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Research article

An investigation of *Panax ginseng* Meyer growth promotion and the biocontrol potential of antagonistic bacteria against ginseng black spot

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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.

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 cinema*, *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 × 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 Hessenmüller 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) × 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′-TACGGCTACCTTGTTACG-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.

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