STUDY ON THE GROWTH-PROMOTING POTENTIAL AND DISEASE RESISTANCE OF THE ANTAGONISTIC BACTERIUM FRANKIA FRANCESE F1 ON GINSENG ROOT ROT AND RUST ROT

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Abstract. To effectively control root rot and rust rot, two major destructive diseases in ginseng production, an antagonist strain F1 was selected for molecular biology identification and showed 100% homology with *Frankia casuarinae* strain Ccl3 of the genus Mucor. The germination test found that the antagonist F1 could fix nitrogen, and secrete 3-indoleacetic acid (IAA) and iron carriers. And the seed germination test found that the germination rate of ginseng seeds diluted 50 times with F1 fermentation solution was 100%. Compared with the blank treatment, the increase in embryo length was 283.33%. The treatment of ginseng seedlings with different concentrations of fermentation solution improved the resistance of ginseng seedlings to root rot and rust rot. Among them, diluting 50 times, F1 fermentation solution significantly increased the height, root length, fresh weight, dry weight, root vigour and chlorophyll content of ginseng plants. It reduced the malondialdehyde content of leaves, significantly affecting ginseng seedlings' growth. At the same time, diluting 50 times, the F1 fermentation solution significantly increased the expression of biological control genes in plant leaves. It reduced the incidence and disease index of ginseng root rot and rust rot. The biological control effect of the F1 fermentation solution on ginseng reached 78.72%.
Keywords: *plant, disease resistance, antagonistic bacteria, promotion of birth, biological control*

Introduction

Ginseng (*Panax ginseng* C.A. Meyer) is a perennial herb of the Wujia family. Ginseng has pharmacological effects such as brightening the eyes, regulating blood pressure, educating the mind, treating physical weakness, improving mental health, and restoring heart function (Irfan et al., 2020) Various valuable bioactive compounds, such as ginsenosides, flavonoids, polysaccharides and amino acids, are present and have been used to treat many diseases (Mancuso and Santangelo, 2017). In recent years, soil-borne illnesses caused by continuous cropping, improper fertiliser application and plant nematode damage have become increasingly common, which has caused severe economic losses to agricultural production. Root rot and rust rot are two common diseases affecting ginseng, leading to a decrease in yield and quality (Li et al., 2020; Kim et al., 2017; Farh et al., 2018). Root rot is a soil-borne disease caused by the fungus *Fusarium solani*, which mainly infects the roots of ginseng and can survive in the soil for more than three years with mycelium and thick wall spores, spreading through rainwater, running water and bacterial compost, leading to softening and rotting of the roots and neck, and finally the ginseng wilts and dies. Rust rot is a soil-borne disease caused by *Cylindrocarpon destructans*. This pathogenic bacterium is highly viable in the soil. It can overwinter in diseased tissues and dirt, not quickly dying (Li et al.2020), causing soil bacteria to affect the planting of the next crop of ginseng, resulting in ginseng crop failure (Li et al., 2018), invading all parts of the ginseng roots during the planting period, with rust-coloured spots appearing on the roots after the onset of the

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disease. Severe rot and death extend from the epidermis to the roots (Wang et al., 2019a). The selection of beneficial microorganisms to control plant diseases can improve the yield and quality of agricultural products, reduce the use of chemical pesticides, and biological means of control have the advantages of being environmentally friendly, safe and efficient for sustainable agricultural development (Bonanomi et al., 2018; Jogaiah et al., 2018).

Currently, there are more studies on plant growth-promoting rhizobacteria (PGPR) at home and abroad. The symbiosis between PGPR and plants in the root system improves soil nutrient indicators, promotes plant growth and enhances plant chlorophyll storage (Khatoon et al., 2020), which is one of the hot spots of research in soil microbiology and microecology. Yuan et al. (2022) obtained 13 PGPR strains containing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase from peony rhizosphere soil, YDSY1, YDSY2 and YDSY4 had a significant growth-promoting ability. Many studies have found that growth indicators such as root length, fresh weight of roots, fresh weight of stems and dry weight of roots increased to varying degrees when strains capable of fixing nitrogen, solving phosphorus, solving potassium and producing plant growth hormones - indoleacetic acid and iron carriers - were applied to plants (Panigrahi et al., 2020). *Frankia* is a group of nitrogen-fixing actinomycetes that can symbiotically nodulate with non-leguminous woody plants (Ghodhbane-Gtari et al., 2013). Marappa et al. (2020) found that *Frankia* could improve the nutrient content of *Casuarina* woodlands. Reddell et al. (1988) found that the organic matter, total nitrogen, fast-acting potassium and fast-acting nitrogen contents of mosaic stands were significantly increased, with the most significant increase in fast-acting N. Ngom et al. (2016) showed that mosaic plants' plant survival and disease resistance were significantly improved after the implantation of Frankia spp.

In this paper, the antagonistic bacterium F1 was screened as an actinomycete with a high inhibitory rate against ginseng root rot and rust rot. After the systematic biological identification of the antagonistic bacterium F1, the growth-promoting ability of the antagonistic bacterium F1 on Ginseng and the effect of different concentrations of fermentation solution and live bacterial bodies on the physiological indicators of ginseng seedlings were investigated.

Materials and methods

Test material

Antimicrobial F1: A strain of antimicrobial F1 with a high inhibition rate against ginseng root rot and rust rot-causing bacteria was isolated from randomly collected ginseng inter-root soil samples in Jing Yu County, Baishan City, Jilin Province.

Pathogenic bacteria: *Fusarium solani* and *Cylindrocarpon destructans*, both highly pathogenic physiological microspecies, were provided by the Microbiological Conservation Centre of Jilin Agricultural University.

Ginseng variety for testing: Western Ginseng susceptible to root rot and rust rot, purchased from seed markets.

Main culture media

(1) Gao's I medium: soluble starch 20.0 g, KNO_3 1.0 g, NaCl 0.5 g, K_2HPO_4 1.0 g, $MgSO_4$ 0.5 g, $FeSO_4$ 0.01 g, pH 7.0~7.2, agar 20.0 g, distilled water 1000 mL.

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(2) Ashby's nitrogen-free medium: mannitol 10 g, K_2HPO_4 0.2 g, $MgSO_4$ 0.2 g, NaCl 0.2 g, K_2SO_4 0.3 g, $CaCO_3$ 5.0 g, agar 18.0 g, distilled water 1000 mL, pH 7.0~7.5.

(3) Inorganic phosphorus medium: glucose 10.0 g, NaCl 0.3 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, $(NH_4)_2SO_4$ 0.5 g, KCl 0.3 g, $Ca_3(PO_4)_2$ 5.0 g, $FeSO_4 \cdot 7H_2O$ 0.003 g, $MnSO_4 \cdot 4H_2O$ 0.003 g, deionised water 1000 mL, agar 20.0 g, pH 7.0~7.5.

(4) Indoleacetic acid (IAA) identification medium: King's medium: glycerol 15 mL, $MgSO_4 \cdot 7H_2O$ 1.5 g, $K_2HPO_4 \cdot 3H_2O$ 1.5 g, peptone 20.0 g, deionised water 1000 mL. Colourimetric solution: $FeCl_3$ 12.0 g dissolved in 300 mL of distilled water and 429.7% H_2SO_4 added slowly, cooled and then fixed to 1 L.

(5) CAS staining medium: 1 mmol/L $CaCl_2$ 0.1 mL, 10% casein 3 mL, 1 mmol/L $MgSO_4 \cdot 7H_2O$ 2 mL, 20% sucrose solution 1 mL, 0.1 mol/L phosphate buffer solution 5 mL, CAS staining solution 5 mL, agar 2 g, deionised water 100 mL.

Molecular biology of antagonistic bacterium F1

The antagonist DNA was extracted by the CTAB method; PCR amplification was performed using 16S universal primers and two × TsingKE MasterMix (Code No.: TSE003), while dH_2O was used as a negative control. PCR amplified the 16S rDNA gene. The PCR reaction procedure was 96 ℃ for 2 min; 96 ℃ for 10 s, 50 ℃ for 10 s, and 60 ℃ for 3 min; 30 cycles. After the PCR reaction, the product was purified, and 2 μL was used for 1% agarose gel electrophoresis at 100 V for 45 min at constant pressure. The PCR products were tested by agarose gel electrophoresis and sent for sequencing. Beijing Allwegene Technology Company did the sequencing of the 16S rDNA gene of the antagonistic bacteria. To determine the position of the strain's species in the taxonomic system, the construction of a phylogenetic tree from the 16S rDNA gene of the strain The developmental tree is built in the following steps: The sequencing results were uploaded to the BLAST function of the National Center for Biotechnology Information (NCBI) website, the sequenced 16S rDNA sequences were homology matched, and a phylogenetic tree of the strains was constructed using MEGA 6.0.

Determination of the growth-promoting properties of the antagonist F1

Determination of the nitrogen fixation characteristics of the antagonistic bacterium F1

Qualitative determination: A small amount of F1 was picked and inoculated onto Ashby nitrogen-free medium plates and incubated for 7 d at 28 °C to see if it could grow in Ashby nitrogen-free medium. If the strain can grow in the test medium, it is tentatively determined to have the ability to fix nitrogen (Liu et al., 2017).

Quantitative assay: The well-purified antagonist F1 was selected by the acetylene reduction method (Luo et al., 2021) and inserted into 6 mL of the nitrogen-free liquid medium in a vial. Sealed with a pressure cap, incubated at 30 ℃, 150 r/min for 24 h. The volume of the vial was 10 mL. The gas was withdrawn using a sterile syringe with a volume fraction of 10%, injected with a volume fraction of 10% high-purity acetylene and the eye of the needle sealed with adhesive tape. The incubation was continued in the incubator for 24-48 h. The amount of ethylene produced was determined by gas chromatography, and the magnitude of nitrogen-fixing enzyme activity (ARA) was calculated according to the following equation:

$$ARA = (Vst \times Cst \times Asa \times Vtu) / (Vsa \times Ast \times H \times 22.4) \quad \text{(Eq.1)}$$

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In the formula: ARA: nitrogenase activity; Vst: standard ethylene injection volume; Cst: standard ethylene concentration; Asa: ethylene peak area; Vtu: volume of the vial; Visa: sample injection volume; Ast: standard ethylene peak area; H: incubation time.

Determination of the phosphorus solubilisation characteristics of the antagonistic bacterium F1

The F1 (8 mm diameter) was inoculated into insoluble inorganic phosphorus medium using the transparent circle method and incubated at 28 ℃ for 5 d. The diameter of soluble phosphorus circles (D) and colonies (d) were measured. The strain's ability to dissolve phosphorus was determined according to whether soluble phosphorus circles could be produced; the strain's ability to dissolve phosphorus was initially determined according to the size of the D/d value, and the colony growth was recorded.

The IAA-producing capacity of antagonistic bacterium F1

Qualitative determination: The Salkawski colourimetric method was used to seed the antagonist F1 in Koch I liquid medium at 150 r/min at 28 °C for three days. 5 mL of seed solution was pipetted into 50 mL of Kim's medium without tryptophan, and Kim's medium containing L-tryptophan at a concentration of 100 mg/L, incubated at 28 °C with shaking at 200 r/min for 48 h and then centrifuged at 8000 r/min for 10 min, using the addition of 5 mL of deionised water as a control. Aspirate 5 mL of supernatant fermentation solution and CK into separate test tubes, add 5 mL of colourimetric solution, leave horizontally for 15 min and compare the colour change of the keys in the test tubes. If the colour is red, the antagonist F1 can produce IAA, and the shade of red proves the amount of IAA secreted by the antagonist F1; if the colour remains red, the antagonist F1 is unable to secrete IAA.

Quantitative determination: The standard curve was prepared using pure 3-IAA (3-indoleacetic acid), and the amount of IAA secreted by the antagonist F1 was determined by reference to the literature (Halda-Alija, 2003; Thakuria et al., 2004). The culture of the strain was centrifuged at low temperature (10000 r/min, 10 min), the supernatant was removed, and 1 mL was taken, added to the colourimetric solution and left to stand in the dark for 30 min, and immediately after completion, absorbance was measured at 530 nm, and the uninoculated IAA assay medium was used as a control.

Iron carrier capacity of antagonistic bacterium F1

Qualitative assay: Using CAS plate assay (Schwyn and Neilands, 1987), F1 bacteriophage cake was spotted on a solid medium with CAS staining solution and incubated for 5 d at 28 °C. *E. coli* that did not produce iron carriers were used as the control, and the colour of the medium around the colony was observed after the antagonist F1 was fully grown. If the halo around the cake changes from blue-green to orange-yellow, the antagonist F1 is able to produce iron carriers, and the ratio between the diameter of the halo and the diameter of the colony is measured. The greater the ratio, the greater the iron carrier activity.

Quantification: The antagonist F1 seed solution was inoculated at 7% inoculum into the quantitative iron production carrier medium, and samples were taken every 12 h, centrifuged at low temperature (4 ℃, 5000 r/min, 10 min), removed the precipitate and left the supernatant, mix the supernatant with CAS assay liquid volume 1:1, and leave for 40 min away from light, immediately after finishing, the absorbance value (As) of

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OD_{630} was measured, and this absorbance value was used as a reference value (Ar) to calculate the relative ferritin content according to the following equation, using the uninoculated iron-producing carrier quantification medium as a control (Machucaet al., 2003).

$$\text{Relative content of siderophore (\%)} = [(Ar - As) / Ar] \times 100\% \quad \text{(Eq.2)}$$

Detection of the promotion effect of the strain on ginseng seed germination

Ginseng seeds with full seeds were selected and surface disinfected twice with 75% alcohol for 3 minutes each time and finally rinsed five times with ultrapure water, respectively, with diluted F1 Fermentation Solution stock, Soak seeds in 10 mL each of 10x (10^{-1}), 50x (5×10^{-1}), 100x (10^{-2}) and 200x (2×10^{-2}) dilutions, after 24 h, the seeds were rinsed three times, and the surface water of the seeds was absorbed by the absorbent paper. The seeds were transferred to two layers of filter paper moistened with sterile water, a group of 10 ginseng seeds were put into test tubes and were cultured alternately between light and dark in an incubator at 28 °C, soaked in sterile water as a CK control, and each treatment was repeated three times. After five days of the germination test, the number of germinated seeds was recorded, the lengths of hypocotyl and radicle were measured, and the germination rate and concise vigour index (SVI) of ginseng seeds were calculated according to the formula.

$$\text{Germination rate (\%)} = (\text{germinating seeds} / \text{number of seeds for testing}) \times 100\% \quad \text{(Eq.3)}$$

$$\text{Increase in embryonic axis length} = \{(\text{length of treated embryonic axis-untreated Embryonic axis length}) / \text{untreated Embryonic axis length}\} \times 100\% \quad \text{(Eq.4)}$$

$$\text{Concise Vitality Index (SVI)} = \text{germination rate\%} \times \text{embryonic axis length} \quad \text{(Eq.5)}$$

A pot experiment on the ability of strains to promote the growth of ginseng seeds

Ginseng soil samples were collected randomly from perennial ginseng cultivation sites in Jingyu County, Baishan City, Jilin Province, China, autoclaved for 2 h, dried at 80 °C until no change in weight and then sieved through 60 mesh. Spore suspensions of two pathogenic bacteria of ginseng root rot and rust rot were concentrated by centrifugation, spore concentrations were determined using microscopes and haematocrit plates, and 10-fold gradient dilutions were performed. Six portions of 0.4 g sterilised ginseng soil were accurately weighed and placed in 2 mL centrifuge tubes with 100 μL of each concentration of spore suspension, a standard ginseng soil with a water content of approximately 20% and a spore concentration of 4 × 101 to 4 × 106 spore-g-1 were prepared and packed into pots (16 cm high and 15 cm diameter at the bottom), with 2 kg of ginseng soil in each pot. Healthy and full ginseng seeds were soaked in water and germinated in the dark at 20 °C. When the ginseng seed shoots were about 2 cm long, they were added to the ginseng-bearing soil and incubated for 48 h. The bearing soil was treated. A total of five treatments were used, divided into a blank control, a chemical treatment and three fermentation solution treatments.

1. T1 treatment: add 50 mL of 10-fold diluted F1 fermentation broth
2. T2 treatment: add 50 mL of F1 fermentation broth diluted 50 times
3. T3 treatment: add 50 mL of F1 fermentation solution diluted 100 times

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4. T4 treatment: addition of 50 mL of the chemical carbendazim liquid
5. CK blank treatment: add 50 mL of distilled water

Each treatment was replicated six times in 18 pots with six ginseng plants per pot, and each pot was watered daily with an equal amount of water, maintained at 25 °C and 75% relative humidity. Pot culture was sampled at 15 d, 30 d and 60 d. Three pots were randomly selected for each treatment.

Effect of fermentation solution treatment on root vigour and chlorophyll of ginseng

Samples of 0.5 cm lateral root segments of ginseng were dug up and rinsed, and the Hoechst of the ginseng root system was determined using the 2,3,5-triphenyl tetrazolium chloride (TTC) method.

To determine the chlorophyll content, 2 g of freshly cut ginseng leaves with the midvein removed were weighed and ground with a small amount of quartz sand and 80% acetone until the tissue turned white, and the filtrate was filtered and centrifuged at 800 r/min for 10 min. After centrifugation, the supernatant was further centrifuged at 3000 r/min for 8 min, and the precipitate was fixed by adding acetone to obtain a precipitate. The absorbance of the precipitated solution was measured at 663 nm and 646 nm to determine the content of chlorophyll a and chlorophyll b, respectively, and total chlorophyll was calculated by this method.

Effect of fermentation solution treatment on the activity of ginseng plant defence enzymes

Determination of enzyme activity after fermentation broth treatment

0.5 g of ginseng leaf fragments were placed in 2 ml of pre-cooled phosphate buffer (pH = 6.8, concentration 0.05 mol/L), ground into a slurry by adding quartz sand in an ice bath and then centrifuged at 1000 r/min for 20 min to obtain the supernatant as the enzyme solution (stored at -20 °C). Superoxide dismutase (SOD) activity was determined using the nitrogen blue tetrazolium (NBT) photoreduction method (Lai et al., 2008), and catalase (CAT) activity was determined by reverse titration of residual H_2O_2 with $KMnO_4$ (Johnson and Temple, 1964). The enzymatic activity of peroxidase (POD) was determined by the guaiacol method (Zieslin et al.1991), polyphenol oxidase (PP0) activity was determined by the o-phenylene dichroism method, and phenylalanine aminolysis (PAL) activity was determined according to the method of Huyskens-Keil et al. (2020).

Pot trials on the efficacy of antagonistic bacterial F1 fermentation solution against root and rust rots of ginseng

Healthy, strong and uniformly sized ginseng seedlings were selected; each ginseng seedling was inoculated by pin-pricking eight wounds and sprayed with a suspension of spores of *Fusarium solani* and *Cylindrocarpon destructans* spores (1 × 106 CFU-mL-1), the causative agents of ginseng root rot and rust rot, and moisturised after inoculation, six ginseng plants were planted in each ginseng pot, and after 36 h the experiment was divided into four treatments, two control and two treatment groups, which were sprayed with the fermentation solution of the strain and the polymyxin solution, with five replications of each treatment. Treatment A: sprayed with 20 mL of antagonist F1 fermentation solution; Treatment B: sprayed with 20 mL of carbendazim

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solution; Treatment C: sprayed with 20 mL of sterile water as a blank control; Treatment D: inoculated with pathogenic bacteria alone. The disease was observed after 30 d and graded according to the percentage of root area covered by the spot. Grading criteria: grade 0, no symptoms; grade 1, mild root discolouration, 0 < root necrosis area/root system area ≤ 30%; Grade 2, roots severely dark brown, 30% < root necrosis area/root system area ≤ 60%; Grade 3, basic necrosis of main roots, reduction of lateral roots, 60% < root necrosis surface/root system area ≤ 100%. Incidence and disease indices were counted, and control effects were calculated (Brunner et al., 2005).

$$\text{Incidence} = \text{number of diseased plants} / \text{total number of plants} \times 100\% \quad \text{(Eq.6)}$$

$$\text{Disease index} = \Sigma\ [(\text{number of diseased plants at each level} \times \text{relative grade value}) / (\text{total number of plants surveyed} \times \text{highest grade representative value})] \times 100 \quad \text{(Eq.7)}$$

$$\text{Effectiveness of prevention and treatment} = (\text{disease index of the control group} - \text{disease index of treatment group}) / \text{disease index of control group} \times 100\% \quad \text{(Eq.8)}$$

Results

Sequence analysis and phylogenetic tree construction of the 16S rDNA strain

The 16S rDNA of the antagonist F1 was homologous to *Frankia casuarinae* strain Ccl3 *(Frankia casuarinae Ccl3*), published in GenBank under sequence accession number GU296535, and 100% homology was achieved by NCBI homology matching and pulling sequences with high similarity. The evolutionary status of the antagonist F1 was analysed using the neighbour-joining method, and the 16S rDNA sequences obtained from sequencing and those of other model bacteria were used for the construction of a phylogenetic tree (see *Figure 1*). The results showed that antagonist F1 was the closest genetic distance to *Frankia strain Ccl3* (*Frankia casuarinae Ccl3*) and, combined with morphological and physiological and biochemical characteristics, inferred that it might be identical to *Frankia strain Ccl3* (*Frankia casuarinae Ccl3*), a genus of Frankia.

Strain promoting activity

Nitrogen fixation by strains of bacteria

Qualitative tests have shown that antagonist F1 normally grows after seven days of inoculation on Ashby's nitrogen-free solid medium (see *Fig. 2*), and continues to grow well after three generations of transfer.

Phosphorus solubilisation by strains

The antagonist F1 did not show a halo in the inorganic phosphorus medium (see *Fig. 3*). *E. coli* as CK also failed to grow normally in the inorganic phosphorus medium, and the strain was tentatively determined to have no inorganic phosphorus solubilising effect.

The IAA-producing activity of the strain

Qualitative testing revealed that after antagonist F1 was cultured in Kim's medium centrifuged at low temperature, the supernatant was retained, a drop of colourimetric solution was added to the supernatant, and the tube containing the fermentation solution

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turned reddish brown (see *Fig. 4*), the colour of the blank control CK-treated tubes changed to pale yellow after the addition of drops of colourimetric solution, indicating that the antagonist F1 can secrete IAA and is a strong IAA producer. A standard curve was prepared by collecting standard samples of IAA, and a linear regression equation was obtained between IAA concentration and 0D530 value, $y = 0.0245x + 0.008$, $R^2 = 0.9986$. The OD value for colourimetry with chromogenic agents in tryptophan-free Kim's culture was 0.628 and 0.215 in tryptophan-free Kim's culture. Based on the standard curve, the mass concentration of IAA secreted by the antagonist bacterium F1 was 25.30 mg/L in the tryptophan-containing Kim's culture and 8.44 mg/L in the tryptophan-free Kim's culture.

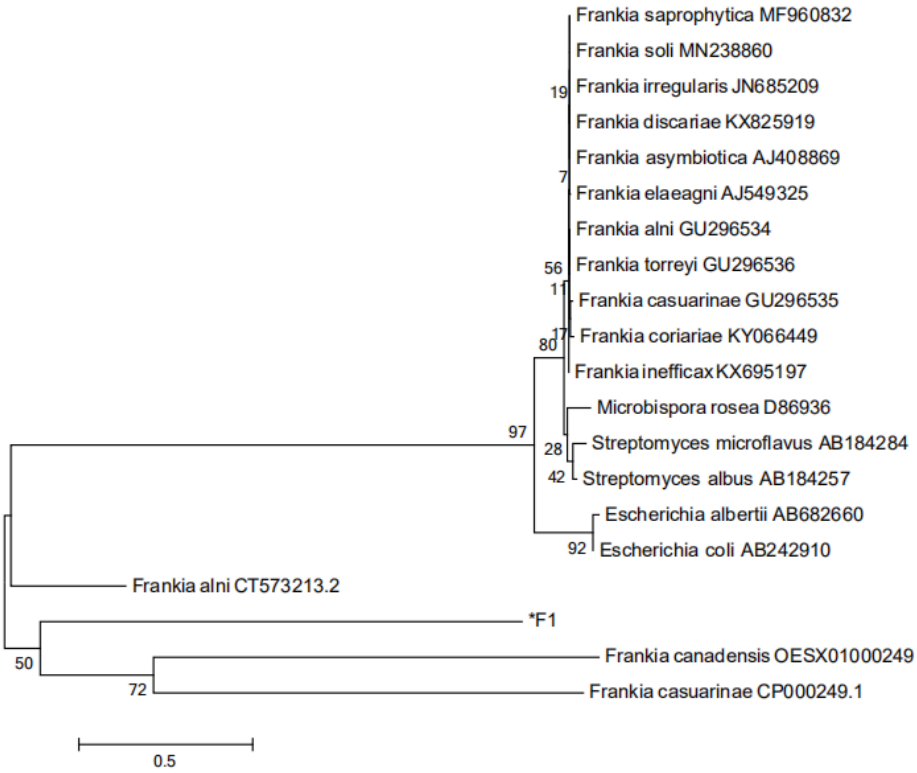

Figure 1. *Phylogenetic tree constructed by antagonistic strain F1 based on 16S rDNA gene sequence*

Figure 2. *Growth state of F1 on nitrogen-fixing medium*

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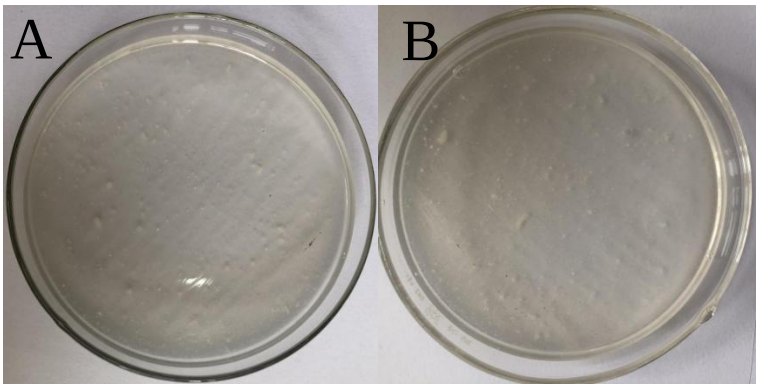

Figure 3. *Growth state of F1 on a medium of phosphate removal*

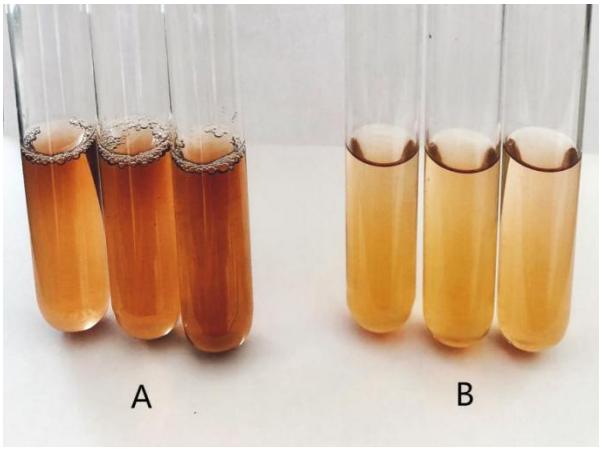

Figure 4. *The reaction of F1 fermentation broth with Salkowsk's chromogenic agent. (A) contains F1 fermentation broth and (B) is sterile water control group CK*

Iron-producing carrier activity of the strain

Microorganisms capable of producing iron carriers were grown in CAS plates, producing iron carriers with high iron chelating capacity using iron ions in the medium. As shown by the results of the medium, the antagonist F1 colonies appeared surrounded by an orange-yellow aperture after 7 d of incubation on CAS medium (see *Fig. 5*), the absence of an orange-yellow aperture around the blank control *E. coli* indicates that the antagonist bacterium F1 is capable of producing iron carriers. The quantitative assay of antagonist F1 was calculated by the formula, and the relative amount of organophilic pigments produced by antagonist F1 was 50.32%.

Effect of strain antagonist F1 fermentation broth on ginseng seed germination

The results showed that the antagonistic bacterium F1 promoted the germination of ginseng seeds, and the concentration of the fermentation solution was positively correlated with the germination rate, embryo axis length increase and the brief vigour index, as shown in *Table 1,* the germination rate was 100%, and the increase in embryo axis length was 283.33% at a concentration of 5×10^{-1} in the fermentation broth of the antagonist F1, the Concise Vitality Index was 8.0% higher than that of the control. All

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other concentration treatments had a pro-germination effect on ginseng seeds, all of which were higher than the CK control. The antagonist F1 in the above growth promotion test secretes the plant growth hormone indoleacetic acid (IAA), which produces iron carriers to increase plant uptake of nutrients. Thus, the antagonistic bacterium F1 was shown to promote the germination of ginseng seeds.

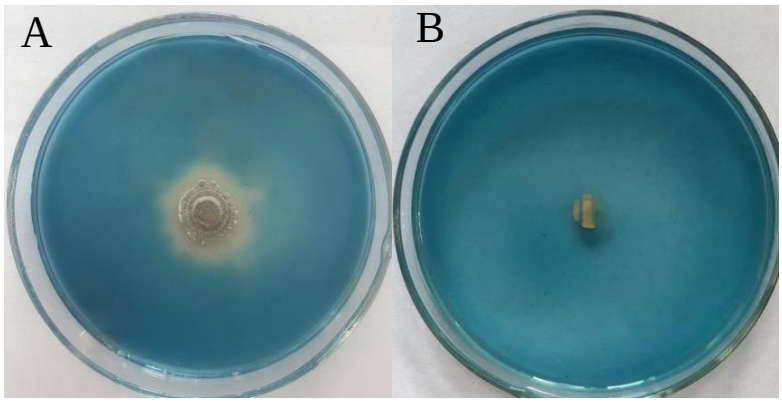

Figure 5. *Growth state of F1 strain on CAS culture medium. (A) Antagonistic bacterium F1; (B) E. coli CK*

Table 1. *Effect of strain F1 sterile filtrate on seed germination of ginseng*

Fermentation broth concentration	Germination rate (%)	Embryonic axis length (mm)	Increase in embryonic axis length (%)	Concise vitality index (SVI) (%)
CK	50	2.4 ± 0.9	-	1.20
Original	70	5.9 ± 1.2	145.83	4.13
10^{-1}	80	8.8 ± 1.3	266.67	7.04
5×10^{-1}	100	9.2 ± 2.1	283.33	9.20
10^{-2}	60	7.2 ± 1.8	200.00	4.32
2×10^{-2}	60	5.1 ± 1.2	112.50	3.06

Effect of fermentation solution treatment on root vigour

The results are shown in *Figure 6*. Ginseng plants showed significant differences ($P < 0.05$) in root vigour from the CK blank control treatment at 15 d, 30 d and 60 d after the T1, T2 and T3 fermentation solution treatments and the T4 chemical treatment. At 15 d, root vigour of ginseng plants was enhanced by 37.50%, 118.18% and 69.32% for T1, T2 and T3 fermentation solution treatments, respectively, and 29.55% for T4 chemical treatment compared to CK blank control. At 30 d, the root vigour of ginseng plants was enhanced by 62.74%, 124.51% and 71.57% for T1, T2 and T3 fermentation solution treatments, respectively, and 15.69% for T4 chemical treatment compared to the CK blank control. At 60 d, root vigour of ginseng plants was enhanced by 37.29%, 117.80% and 61.02% for T1, T2 and T3 fermentation solution treatments, respectively and 20.34% for T4 chemical treatment compared to CK blank control. From the four treatments compared to the CK blank control increase, it was found that the T2 fermentation solution treatment significantly enhanced the root vigour of ginseng plants at 15 d, 30 d and 60 d.

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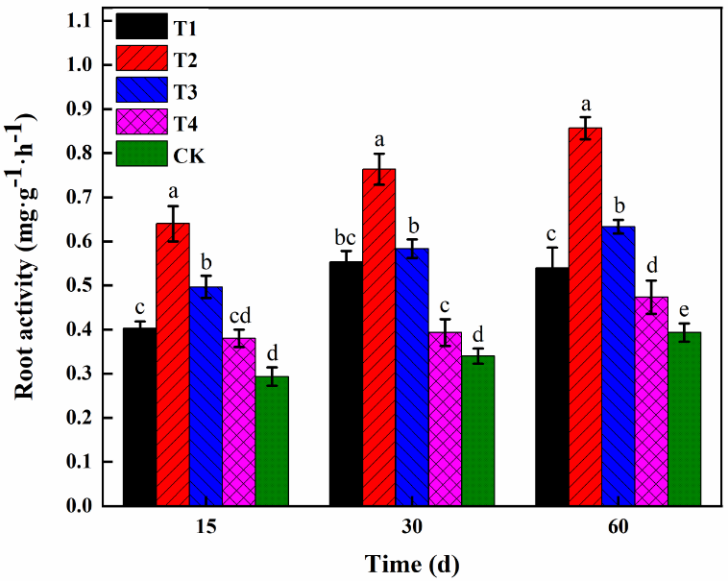

Figure 6. *Effects of different treatments on the root activity of Ginseng plants. The same letter in each column of the graph indicates that the difference does not reach a significant level, while different letters indicate that the difference reaches a significant level ($p < 0.05$)*

Effect of fermentation solution treatment on chlorophyll content

The results are shown in *Table 2*. Statistical analysis of the measured chlorophyll content revealed that the T1, T2, T3 and T4 treatments all increased the chlorophyll a content, chlorophyll b content and total chlorophyll content in the leaves to some extent, with significant differences, compared to the control ($P < 0.05$). At 15 d, T1, T2, and T3 fermentation broth treatments and T4 chemical treatments increased chlorophyll content by 50.0%, 75.0%, 25.0% and 16.67%, respectively, compared to the CK blank control; chlorophyll b content increased by 94.74%, 152.63%, 68.42% and 47.37% and total chlorophyll content increased by 65.45%, 101.82%, 40.0% and 27.27%, respectively. At 30 d, the T1, T2 and T3 fermentation broth treatments and the T4 chemical treatment increased chlorophyll content by 45.50%, 72.50%, 40.0% and 7.5%, respectively, compared to the CK blank control; chlorophyll b content increased by 57.14%, 161.90%, 61.90% and 85.71%, respectively, and total chlorophyll content increased by 47.54%, 103.28%, 47.54% and 34.43% respectively. At 60 d, the T1, T2 and T3 fermentation broth treatments and the T4 chemical treatment increased chlorophyll content by 48.78%, 82.93%, 41.46% and 36.59%, respectively, compared to the CK blank control; chlorophyll b content increased by 54.17%, 75.0%, 41.67% and 29.17%, respectively, and total chlorophyll content increased by 50.77%, 80.0%, 41.54% and 33.85%, respectively, in total chlorophyll content. The most significant increase in chlorophyll content, chlorophyll b content and total chlorophyll content in the leaves of ginseng plants was found to be the result of T2 fermentation solution treatment at different sampling periods of 15, 30 and 60 d.

Effect of fermentation solution treatment on the MDA content of plant leaves

The results are shown in *Figure 7*, and statistical analysis of the measured MDA levels showed significant differences between the T1, T2 and T3 treatments compared

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to the control group ($p < 0.05$). At 15 d, compared to the CK blank control, the T1, T2, and T3 fermentation solution treatments decreased the leaf MDA content of ginseng plants by 10.73%, 12.88% and 12.45%, respectively, and the T4 chemical treatment decreased the leaf MDA content of ginseng plants by 5.15%. At 30 d, compared to the CK blank control, the T1, T2, and T3 fermentation solution treatments decreased the leaf MDA content of ginseng plants by 9.20%, 19.54% and 13.41%, respectively, and the T4 chemical treatment decreased the leaf MDA content of ginseng plants by 12.64%. At 60 d, compared to the CK blank control, the T1, T2, and T3 fermentation solution treatments decreased the leaf MDA content of ginseng plants by 7.06%, 29.41% and 12.16%, respectively, and the T4 chemical treatment decreased the leaf MDA content of ginseng plants by 4.31%. From the decrease of the four treatments compared to the CK blank control, it was found that the T2 fermentation solution treatment could significantly reduce the MDA content of the leaves of ginseng plants at 15 d, 30 d and 60 d.

Table 2. *Effects of different treatments on chlorophyll content in Ginseng leaves, the same letter in each column indicates that the difference does not reach a significant level, while different letters indicate that the difference reaches a significant level ($p < 0.05$)*

Time (d)	Process	Chl_a (mg·g^{-1})	Chl_b (mg·g^{-1})	Chl_a / Chl_b	Total chlorophyll (mg·g^{-1})
15 d	T1	0.54 ± 0.12 b	0.37 ± 0.04 b	1.46 ± 0.13 c	0.91 ± 0.16 b
	T2	0.63 ± 0.09 a	0.48 ± 0.10 a	1.31 ± 0.11 d	1.11 ± 0.19 a
	T3	0.45 ± 0.15 c	0.32 ± 0.04bc	1.41 ± 0.13cd	0.77 ± 0.19 d
	T4	0.42 ± 0.08cd	0.28 ± 0.04 c	1.50 ± 0.12 b	0.70 ± 0.12 c
	CK	0.36 ± 0.10 d	0.19 ± 0.06 d	1.89 ± 0.10 a	0.55 ± 0.16 e
30 d	T1	0.57 ± 0.14 b	0.33 ± 0.04bc	1.73 ± 0.09 b	0.90 ± 0.18 b
	T2	0.69 ± 0.13 a	0.55 ± 0.12 a	1.25 ± 0.14 d	1.24 ± 0.25 a
	T3	0.56 ± 0.10bc	0.34 ± 0.11bc	1.65 ± 0.12 c	0.90 ± 0.21 b
	T4	0.43 ± 0.12 c	0.39 ± 0.04 b	1.10 ± 0.11 e	0.82 ± 0.16 c
	CK	0.40 ± 0.09cd	0.21 ± 0.03 c	1.90 ± 0.15 a	0.61 ± 0.12 d
60 d	T1	0.61 ± 0.10 b	0.37 ± 0.06ab	1.65 ± 0.13 c	0.98 ± 0.16 b
	T2	0.75 ± 0.21 a	0.42 ± 0.08 a	1.79 ± 0.22ab	1.17 ± 0.29 a
	T3	0.58 ± 0.12bc	0.34 ± 0.07bc	1.71 ± 0.13 b	0.92 ± 0.19 c
	T4	0.56 ± 0.07bc	0.31 ± 0.05 b	1.81 ± 0.12 a	0.87 ± 0.12cd
	CK	0.41 ± 0.11 c	0.24 ± 0.04 c	1.71 ± 0.11 b	0.65 ± 0.15 d

Effect of fermentation solution treatment on the amount of biocontrol gene expression in ginseng plants

The results showed (*Fig. 8*) that SOD activity, POD activity, PPO activity, PAL activity and CAT activity at 15 d, 30 d and 60 d were higher in T1, T2 and T3 different fermentation broth treatments and T4 chemical treatment than in CK blank control treatment, with significant differences ($P < 0.05$). The defence enzymes SOD activity, POD activity, and PPO activity in ginseng plants were significantly stronger at 15 d, 30 d and 60 d after T1, T2 and T3 fermentation solution treatments than the T2 chemical treatment. The increase in plant defence enzymes was greater after fermentation broth treatment and less after chemical treatment. Of the three fermentation broth concentrations, the T2 treatment fermentation broth concentration had a more pronounced effect on the increase in defence enzymes in the leaves of ginseng plants at 60 d. SOD activity was highest in the T2 treatment at 60 d, reaching

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196.60 U/g/min, higher than the T1 treatment at 48.37 U/g/min, higher than the T3 treatment at 54.63 U/g/min, higher than the T4 treatment at 74.73 U/g/min and higher than CK treatment 109.10 U/g/min respectively. POD activity at 60 d was highest in the T2 treatment at 1739.33 U/g/min, higher than the T1 treatment at 400.67 U/g/min, higher than the T3 treatment at 480.67 U/g/min, higher than the T4 treatment at 856.67 U/g/min and higher than the CK treatment at 1037.33 U/g/min. PAL activity at 60 d was highest in the T3 treatment at 5837.33 U/g/h, higher than the T1 treatment at 984.67 U/g/h, higher than the T3 treatment at 1445.0 U/g/h, higher than the T4 treatment at 2194.67 U/g/h and higher than the CK treatment at 2702.0 U/g/h respectively. PPO activity at 60 d was highest in the T2 treatment at 79.17 U/g/min, higher than the T1 treatment at 13.77 U/g/min, higher than the T3 treatment at 14.93 U/g/min, higher than the T4 treatment at 19.93 U/g/min and higher than the CK treatment at 35.10 U/g/min。 CAT activity at 60 d was highest in T2 treatment at 34.57 U/g/min, and CAT activity was higher than T1 treatment at 4.33 U/g/min, higher than T3 treatment at 5.19 U/g/min, higher than T4 treatment at 3.50 U/g/min and higher than CK treatment 10.87 U/g/min.

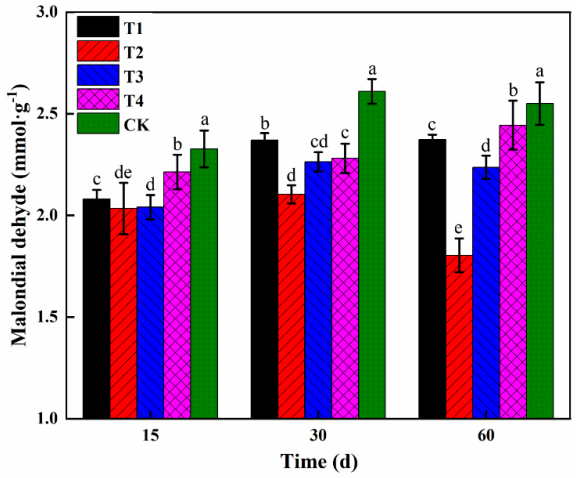

Figure 7. *Effects of different treatments on MDA content in ginseng leaves, the same letter in each column of the graph indicates that the difference does not reach a significant level, while different letters indicate that the difference reaches a significant level ($p < 0.05$)*

Greenhouse pot trials of fermented liquid for the control of root and rust rot of ginseng

Observation 30 d after inoculation (see *Table 3*) of potted ginseng plants sprayed with the causative agents of ginseng root rot and rust rot, *Fusarium solani* and *Cylindrocarpon destructans*. After treatment with the F1 fermentation solution, the incidence and disease index was significantly reduced, with a relative efficacy of 78.72% and a disease index of 11.21. The ginseng plants developed and grew normally after treatment. The relative efficacy of the treatment with carbendazim solution against root and rust rot of ginseng was 63.19%, which was lower than the relative efficacy of the fermentation solution of the fungus F1 against Ginseng, with a disease index of 19.39. The blank control ginseng plants were almost completely diseased with a disease index of 52.67; growth and development were affected, the roots rotted, and the whole was wilted and withered. The condition index for the treatment was 60.89 using spore

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suspensions of *Fusarium solani* and *Cylindrocarpon destructans* alone. Biofungus F1 was not only effective against root rot and rust rot of ginseng but also significantly promoted the growth of plant height and root length, with higher fresh and dry weights than the other three treatments. It showed that Biofungus F1 was more effective in controlling the root rot and rust rot of ginseng, and its control effect was significantly higher than that of the pesticide treatment group ($P < 0.05$).

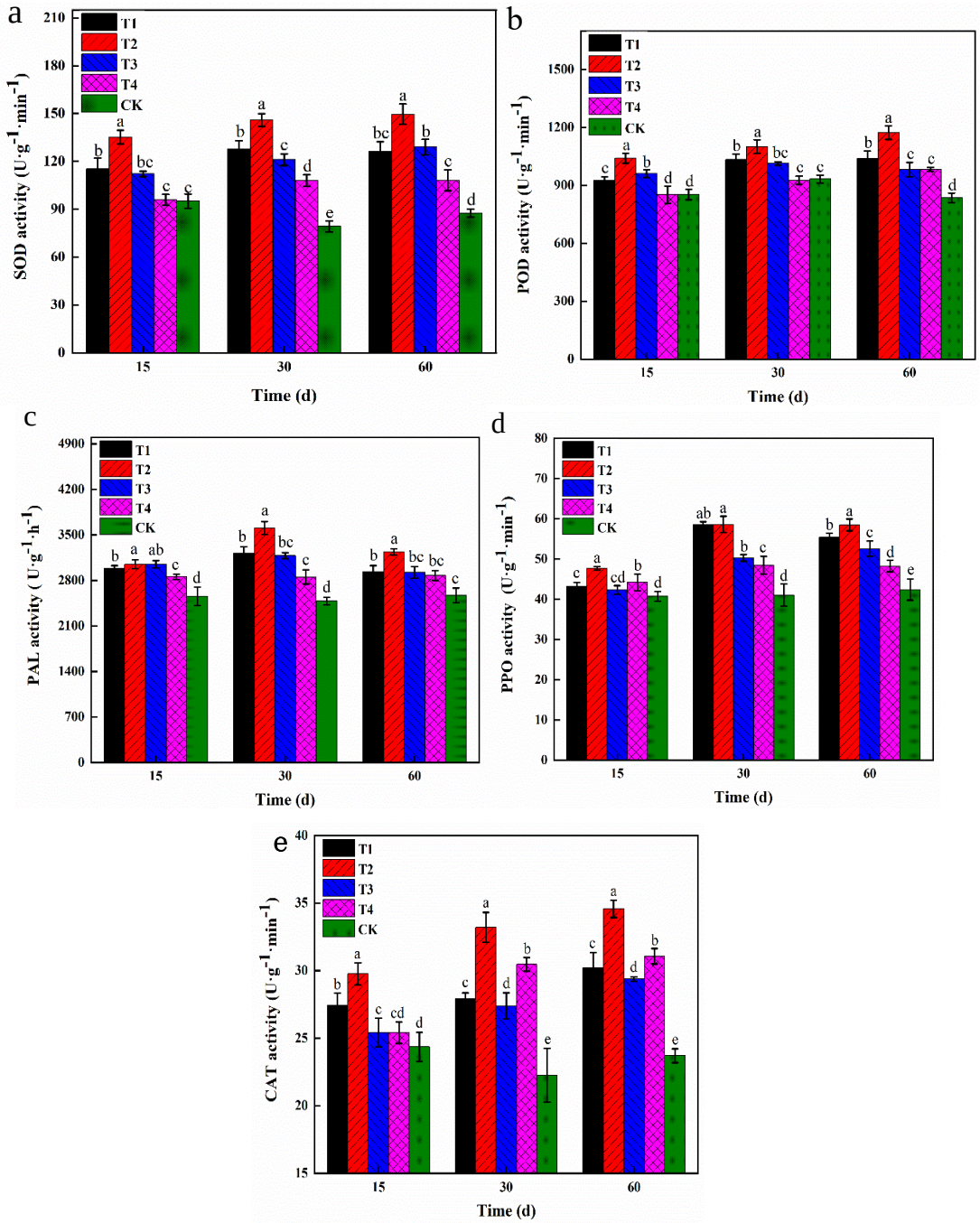

Figure 8. *Influence of different treatments on the expression level of anti-growth genes in ginseng plants after treatment, (a) SOD; (b) POD; (c) PAL; (d) PPO; (e) CAT. The same letter in each column of the graph indicates that the difference does not reach a significant level, while different letters indicate that the difference reaches a significant level ($p < 0.05$)*

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Table 3. *Effect of biocontrol F1 on ginseng root rot and rust rot in greenhouse*

Treatment	Plant height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Disease index	Prevention and treatment effect (%)
A	19.72 ± 0.21a	10.21 ± 0.23a	5.39 ± 0.13a	0.50 ± 0.01a	11.21 ± 1.27d	78.72 ± 2.29a
B	14.29 ± 0.18b	8.63 ± 0.12b	5.01 ± 0.11ab	0.46 ± 0.03ab	19.39 ± 0.63c	63.19 ± 2.48b
C	12.13 ± 0.32c	6.01 ± 0.19c	4.61 ± 0.19b	0.39 ± 0.05b	52.67 ± 0.82b	-
D	10.02 ± 0.24d	5.14 ± 0.20d	4.30 ± 0.21bc	0.32 ± 0.01bc	60.89 ± 1.17a	-

Treatment A: sprayed with 20 mL of fermentation solution of antagonist F1; Treatment B: sprayed with 20 mL of carbendazim solution; Treatment C: sprayed with 20 mL of sterile water as a blank control; Treatment D: inoculated with pathogenic bacteria alone. The same letter in each column indicates that the difference did not reach a significant level, while different letters indicate that the difference reached a significant level ($P < 0.05$)

Discussion

Bacteria are abundant microorganisms in the soil, many of which have plant growth-promoting properties known as PGPR (Lugtenberg et al., 2009). Their mechanisms of action include the production of indole-3-acetic acid (IAA), nitrogen fixation, solubilisation of soil phosphorus and the production of various nutrients as well as the production of iron carriers, cellulose, proteases, antibiotics and cyanide as antagonists to pathogens (Kumar et al., 2012). Plant inter-rooted growth promoters not only promote plant growth, thus reducing the use of chemical fertilisers and reducing environmental pollution hazards. Screening of antagonistic strain F1 from ginseng inter-rhizosphere soil for antagonism to ginseng root rot and rust rot and exploring whether this strain is a plant inter-rhizosphere promoting bacterium. In this study, the nitrogen fixation rate of the antagonist F1 was found to be 76.11 nmol/(h·ml). The ability of the antagonist F1 to produce indoleacetic acid IAA and iron carriers was determined using a spectrophotometer, with a secreted indoleacetic acid content of 25.30 mg/L and a relative ironophilic content of 50.32%. Seed germination tests showed that F1 fermentation solution at a concentration of 5×10^{-1} resulted in 100% germination of ginseng seeds, a 283.33% increase in embryo axis length and a brief vigour index 8.0% higher than that of the control. Himaman et al. (2016) screened three strains of *Streptomyces Actinomycetes* with a high broad spectrum of inhibition in soil roots, all producing IAA and iron carriers. Montanez et al. (2012) found that iron carriers produced by actinomycetes focused on promoting plant growth by trapping iron in the soil, stimulating plant growth capacity through their specific uptake system. Myo et al. (2019) reported that the plant growth hormone IAA, which can be produced by microorganisms through a tryptophan-dependent biosynthetic pathway, was found to have growth-promoting effects on plants. The results of the pot test for growth promotion capacity revealed that the root vigour of ginseng seedlings inoculated with F1 at different fermentation solution concentrations T1, T2 and T3 were enhanced by 37.29%, 117.80% and 61.02%, respectively, and the root vigour of ginseng seedlings treated with T4 chemical was enhanced by 20.34% compared with the control treatment; The total chlorophyll content of ginseng seedlings inoculated with F1 at different fermentation solution concentrations T1, T2 and T3 increased by 50.77%, 80.0% and 41.54%, respectively, while the total chlorophyll content of ginseng seedlings treated with T4 chemicals increased by 33.85%, the leaf MDA content of ginseng seedlings inoculated with different fermentation solution concentrations of F1 decreased by 7.06%, 29.41% and 12.16% for T1, T2 and T3 treatments respectively, while T4 chemical treatment decreased the leaf MDA content of ginseng plants by 4.31%. This indicates that inoculation with F1 fermentation broth liquid treatment has a

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protective effect on plants against photooxidative damage and inhibition (Cao et al., 2016). Toral et al. (2020) found that the biocontrol bacteria improved plant resistance to grey mould and reduced the MDA content by inducing a plant resistance response, which is consistent with the results of this study. The study also showed that the F1 fermentation solution had a significant pro-growth effect on ginseng seedlings.

Studies have shown that SOD, POD, CAT, PPO and PAL are closely related to plant disease resistance (Yan et al., 2021; Qin and Tian et al., 2005; Zhao et al., 2005). SOD and POD are the main protective enzyme systems in plants, involved in lignin synthesis, scavenging of free radicals, etc. An increase in their activity marks the accelerated synthesis of lignin and the development of resistance, and they are important enzymes associated with plant disease resistance (Wu et al., 2019). PAL is the key enzyme that catalyses the metabolism of phenylpropanoids, during which a series of secondary metabolites are formed to inhibit the invasion of pathogenic bacteria (Dinango et al., 2022). PPO is an enzyme that can oxidise phenols to quinones, which have toxic effects on the growth of pathogenic fungal hyphae (Boeckx et al., 2015). CAT is the main enzyme for the degradation of H_2O_2 in plants and maintains the in vivo balance of reactive oxygen species during plant defence reactions (Mehdy et al., 1994). The F1 fermentation solution was found to increase the activities of defence enzymes (SOD, POD, PAL, PPO and CAT) in the leaves of ginseng plants from pot experiments, and the most significant increase in the activities of SOD, POD, and PAL, PPO and CAT were observed at 60 d after treatment with the F1 fermentation solution of T2 antagonistic bacteria, which was significantly different from the control group, it was shown that the F1 fermentation broth T2 concentration treatment could act as an inducing factor to activate and enhance the activity of defence enzymes in ginseng plants, thereby enhancing the plant's resistance genes to root rot and rust rot.

Wu et al. (2022) studied the use of *Piriformospora indica* for promoting the growth of gerbera and enhancing root rot resistance, and fungal inoculation reduced MDA and H_2O_2 content in plant roots, which could retard MDA accumulation by preventing excessive ROS formation. After *P. indica* colonisation mitigated the damaging infection caused by *P. cryptogea*, the defence response of gerbera was enhanced by increasing the activity of SOD, CAT and POD, with POD appearing to play a major role. Yan et al. (2022) found that *Bacillus velezensis YYC* could enhance basal immunity in plants by increasing the activity of defence-related enzymes (e.g. PAL, POD and SOD). Xiao et al. (2022) effectively increased the content of PPO, POD, PAL, total phenols and flavonoids in grapes through GABA treatment of *S. pararoseus Y16*, thereby improving disease resistance and pathogen resistance. Wang et al. (2019b) found that treatment with *Yarrowia lipolytica* enhanced the activity of PPO, POD, CAT and PAL and improved the disease resistance of grapevines. Their results were consistent with the present study.

In greenhouse pot trials, the relative efficacy of F1 fermentation solution against ginseng root rot and rust rot reached 78.72%, with a disease index of 11.21. The relative efficacy of F1 fermentation solution against ginseng root rot and rust rot was 63.19% compared with that of the commonly used chemical pesticides, which was lower than that of the antagonistic bacterium F1 fermentation solution against ginseng, with a disease index of 19.39, The F1 fermentation solution was not only effective against root and rust rot of ginseng but also significantly promoted plant height and root length growth, with higher fresh and dry weights than the chemical and blank control treatments. The antagonistic bacterium F1 was effective against ginseng root rot, and its

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relative efficacy was 15.53% higher than that of the chemical group, thus verifying that the antagonistic bacterium F1 in the ginseng inter-root soil has anti-disease and growth-promoting effects and is an excellent strain for the development of antimicrobial agents. This study elucidates the growth-promoting mechanism of antagonistic bacteria F1 and provides a theoretical basis for the development of new microbial fertilisers or pesticides.

Conclusion

The antagonistic bacterium F1 Frankia, isolated and screened from ginseng inter-root soil, was able to fix nitrogen, secrete indoleacetic acid (IAA), a plant growth hormone, and an iron-producing carrier. F1 fermentation solution concentration had a germination rate of 100% on ginseng seeds, increased the root vigour and chlorophyll content of ginseng plants, reduced the content of malondialdehyde, and induced. The antagonistic bacterium F1 has a greater potential for biocontrol in promoting disease resistance.

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