
Oxidase test	+	Oxidative/fermentative test	–
L-Arabinose	+	Casein hydrolysis test	–
D-Xylose	–	Decomposition of tyrosine	–
α-D-Glucose	+	Hydrolyzation of amyllum	+
Lipase	–	Voges–Proskauer	–
D-Mannitol	+	Growth in 2% NaCl	+
Nitrate reduction	+	Growth in 5% NaCl	+
Catalase	+	Growth in 7% NaCl	±
Citrate utilization	–	Growth in 10% NaCl	–

“+”, positive (growth or reaction); “–”, negative (unavailable no growth or no reaction), “±”, weak or delay

Pseudomonads are widely distributed throughout diverse agricultural ecosystems [35], and the broad-spectrum activity of *Pseudomonas* spp. contributes to their *in vitro* antifungal activity and *in vivo* disease control [36]. Lee et al. [37] isolated a new antibiotic called aerugine, which has protective activity against pepper *Phytophthora* disease and cucumber anthracnose from the culture filtrates of *Pseudomonas fluorescens* MM-B16. Zeng et al. [38] isolated H-6 endophytic bacteria of *Burkholderia* spp. from the leaves of *Huperzia serrata* (Thunb.) Trevis. These bacteria showed a strong antagonistic effect against *F. graminearum* and *Sclerotinia*

scleroliorum. In the present study, by using confronting incubation, we have shown that strain SZ-22 exhibits different degrees of inhibition for *A. panax*, *F. graminearum*, *B. maydis*, and other nine plant pathogenic fungi. After repeated verification, the antifungal effect was relatively stable, showing broad-spectrum antifungal activity.

In recent years, research on rhizosphere soil bacteria, *Pseudomonas*, *Bacillus*, *Agrobacterium*, and other dominant microbial populations in the plants' rhizosphere soil showed they can prevent certain diseases and promote plant growth. The disease prevention mechanisms of rhizosphere soil have been summarized as competition, antagonism, and induced plant resistance [39]. Based on this theory, *B. subtilis* and *P. fluorescens* have been developed and widely used in a variety of agricultural economic crops [40,41]. Nevertheless, the biocontrol of fungal diseases of ginseng has been little reported. Bae et al. [42] found that *Bacillus* spp. can be used to prevent and cure root rot resulting from *Fusarium solani* and *Phytophthora* blight resulting from *Phytophthora cactorum*. Jang et al. [43] found that *B. subtilis* and *Bacillus amyloliquefaciens* can effectively control ginseng root rot resulting from *C. destructans* (Zins.). Results of the present study showed that SZ-22 bacterial suspension not only has a control effect against ginseng black spot but also has an adequate promoting effect on the growth of ginseng plants, indicating the potential of this bacterium for applications related to the prevention and treatment of ginseng diseases. Through comprehensive morphological, physiological, and biochemical

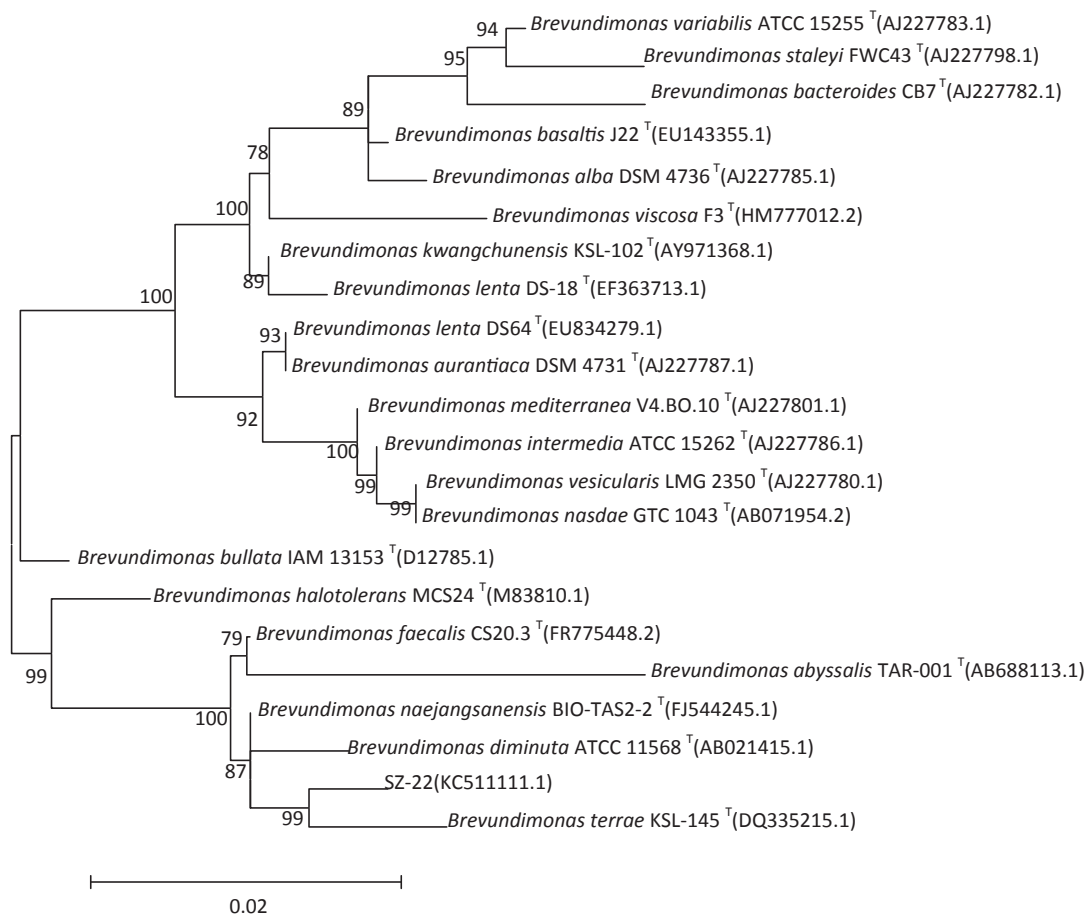


Fig. 5. Phylogenetic tree based on the *gyrB* sequences of the antagonistic bacteria. The scale bar indicates the distance in substitutions per nucleotide. The length of each pair of branches represents the distance between sequence pairs, whereas the units at the bottom of the tree indicate the number of substitution events.

discrimination, as well as using 16S rDNA and *gyrB* gene sequence alignment, the bacterial strain SZ-22 was observed to be identical to *B. terrae*. Currently, there are no reports providing similar results. The strain SZ-2 was determined as *Bacillus methylotrophicus* by our laboratory [19]. Moreover, Shanmugam et al. [44] found that the biocontrol strain mixture of *B. cepacia* TEPF-Sungal and *Trichoderma harzianum* S17TH could effectively control rhizome rot of ginger caused by *Pythium myriotylum*; however, Liu et al. [45] found that individual antagonistic strains performed better than strain combinations, and strains differed significantly in the levels of biocontrol achieved. Therefore, because of the competition between antifungal strains, it might play a role in some negative interaction effects. The inhibition efficacies of SZ-2 and SZ-22 strain mixture against ginseng black spot disease resulting from *A. panax* still warrant further study.

The fermentation filtrate of SZ-22 showed a significant inhibitory effect against ginseng root rot, suggesting that the antimicrobial substance secreted by *B. terrae* plays a role in the prevention and treatment of ginseng diseases. This finding might reflect the fact that the pot experiment used in the present study contained a bacterial suspension of SZ-22, but not the fermentation broth strain. The results have also demonstrated the ability of *B. terrae* to prevent disease and promote growth, suggesting that *B. terrae* uses “competition as the main, antagonistic and induction as supplement” as a disease prevention mechanism. By occupying a favorable survival locus of the plants and soil, *B. terrae* strengthens the relationship with plants and indirectly improves the competitive ability of pathogenic bacteria to decrease these microbes in response to limited living space. Moreover, the antagonism of this bacterium combines with the plant’s ability to prevent disease and achieve disease prevention.

5. Conclusion

B. terrae SZ-22, which was isolated as an antifungal bacterium, showed broad-spectrum antifungal ability and exhibited good biocontrol efficacy *in vitro*. Moreover, the SZ-22 bacterial suspension led to ginseng plant growth promotion. These results indicate that *B. terrae* SZ-22 can be used as a promising biocontrol agent against the phytopathogenic fungi of *P. ginseng*. In-depth studies should be carried out in different and complex field conditions to evaluate its biocontrol efficacy and influence on indigenous microbial communities, as well as the effect of agro approaches (chemical fertilizers, pesticides, fungicides, etc.) on *B. terrae* SZ-22.

Conflicts of interest

All the authors declare that there are no conflicts of interest.

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