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Potential biocontrol and plant growth promotion of an endophytic bacteria isolated from *Glycyrrhiza uralensis* seeds

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Abstract

Background: Endophytic bacteria have been demonstrated to be one of the most potentially important biocontrol agents for their efficiently improved plant growth and protection of host plants from infection by phytopathogens.

Results: Nine strains of endophytic bacteria isolated from *Glycyrrhiza uralensis* Fisch. (*G. uralensis*) seeds were screened for the inhibitory effect against 5 fungal phytopathogens by using dual culture method. Among which, the isolate FT₂ showed the highest percentage of inhibition to all the test fungal phytopathogens. Detached root assay showed that FT₂ significantly reduced disease index and rotted area on *Angelica sinensis* root slices caused by *Fusarium acuminatum*. FT₂ also exhibited multiple plant growth promotion traits by qualitative analytical method. Based on phenotypic, physiological and biochemical characterization and genotypic characterization, the FT₂ strain was identified as *Stenotrophomonas rhizophila*. Application of strain FT₂ reduced the disease index of Cucumber Fusarium Wilt, and the biocontrol effect was equal to chemical fungicide, carbendazim. Moreover, strain FT₂ increased the plant height, stem diameter, leaf number and fresh weight of cucumber seedlings and the activities of the defense-related enzymes including phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase. In addition, strain FT₂ could promote seed germination and seedling growth of *G. uralensis* and increase enzyme activities, available nutrient contents and bacterial count in *G. uralensis* rhizosphere soil.

Conclusions: The results demonstrated that the strain FT₂ could be used to develop environmentally friendly microbial products to improve the activity against pathogenic and promote plant growth.

Keywords: *Stenotrophomonas rhizophila*, Biocontrol, Growth promoting, *Glycyrrhiza uralensis* seeds, Endophytic bacteria

Background

Chemical pesticides play an important role in agriculture for crop losses due to fungal disease, long term or excessive use of these chemical pesticides which can lead to some negative impacts, such as environmental pollution, the emergence of drug-resistant pathogens and the

defect of human health. In order to eliminate the negative effects of chemical pesticides, it is necessary to find alternative approaches to control fungal diseases. The use of endophytic microorganisms to promote crop growth and biocontrol of fungal diseases has become a powerful tool and has attracted more and more attention from all over the world (Zhu et al. 2020).

Endophytic bacteria come from all plant tissues including roots, stems, leaves, fruits, seeds and meristems that is characterized by colonization within plant tissues and will not cause substantial damage to plants. Endophytic bacteria have been proven to be one of the most promising biocontrol agents because they can effectively

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improve plant growth and protect host plants from pathogens (Prasom et al. 2017). More recent reports have found that endophytic bacteria can effectively improve seed germination rate (Li et al. 2021), plant growth (Mohamed et al. 2020) and biomass accumulation (Ahmed et al. 2022). Moreover, endophytic bacteria settle in niches similar to plant pathogens and can communicate and interact with plants more effectively than rhizosphere bacteria, which make them suitable as biocontrol agents.

Plant seeds are not only important reproductive organs of plants, but also play an important role in agricultural production; compared with other parts, they are also characterized by carrying beneficial bacteria and pathogens (Shahzad et al. 2018). Studies have confirmed that there are abundant microbial populations on and inside seeds (Guo et al. 2021). Endophytic bacteria from seeds establish a strong connection with plants and have shown the beneficial effects on host plants (Adam et al. 2018). One of the main functions of seed endophytic bacteria was to protect plants from various pathogens (White et al. 2018). Moreover, they were reported to promote plant growth through direct and indirect effects. Direct effects refer to the capacity of nitrogen fixation, NH_3 production, dissolving organic or inorganic phosphorus and potassium and producing plant hormones such as indole acetic acid (IAA). Indirect effects refer to the growth metabolism secretion of extracellular enzyme, siderophores and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Although there have been several research reports on seed endophytic bacteria, the endophytic bacteria in seed are still not well understood than those in nutritive organs of the plant.

This study aimed to identify and screen the potential endophytic bacterial strain isolated from *G. uralensis* seeds with the strongest antagonistic activity in vitro, to study the plant growth promotion traits of potential strain and to evaluate the biocontrol efficiency and plant growth promotion effect of the strain, which will promote the development of seed endophytic bacteria in agricultural production.

Methods

Plant material

Glycyrrhiza uralensis seeds were collected from wild *G. uralensis* plants in Urad front flag (40°72'N; 108°65'E), Inner Mongolia, China, in September 2019. Healthy seeds were selected and stored in kraft paper bag at 4 °C until use.

Fungal phytopathogens

The five fungal phytopathogens used in this study, *Phyllosticta* sp., *Fusarium acuminatum*, *Botrytis cinerea* Pers.,

Fusarium oxysporum Sacc and *Scutellariae botrytis*, were all provided by Associate Professor Cuiyun Zeng, Gansu University of Chinese Medicine. They were isolated from *Dioscorea oppositifolia* L., *Anemarrhena asphodeloides*, *Radix Scutellariae*, *Cucumis sativus* L. and *Angelica sinensis*, respectively, and maintained as 50% (v/v) glycerol at − 70 °C for further use.

Isolation of endophytic bacteria

Glycyrrhiza uralensis seeds were steeped with 85% (v/v) H_2SO_4 for 45 min, sterilized with 0.1% (v/v) H_2O_2 for 10 min, rinsed in distilled water for 3 times and soaked in distilled water for more than 3 h at room temperature (Zhang et al. 2020b). Seeds were surface sterilized by stepwise washing in 75% (v/v) ethanol for 5 min, 30 s wash in 1% (v/v) H_2O_2 , 10 s wash in 5% (v/v) NaClO and subsequent rinsing in sterile distilled water for ten times. To evaluate the success of sterilization, 100 µl of the water from the last rinse was inoculated on NA medium (nutrient agar medium; beef extract 3.0 g, proteose peptone 10.0 g, NaCl 5.0 g, agar 20 g, d.H₂O 1 l, pH 7.2) at 28 °C for 2–7 days. No microbial growth on NA medium indicated that surface sterilization was efficient. The surface-sterilized seeds were ground in a mortar and diluted up to 10 ml in sterile water. Tissue particles were left for 30 min at 4 °C, and the obtained suspension (200 µl) was spread onto NA medium. After culturing for 2–7 days at 28 °C, single colonies were picked and purified by repeated streaking and microscopic examination. The pure cultures were maintained in NA medium slants at 4 °C and as 50% (v/v) glycerol at − 80 °C for further use.

In vitro antagonistic activity

Dual culture assay

In vitro antagonistic activity of bacterial isolates was evaluated by dual culture method on PDA medium (potato dextrose agar medium; potato infusion 200 g, dextrose 20 g, agar 20 g, d.H₂O 1 l). A 6-mm mycelial plug was removed from an actively growing plate of each fungal phytopathogen and placed on the center of a fresh PDA medium plate (90 mm in diameter). Approximately a 1.5 cm from the plug, a 2-day-old bacterial isolate was streaked on opposite sides of the mycelial plug. Control group plates inoculated only with each fungal phytopathogen. Dual culture plates and control plates were incubated at 28 °C up to 5–7 days. The percentage of inhibition was calculated by following formula:

$$\text{Inhibition (\%)} = (R_1 - R_2) / R_1 \times 100$$

where R_1 is the radius of control plate and R_2 is the radius of dual culture plate. The experiment was repeated 3 times with 3 independent replications.

A bacterial isolate with the highest percentage of inhibition against 5 fungal phytopathogens was evaluated using the method described above and selected for all subsequent studies. The experiment was repeated 3 times with 3 independent replications.

Detached root assay against *F. acuminatum*

Angelica sinensis is an herbaceous perennial plant with high medicinal value. Root rot, caused by fungi pathogens such as *Fusarium* spp., is a ubiquitous disease that seriously harms the output and quality of *A. sinensis* (Mi et al. 2017). The bacterial isolate FT₂ showed the strongest antagonistic activity in the dual culture assay, and thus, detached root assay was performed to evaluate the antagonistic activity of FT₂ against *A. sinensis* root rot caused by *F. acuminatum*.

Fusarium acuminatum was activated on PDA medium and cultured in the dark at 22 °C for 7 days. The spores were washed with sterile water and made into a suspension of 10⁶ cfu/ml (Mi et al. 2017). The bacterial isolate FT₂ was incubated in NA liquid medium at 28 °C for 48 h. The resultant bacterial culture was centrifuged (10 min at 9000 rpm) in sterile distilled water and then re-suspended in sterile distilled water to a final OD₆₀₀ of 1.0, which is approximately to a final concentration of 10⁸ cfu/ml.

For surface sterilization, the healthy roots of *A. sinensis* were washed with running water for 30 min, soaked in 70% (v/v) ethanol for 30 s and then in 3% (v/v) sodium hypochlorite for 3 min and washed with sterile distilled water for 5 times. The surface-treated roots were cut into 5 mm slices with a sterile scalpel and placed on plates containing filter paper moistened with 1 ml sterilized water (Mi et al. 2017). Five treatments were included: (i) Control, roots slices were inoculated with sterilized water only; (ii) Fa only, roots slices were inoculated with *F. acuminatum* only; (iii) FT₂ only, roots slices were inoculated with the bacterial isolate FT₂ only; (iv) Fa + FT₂, roots slices were inoculated with a mixture of the bacterial isolate FT₂ and *F. acuminatum*; and (v) Fa + Pre-FT₂, roots slices were pre-inoculated with the bacterial isolate FT₂, followed by inoculation of *F. acuminatum* after 24 h of incubation. A 40 µl spore suspension or bacterial suspension was applied to the roots slices, and control was treated with the same amount of distilled water and then incubation at 22 °C, 50% RH for 3 days. There were 4 slices per plate and 3 plates for each treatment. The assay was performed with 3 replicates.

Disease severity was recorded on a 0–5 visual scale according to Tian et al. (2021), which 0=no symptoms, 1=less than 20% root slice area rotted, 2=21%–40% root slice area rotted, 3=41%–60% root slice area rotted, 4=61%–80% root slice area rotted, and 5=more

than 80% root slice area rotted. The disease index (DI) and biocontrol effect (BE%) were calculated by following formula:

$$DI (\%) = \sum (ab)/AB \times 100$$

where *a* represents the number of root slices with the same disease severity scale, *b* represents the disease severity scale, *A* represents the total number of root slices, and *B* represents the highest disease severity scale.

$$BE (\%) = (PT - BT) / PT \times 100$$

where PT represents the disease index for the pathogen treatment and BT represents the disease index for the bacterial isolate plus pathogen treatment.

Identification of endophytic bacteria

Phenotypic and physiological characterization

The bacterial isolate from overnight raised culture in NA medium was streaked in LB medium (Luria–Bertani medium; tryptone 10 g, yeast extract 5 g, NaCl 10 g, agar 20.0 g, d.H₂O 1 l, pH 7.4) and incubated for 2–4 days at 28 °C. Colony morphology was analyzed based on their shape, color and other characteristics (form, margin and transparency) on LB medium.

Growth tests for pH range were carried out by using NA medium adjusting the pH to 2, 5, 7, 9, 11 with 1 mol/l HCl or 1 mol/l KOH after sterilization. In order to study the temperature growth range, the overnight culture was spotted on the NA medium plates and incubated at different temperatures (4, 10, 15, 20, 28, 37 and 42 °C) for 2–4 days and then observed for positive growth. NaCl tolerance test for growth was performed using NA medium supplemented with different concentrations of NaCl (0, 3, 5, 7, 10 and 15%, w/v) and incubated at 28 °C for 2–4 days. Furthermore, the utilization of sole carbon sources, such as D-mannitol, D-galactose, sucrose, D-maltose, D-glucose, D-fructose, D-xylose and D-sorbitol, and the utilization of sole nitrogen sources, such as glutamine, histidine, urea, glycine and ammonium sulfate, were analyzed as the described method (Abo-Elyousr et al. 2021). All the experiments were performed in triplicate.

Biochemical characterization

Urease activity and lipase activity were assayed according to method reported by Guenoun et al. (2018). Peptone and coagulation of milk (20%, w/v skimmed milk), liquefaction of gelatin (20%, w/v) and production of H₂S were examined as described previously (Chen et al. 2021a). Indole test was performed according to the previous description (Avinash and Rai 2014). Citrate utilization, starch hydrolysis and oxidase activity were also carried

out. Other biochemical characteristics were done by following Bergey's Manual of Determinative Bacteriology (Holt et al. 1994). All tests were carried out in triplicate.

Genotypic characterization and identification

DNA extraction and PCR conditions

Genomic DNA was extracted using the genomic DNA extraction kit from Shanghai Sangon Biotech. 16S rRNA sequence of the extracted DNA was amplified by using the universal primer 27F (AGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT). Final PCR reaction was carried out in 50 µl reaction mixture containing 20–100 ng DNA template, 0.5 U Taq DNA polymerase, 5 µl 10 × buffer, 2 µl dNTP Mix (10 mM) and 2 µl of each primer (10 µM). Amplification of the 16S rRNA genes was performed in a thermal cycler according to the following steps: 94 °C initial denaturation for 4 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR product was analyzed by gel electrophoresis on 1.0% (w/v) agarose gel.

16S rRNA sequencing and phylogenetic analysis

The PCR product electrophoresis band cut the desired DNA target electrophoresis band, and the PCR product was directly sequenced with PCR primers. The 16S rDNA sequence was aligned on the ribosome database, and the obtained sequence was submitted to the National Centre for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>). The BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>) on the NCBI database was used to compare the obtained sequence with the sequence of the reference strain stored in the public database. The computer software package ClustalW was used for sequence alignment,

and the maximum compound likelihood model was used to analyze by the neighbor-joining method, and finally, the tree constructions were performed with the MEGA 7.0 version software.

Screening for plant growth-promoting traits

Plant growth-promoting traits of the isolated strain FT₂ were studied through the following standard tests. All tests were performed in triplicate.

IAA production

The production of IAA was examined by Salkowski's reagent. The test bacterial strain was inoculated into 250 ml NA liquid medium containing L-tryptophan (0.5 mg/ml) and cultured at 28 °C and 180 r/min for 3 days. After the bacterial culture was centrifuged at 9000 rpm for 10 min, 2 ml of the supernatant was mixed with 4 ml Salkowski's

reagent (0.5 mM FeCl₃ in 50 ml H₂SO₄) and incubated at room temperature in dark condition for 30 min. The appearance of the red color indicated the production of IAA, which was quantified using an ultraviolet spectrophotometer at 530 nm. Un-inoculated medium was used as control. A calibration curve was established using pure IAA and expressed as milligram per liter.

Siderophore production

The production of siderophore was qualitatively estimated on Chrom Azurol S (CAS) agar medium. The bacterial strain was spotted on CAS agar medium and incubated at 28 °C for 2–3 days. Appearance of yellow-orange halos around the colonies was considered to be a positive test for siderophore production.

Nitrogen fixation

The Ashby nitrogen-free solid medium was inoculated with strain grown on NA medium for 2 days and cultured in a constant temperature incubator at 28 °C for 1–2 days. The strain could grow on selective medium and proved that it had the ability to fix nitrogen.

Phosphate solubilization

A two-day-old bacterial strain was spot inoculated on inorganic phosphorus medium containing 0.05% (NH₄)₂SO₄, 0.03% NaCl, 0.03% MgSO₄·7H₂O, 0.003% MnSO₄, 0.03% KCl, 0.003% FeSO₄·7H₂O, 0.5% Ca₃(PO₄)₂, 1% glucose and 2% agar and organic phosphorus medium containing 0.05% (NH₄)₂SO₄, 0.03% NaCl, 0.03% MgSO₄·7H₂O, 0.003% MnSO₄, 0.03% KCl, 0.003% FeSO₄·7H₂O, 1% glucose, 0.5% CaCO₃, 0.02% lecithin and 2% agar. The formation of a clear zone around the bacterial colonies indicated the ability to solubilize phosphates (inorganic/organic P).

Potassium solubilization

Potassium solubilization was tested by spotting the strain on potassium solubilization agar medium containing 0.5% sucrose, 0.2% Na₂HPO₄, 0.05% MgSO₄·7H₂O, 0.0005% FeCl₃, 0.01% CaCO₃, 0.1% potassium feldspar powder and 1.8% agar. After culturing at 28 °C for 5–7 days, a transparent hydrolysis circle around the bacteria means the result was positive.

NH₃ production

The bacterial strain was inoculated into 100 ml of peptone water (peptic digest 10 g, NaCl 5 g, d.H₂O 1 l, pH 7.6) and cultured at 28 °C for 2 days, and then, 0.5 ml of Nessler's reagent (K₂HgI₄ and NaOH) was added. The color change of peptone water (from brown to yellow) indicated the production of NH₃.

ACC deaminase production

The ability of the bacterial strain to utilize ACC as the sole nitrogen source was analyzed. The strain was inoculated on Dworkin and Foster (DF) salt minimal medium, which contained 4 g KH_2PO_4 , 6 g Na_2HPO_4 , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 μg H_3BO_3 , 10 μg MnSO_4 , 70 μg ZnSO_4 , 50 μg CuSO_4 , 10 μg MoO_3 , 2 g glucose–gluconic acid, 2 g citric acid, 2 g $(\text{NH}_4)_2\text{SO}_4$ and 20 g agar. The presence of bacterial growth on the media was deemed positive for ACC deaminase production.

Extracellular enzyme production

Strain was tested further for its ability to produce the following enzymes:

Protease Protease activity was assayed by spotting the bacterial strain on Skim Milk Agar medium (skim milk powder 15 g, agar 20 g, $\text{d.H}_2\text{O}$ 1 l). After 2 days incubation at 28 °C, a clear zone around the colony indicated positive result (Abdel-Kareem et al. 2021).

Cellulase Cellulase activity was determined using CMC agar medium (CMC-Na 10 g, KNO_3 2 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g, NaCl 0.5 g, K_2HPO_4 1 g, agar 20 g, ddH_2O 1 l). After culturing at 28 °C for 2 days, the plates were stained with 5 ml of 0.1% (w/v) Congo red for 1 h at room temperature. Then, the plates were washed twice with 1 M NaCl for 10 min. A clear hydrolysis zone around the colony was deemed positive for cellulase production.

Biocontrol of Cucumber *Fusarium* Wilt

Cucumber (*Cucumis sativus* L.) is an important vegetable crop. However, the growth of this crop is often threatened by Cucumber *Fusarium* Wilt, which is a common fungal disease caused by *Fusarium* spp. (Zhao et al. 2012). A pot experiment was designed to assess the biocontrol efficiency of strain FT₂ against Cucumber *Fusarium* Wilt caused by *F. oxysporum* Sacc.

Preparation of cucumber seedlings and spore suspension

One-month-old cucumber seedlings of susceptible cultivar Jinchun No. 4 were used in this experiment. Cucumber seedlings with similar size were transplanted to pots (12.5 × 8.5 cm) filled with a mixture of sterilized sand, coconut fiber, perlite and peat (2:1:1:1, w/w). These pots, each containing one transplant, were placed in a climate chamber at 25 °C, with 50% RH, and a photoperiod of 12/12 h (light/dark). Seedlings were watered when needed.

Fusarium oxysporum Sacc was grown on PDA medium at 25 °C for 7 days, and the grown mycelium was washed

with 10 ml sterile water to create spore suspension. Spore suspension was calculated by using a hemocytometer and adjusted for 10⁷ spores/ml by serial dilutions with sterile water.

Experimental design

In this experiment, cucumber seedlings with fully expanded true leaves were used. The experiment was adopted a completely randomized designs, with 4 treatments and four replicates. The 4 treatments were as follows: (i) Untreated control (UC), plants were inoculated with distilled water only; (ii) Pathogen control (PC), plants were inoculated with pathogen only; (iii) Pathogen + chemical fungicide (PF), plants were inoculated with pathogen and the chemical fungicide; and (iv) Pathogen + FT₂ (PT), plants were inoculated with pathogen and bacterial isolate FT₂. 50 ml of *F. oxysporum* spore suspension was pre-inoculated in PC, PF and PT treatments. Two days after the pathogen incubation, 50 ml of FT₂ bacterial suspension (10⁸ cfu/ml) or the chemical fungicide (carbendazim, 1.25 g/l) was applied to the roots by drenching, and untreated control plants were treated with the same amount of distilled water (Zhang et al. 2021). During the inoculation, plant roots were slightly injured by a sterile needle.

Disease assessment

The development of *Fusarium* wilt on seedlings was evaluated 30 days after pathogen inoculation. The wilting development of each cucumber plant was rated on a scale of 4 (Chen et al. 2012): 0 = whole plant was healthy; 1 = less than 25% of leaves wilted; 2 = 26–50% of leaves wilted; 3 = more than 50% of leaves wilted; and 4 = whole plant died. Disease severity index (DSI) and biocontrol effect (BE) were calculated using the following formula (Chen et al. 2012):

- Disease severity index (%) = $\left[\frac{\sum (\text{scale} \times \text{number of plants with the same scale})}{(\text{total number of plants} \times \text{highest scale})} \right] \times 100$
- Biocontrol effect (%) = $\left(\frac{\text{disease severity index of pathogen control} - \text{disease severity index of the treatment}}{\text{disease severity index of pathogen control}} \right) \times 100$

Growth parameters determination

Destructive sampling was carried out after disease assessment. The plants under different treatments were cleaned with tap water and absorbed surface water with filter paper, and the plant height, stem diameter, leaf number and fresh weight were measured. The plant height was measured with a ruler, and the stem diameter was

measured with a vernier caliper. An electronic scale was used to measure the fresh weight.

Defense-related enzyme activities determination

At the end of the experiment, healthy leaves with different treatments were collected to determine the defense-related enzyme activities. Phenylalanine ammonia-lyase (PAL) activity was assayed by following the method described by Liu et al. (2017) with some modifications. The reaction mixture containing 0.02 M L-phenylalanine 1 ml, 0.1 M borate buffer (pH 8.8) 2 ml and enzyme extract 0.1 ml was incubated at 30°C for 30 min. 200 μ l 6 M HCl was added to terminate the reaction, and the absorbance of the solution was measured at 290 nm. One unit represented the conversion of 1 μ mol L-phenylalanine to cinnamic acid for 1 g of fresh weight per minute.

Polyphenol oxidase (PPO) activity was measured as described by Feng et al. (2021) with a slight modification. The reaction consisted 0.1 ml enzyme extract, 3.9 ml 0.05 mM phosphate buffer (pH 5.5) and 1 ml 0.1 M catechol. 2 ml 20% (w/v) TCA was added to terminate the reaction, and the absorbance of solution was measured at 525 nm. One unit of PPO activity was presented as the change of 0.01 in absorbance at 525 nm for 1 g fresh weight per minute.

Peroxidase (POD) activity was measured as described by Fang et al. (2018) with some modification. The reaction mixture containing 19 μ l 50 mM guaiacol, 50 ml 0.2 M sodium phosphate buffer (pH 6.0), 28 μ l 30% (v/v) H₂O₂ and 1 ml enzyme extract. One unit of enzyme activity was defined by a change in absorbance of 0.01 at 470 nm for 1 g of fresh weight per minute.

Plant growth promotion

Glycyrrhiza uralensis, as a perennial herb of the genus *Leguminosae*, is a commonly used Chinese herbal medicine. The strain FT₂ was evaluated for its growth promotion effect on *G. uralensis* by the following experiments.

Seed germination experiment

Glycyrrhiza uralensis seeds were steeped with 85% H₂SO₄ for 2.5 h, soaked in 0.1% (v/v) H₂O₂ for 10 min, washed 10 times with sterile distilled water and air-dried. Seeds were soaked in the bacterial suspension of strain FT₂ for 6 h (control group seeds were soaked in sterile distilled water for the same period of time), placed in Petri dishes (diameter 90 mm) containing filter paper moistened with sterilized water and incubated at room temperature. Treated seeds were watered regularly until germination was completed. The number of germinated seeds was recorded every 24 h from the 2nd day, and the germination number remaining unchanged for 2 consecutive days was determined as the time germination was

completed. Each treatment was replicated 4 times with 30 seeds constituting one replication in the germination experiment. Germination rate, germination energy, germination index and seedling vigor index were calculated using the following formula:

- Germination rate (%) = $n/N \times 100$, where n is the number of germinated seeds and N represents the total number of tested seeds
- Germination energy (%) = (the number of seeds germinated that reach the highest peak) / (the number of seeds at the beginning of the experiment) $\times 100$
- Germination index = $\sum(Gt/Dt)$, where Gt is the number of seeds germinated at time t, and Dt represents the corresponding germination to date
- Seedling vigor index = seedling length (cm) \times germination index

Seedling growth experiment

This used the same procedure above for seed surface disinfection. The seeds were immersed in sterile water for 6 h and evenly sown in boxes (12 \times 12 \times 6 cm) filled with 630 g high-pressure sterilized and fully dried sand medium collected from native desert regions which do not contain any nutrients and pre-irrigated with 100 ml of distilled water. After growth for 20 days, seedlings were inoculated with 50 ml of bacterial suspension of strain FT₂. Sterile water was used as control. Each treatment had 5 repeats with 20 seeds per repeat. Boxes were positioned in a climate chamber (28 °C, 12/12 h light/dark, RH 50%). Seven days after the inoculation, growth parameters of seedlings, enzyme activities, available nutrient contents and bacterial count in rhizosphere soil were analyzed.

Growth parameters determination The stem and root lengths were measured by a ruler, and the stem and root diameters were measured by a vernier caliper. After placing them in a 55 °C oven for 48 h, their dry weights were also recorded using an analytical balance.

Soil enzyme activity determination The rhizosphere soil samples were sieved (1-mm) before analyzed. The activities of soil urease, phosphatase, saccharase and catalase were separately measured.

Saccharase activity was assessed by Guan (1986) technique, with slight changes. Briefly, 5 g of soil was mixed with 15 ml 8% (w/v) sucrose, 5 ml phosphate buffer (pH 5.5) and 0.25 ml toluene. The mixture was incubated at 37 °C for 24 h and then measured on a spectrophotometer at 508 nm. The results were expressed as mg of glucose released by 1 g of soil in 24 h.

Soil urease activity was assayed according to the method by Guan (1986), and the results were expressed as mg of released $\text{NH}_3\text{-N}$ by 1 kg of soil per hour at 37 °C.

Catalase activity was determined using the technique of Guan (1986) with slight changes. Briefly, 5 g of soil with 40 ml distilled water and 5 ml 0.3% H_2O_2 (v/v) was added into centrifuge tubes and then shaken for 30 min. The filtrate was titrated with 0.1 mol/l KMnO_4 . The results were expressed as 0.1 mol/l KMnO_4 /100 g/hrs.

Acid phosphatase activity was assessed with minimal changes, according to Guan (1986). Briefly, 5 g of soil was incubated in acetate buffer (pH 5.0) at 37 °C for 12 h. 5 ml buffer solution and a 2 ml filtrate were transferred into a 50-ml volumetric flask and then diluted to 50 ml with distilled water, and the phenol released at 600 nm was measured by a spectrophotometer. Acid phosphatase activity was expressed as mg hydrolyzed phenol by 1 kg soil per hour at 37 °C.

Soil available nutrient content and bacterial count determination The available nitrogen was determined by the alkali diffusion method, and available phosphorus was measured by sodium bicarbonate extraction–molybdenum blue method (Chen et al. 2021b). The rhizosphere soil bacterial count was determined by the dilution plate method of Lin (2010).

Statistical analysis

SPSS 26.0 was used for statistical analysis of the data. Differences among means were tested using the least significant difference (LSD) test, $P < 0.05$ representing a significant difference. Mean values and standard errors (SE) were shown in figures and tables. Bar diagrams were prepared using Microsoft Excel.

Results

Evaluating antagonistic activity of endophytic bacteria isolated from *G. uralensis* seeds in vitro

No microbial growth was observed on the control plate, indicating that the surface sterilization procedure was efficient. A total of 9 morphologically distinct bacterial isolates (designated as strain FZ₁, FZ₂, FZ₄, FZ₅, FG₂, FG₄, FG₅, FG₆ and FT₂) were successfully obtained from the *G. uralensis* seeds. Of the 9 isolates screened by using dual culture method, FT₂ exhibited the highest percentage of inhibition against 5 fungal phytopathogens. The percentages of the mycelial growth inhibition were 68.57, 88.57, 55.56, 82.50 and 87.10% toward *Phyllosticta* sp., *Fusarium acuminatum*, *Botrytis cinerea* Pers., *Fusarium oxysporum* Sacc and *Scutellariae botrytis*, respectively (Table 1). FT₂ was evaluated again, and the result showed that it had positive inhibitory effects on mycelial growth of all the test fungal phytopathogens to more than 60% (Fig. 1). Based on this result, the bacterial isolate FT₂ was used for all subsequent studies.

Fusarium acuminatum caused rotting of all the detached root slices at 3 days after inoculation. The rotted area on *A. sinensis* root slices was significantly reduced in treatments of FT₂ combined application or pre-application compared with the treatment of *F. acuminatum* when was applied only (Fig. 2). Moreover, pre-application of FT₂ produced stronger antagonistic activity against *F. acuminatum* where the disease index and biocontrol effects were 6.67 and 93.33%, respectively (Table 2). No rotted root slices were observed when FT₂ was applied alone, and no rot developed in sterilized water treatment (Fig. 2).

Table 1 In vitro antagonistic activity of 9 endophytic bacteria isolated from *Glycyrrhiza uralensis* seeds against 5 fungal phytopathogens

| Strain | Percentage of inhibition | | | | |
|-----------------|--------------------------|----------------------------|-------------------------------|--------------------------------|------------------------------|
| | <i>Phyllosticta</i> sp. | <i>Fusarium acuminatum</i> | <i>Botrytis cinerea</i> Pers. | <i>Fusarium oxysporum</i> Sacc | <i>Scutellariae botrytis</i> |
| FZ ₁ | 51.43 c | – | 35.56 cd | 52.50 e | – |
| FZ ₂ | 51.43 c | 45.71 e | 37.78 c | 80.00 b | 87.10 a |
| FZ ₄ | 48.57 cd | 40.00 f | 35.56 cd | 35.00 f | 83.87 ab |
| FZ ₅ | 45.71 d | – | 42.22 b | – | – |
| FG ₂ | 57.14 bc | 82.86 b | 53.33 ab | 82.50 ab | 83.87 ab |
| FG ₄ | 60.00 b | 74.29 c | 40.00 bc | 65.00 c | 74.19 b |
| FG ₅ | 51.43 c | 71.43 cd | 37.78 c | 85.00 a | 67.74 c |
| FG ₆ | 57.14 bc | 65.71 d | 35.56 cd | 57.50 d | 70.97 bc |
| FT ₂ | 68.57 a | 88.57 a | 55.56 a | 82.50 ab | 87.10 a |

“–” denotes no activity. Means followed by the different letters within each column are significantly different at $P < 0.05$

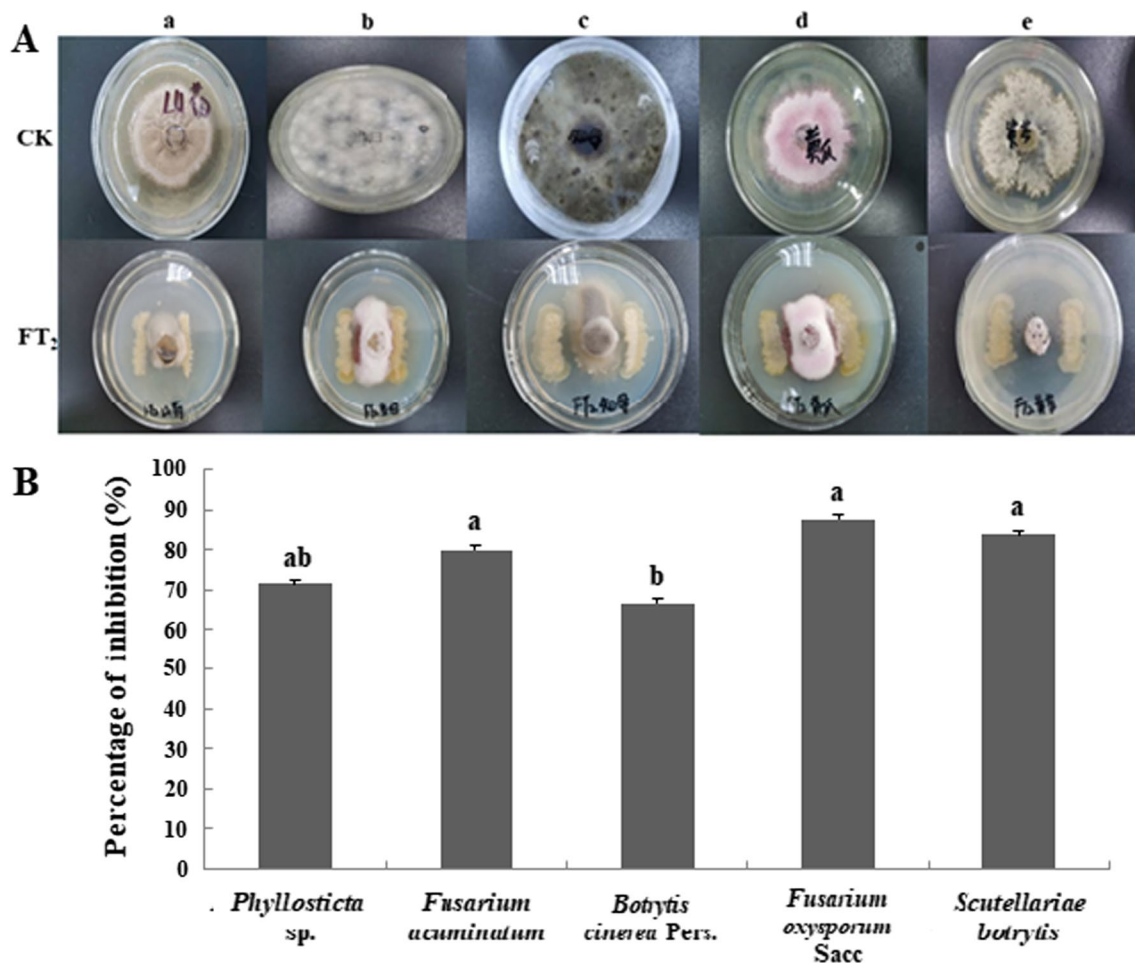


Fig. 1 In vitro antagonistic activity of strain FT₂ against 5 fungal phytopathogens. **A:** Inhibitory effect of strain FT₂ on mycelial growth of 5 fungal phytopathogens on the dual culture plates. **a:** *Phyllosticta* sp.; **b:** *Fusarium acuminatum*; **c:** *Botrytis cinerea* Pers.; **d:** *Fusarium oxysporum* Sacc; and **e:** *Scutellariae botrytis*. CK, control; FT₂, strain FT₂. **B:** Percentage of inhibition of strain FT₂ against 5 fungal phytopathogens. In the same parameter, the different letters within the different treatment represent significant differences at $P < 0.05$

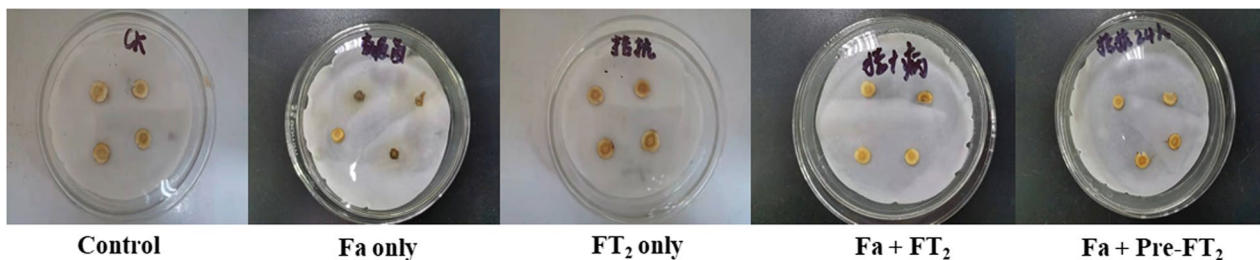


Fig. 2 Rotting symptoms of *Angelica sinensis* detached root slices under different treatments. Control, roots slices were inoculated with sterilized water only; Fa only, roots slices were inoculated with *Fusarium acuminatum* only; FT₂ only, roots slices were inoculated with the bacterial isolate FT₂ only; Fa + FT₂, roots slices were inoculated with a mixture of the bacterial isolate FT₂ and *F. acuminatum*; and Fa + Pre-FT₂, roots slices were pre-inoculated with the bacterial isolate FT₂ followed by inoculation of *F. acuminatum* after 24 h of incubation. The experiment was performed with 3 replicates

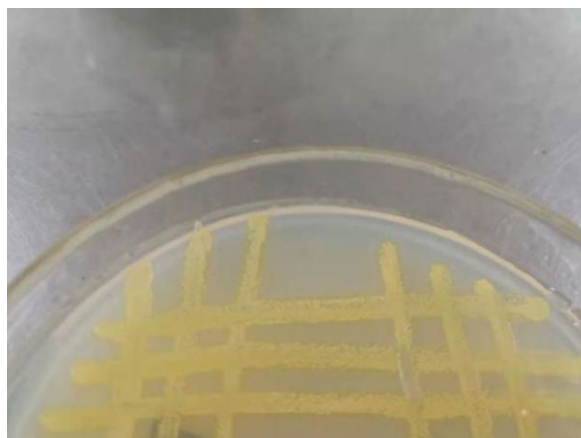
Table 2 Evaluation of antagonistic activity of bacterial isolate FT₂ against *Fusarium acuminatum* on detached *Angelica sinensis* root slices

| Treatment | DI (%) | BE (%) |
|--------------------------|---------|---------|
| Control | 0.00 | – |
| Fa only | 100.00 | 0.00 |
| FT ₂ only | 0.00 | – |
| Fa + FT ₂ | 13.33 a | 86.67 b |
| Fa + Pre-FT ₂ | 6.67 b | 93.33 a |

Control, roots slices were inoculated with sterilized water only; Fa only, roots slices were inoculated with *F. acuminatum* only; FT₂ only, roots slices were inoculated with the bacterial isolate FT₂ only; Fa + FT₂, roots slices were inoculated with a mixture of the bacterial isolate FT₂ and *F. acuminatum*; and Fa + Pre-FT₂, roots slices were pre-inoculated with the bacterial isolate FT₂ followed by inoculation of *F. acuminatum* after 24 h of incubation. DI, disease index; BE, biocontrol effect. –, no rotted root slices were recorded in the treatment. The experiment was performed with 3 replicates, and different letters in the same column represent significance at $P < 0.05$

Identification of the isolated bacterial strain FT₂

For the isolated bacterial strain FT₂, the phenotypic, physiological and biochemical characteristics were described as follows: faint yellow, round to irregular colony shape with rough and opaque surface (Fig. 3). The pH range was 5.0–9.0, and the optimum growth at pH 7.0. Growth occurred at 15–37 °C, and the optimum growth at 28 °C. Tolerate of NaCl was up to 15%. The utilization of D-mannitol, D-galactose, sucrose, D-maltose, D-glucose and D-fructose had the reaction of positive, and D-xylose and D-sorbitol had the reaction of negative; the utilization of glutamine and histidine had the reaction of positive, and urea, glycine and ammonium sulfate had the reaction of negative. Positive reactions were observed for urease, catalase and oxidase, and negative reactions were observed for lipase and amylase production. H₂S production, citric acid and malonic acid had the activities

**Fig. 3** Colony morphology of the isolated bacterial strain FT₂

of positive, and gelatin liquefaction, nitrate reduction, peptone and coagulation of milk had negative (Table 3).

Moreover, part of the 16S rRNA gene of the bacterial isolate FT₂ was also amplified, and the amplified product was 1481 bp. BLASTn search showed that the similarity between FT₂ and *Stenotrophomonas rhizophila* strain DSM14405 was 100% (GenBank accession number CP007597). A phylogenetic tree was constructed by using the NJ method with MEGA7.0 software. The result showed that FT₂ belonged to the genus, *Stenotrophomonas* (Fig. 4). Therefore, this bacterial isolate was named as *Stenotrophomonas rhizophila* FT₂, and the partial 16S rRNA sequence was submitted to the GenBank database (<http://www.ncbi.nlm.nih.gov/GenBank>) under accession number No. MW821464.

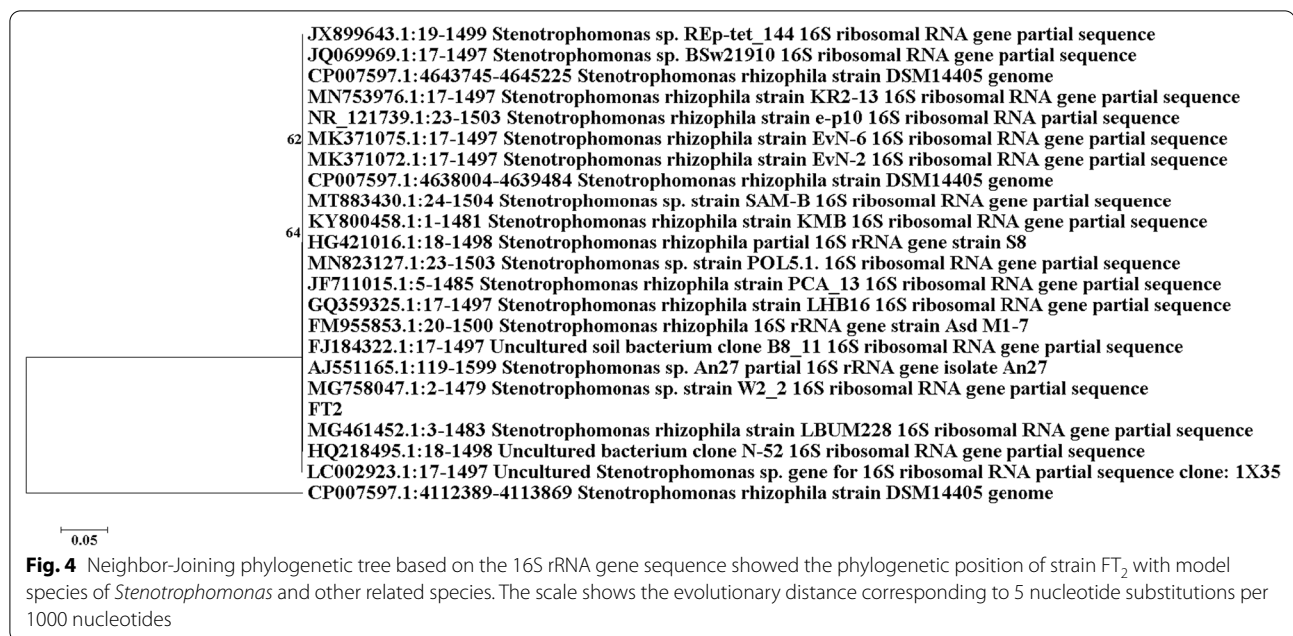
Plant growth-promoting traits of *S. rhizophila* FT₂

The screening results of plant growth-promoting traits are shown in Table 4. *S. rhizophila* FT₂ was positive for production of IAA (8.732 mg/l) and siderophore. *S. rhizophila* FT₂ could grow on a Ashby nitrogen-free solid medium and grow well, indicating that the strain had a certain nitrogen-fixing ability. *S. rhizophila* FT₂ had obvious transparent circles on the inorganic phosphorus medium, indicating that the strain had a strong ability to dissolve inorganic phosphorus, and there was no obvious

Table 3 Physiological and biochemical characteristics of the bacterial strain FT₂ isolated from *Glycyrrhiza uralensis* seeds

| Characteristic | Result | Characteristic | Result |
|---------------------------------|--------|-----------------------------|--------|
| pH range | 5–9 | Carbon source utilization | |
| Optimum pH | 7.0 | D-mannitol | + |
| NaCl range (% w/v) | 0–15 | D-galactose | + |
| Temperature range (°C) | 15–37 | D-xylose | – |
| Optimum temperature (°C) | 28 | Sucrose | + |
| Lipase | – | D-maltose | + |
| Urease | + | D-glucose | + |
| Catalase | + | D-fructose | + |
| Oxidase | + | D-sorbitol | – |
| Amylase | – | Nitrogen source utilization | |
| Gelatin liquefaction | – | Histidine | – |
| Nitrate reduction | – | Glutamine | + |
| H ₂ S production | + | Glycine | – |
| Citrate utilization test | + | Urea | – |
| Peptone and coagulation of milk | – | Ammonium sulfate | + |
| Malonate utilization test | + | | |
| Methyl red test | – | | |
| Indole test | + | | |
| Voges–Proskauer test | – | | |

"+" denotes positive results; "–" denotes negative results

**Table 4** Plant growth-promoting traits of *Stenotrophomonas rhizophila* FT₂

| Plant growth-promoting traits | | | | | | | | | |
|-------------------------------|---------------|------------------------|----------------------------|--------------------------|------------------------------------|----------------------------------|-------------------|----------------------|---------------------|
| IAA* (mg/l) | ACC deaminase | Siderophore production | NH ₃ production | Potassium solubilization | Inorganic phosphate solubilization | Organic phosphate solubilization | Nitrogen fixation | Cellulase production | Protease production |
| 8.732 ± 0.02 | + | + | + | + | — | + | + | + | + |

*IAA production is expressed as mean ± standard error with 3 repeats. "+" indicates positive reaction; "—" indicates negative reaction

transparent circle on the organic phosphorus medium, indicating that the strain had no strong ability to dissolve organic phosphorus. *S. rhizophila* FT₂ was able to solubilize potassium and was positive for ACC deaminase, NH₃ and extracellular enzyme production (Fig. 5).

Biocontrol efficiency of *S. rhizophila* FT₂ against *Cucumber Fusarium Wilt*

Pot experiment demonstrated that inoculation of cucumber seedlings with *F. oxysporum* significantly decreased all tested growth parameters. Compared to the untreated control and fungicide, the stem diameter, plant height, leaf number and fresh weight of seedlings treated with *S. rhizophila* FT₂ were not significantly changed (Table 5).

Experiment carried out in cucumber plants also revealed the suppression effect of *S. rhizophila* FT₂ on *Cucumber Fusarium Wilt* caused by *F. oxysporum*. On plants of pathogen control, the initial symptoms appeared on the stems and then quickly spread to the aboveground part of the plants, accompanied by wilting of the whole plants, where the disease severity index was 88.7%. However, in plants under *S. rhizophila* FT₂ treatment, the

disease severity index was decreased, and the biocontrol effect reached 60.5%. The disease severity index and biocontrol effect of *S. rhizophila* FT₂ treatment were equal to that of fungicidal treatment (Fig. 6 and Table 5).

To achieve the effect of *S. rhizophila* FT₂ in suppression of *Cucumber Fusarium Wilt* one step further, the activities of defense-related enzymes PAL, PPO and POD were investigated. Activities of PAL, PPO and POD in cucumber seedlings treated by *S. rhizophila* FT₂ were significantly increased in comparison with untreated seedlings. *S. rhizophila* FT₂-treated seedlings showed a significant increase in PAL and POD enzyme activities when compared to pathogen control and fungicide. POD enzyme activity was enhanced almost equally in *S. rhizophila* FT₂, fungicide and pathogen control in comparison with untreated control (Fig. 7).

Effect of *S. rhizophila* FT₂ on *G. uralensis* seed germination and seedling growth

As shown in Fig. 8, the germination rate, energy, index and seedling vigor index of *G. uralensis* seeds

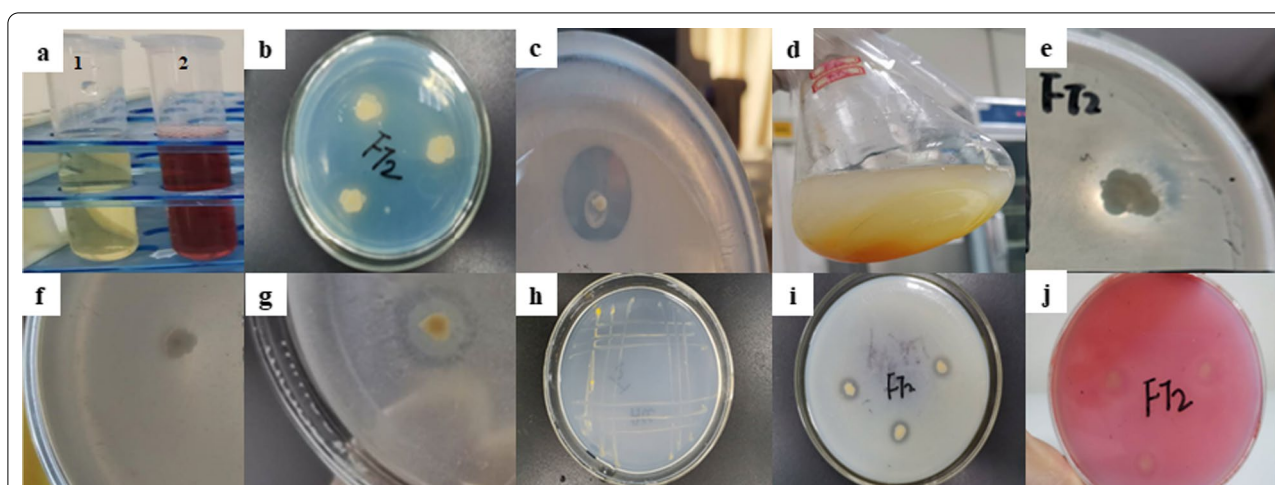


Fig. 5 Plant growth-promoting traits of *Stenotrophomonas rhizophila* FT₂. **a**: The supernatant was mixed with Salkowski's reagent; the appearance of red indicated IAA production (tube 1: un-inoculated strains; tube 2: *S. rhizophila* FT₂); **b**: Yellow-orange halos in CAS agar medium indicated siderophore production; **c**: growth on Ashby nitrogen-free solid medium proved the ability to fix nitrogen; **d**: Nessler's reagent was added in peptone water, and the development of brown to yellow color was the evidence of NH₃ production; **e, f, g**: clear zone formation around the colony in inorganic phosphorus, organic phosphate and potassium agar medium indicated the ability of solubilize inorganic phosphorus, organic phosphate and potassium; **h**: capacity to use ACC as a sole nitrogen source was the presence of bacterial growth in the DF salt minimal medium; and **i, j**: capacity to product protease and cellulase was evident as a clearing zone on Skim Milk Agar medium and CMC agar medium

Table 5 Effect of different treatments on the growth parameters and *Fusarium* wilt of cucumber seedlings

| Treatment | Plant height (cm) | Stem diameter (mm) | Leaf number (number plant ⁻¹) | Fresh weight (g pot ⁻¹) | DSI (%) | BE (%) |
|-----------|-------------------|--------------------|---|-------------------------------------|---------|--------|
| UC | 9.08 a | 3.94 a | 3.67 a | 15.82 a | 50.0 b | – |
| PC | 6.89 b | 2.02 b | 2.00 b | 10.94 b | 88.7 a | – |
| PF | 9.94 a | 4.10 a | 4.00 a | 15.93 a | 33.3 c | 62.5 a |
| PT | 8.94 a | 4.13 a | 3.00 a | 14.81 a | 35.0 c | 60.5 a |

UC, plants were inoculated with distilled water only; PC, plants were inoculated with pathogen only; PF, plants were inoculated with pathogen and the chemical fungicide; and PT, plants were inoculated with pathogen and *Stenotrophomonas rhizophila* FT₂. DSI, disease severity index; BE, biocontrol effect. Means followed by the different letters within each column are significantly different at $P < 0.05$

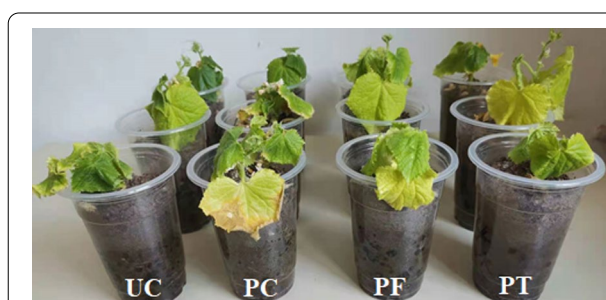
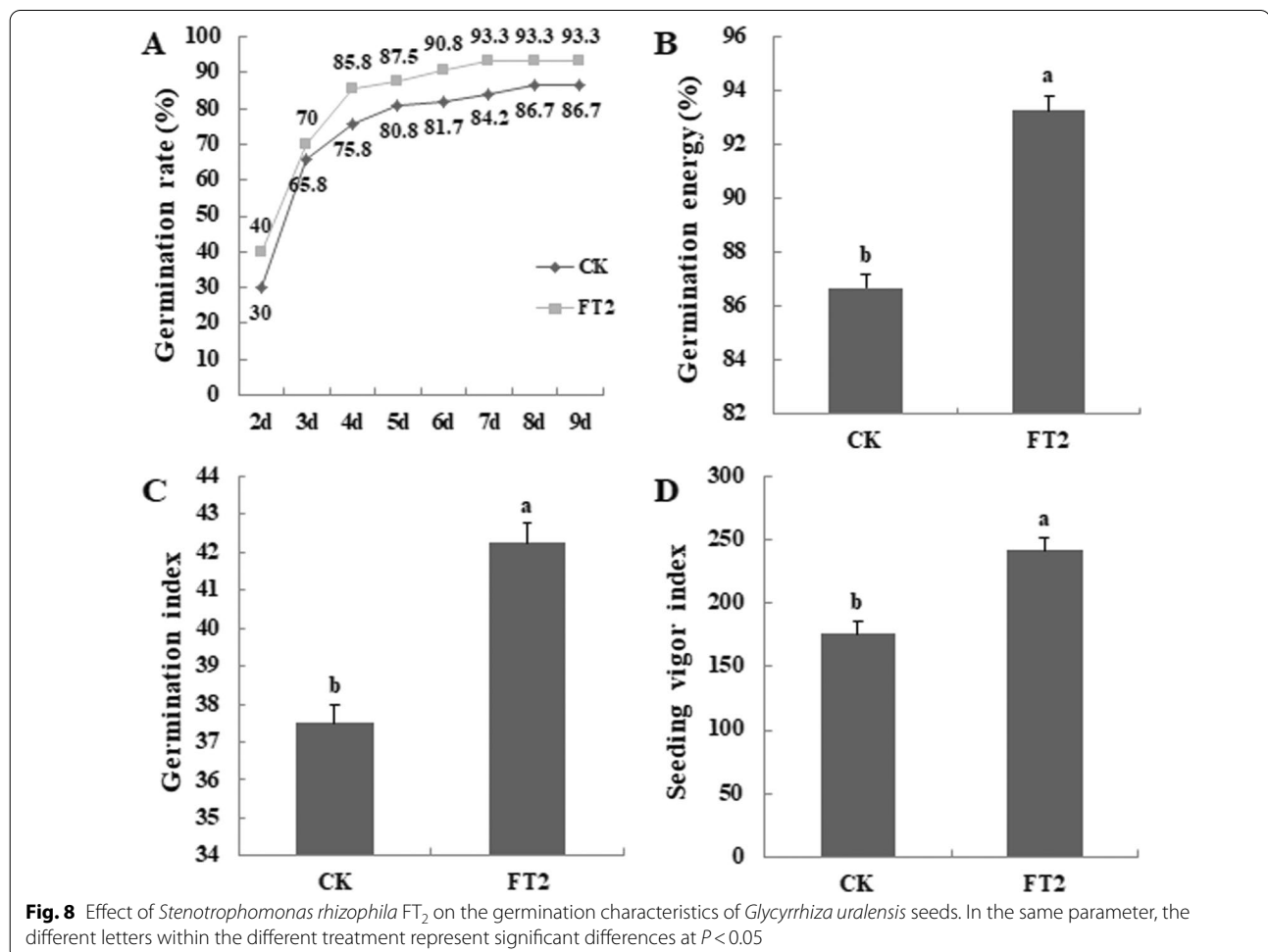
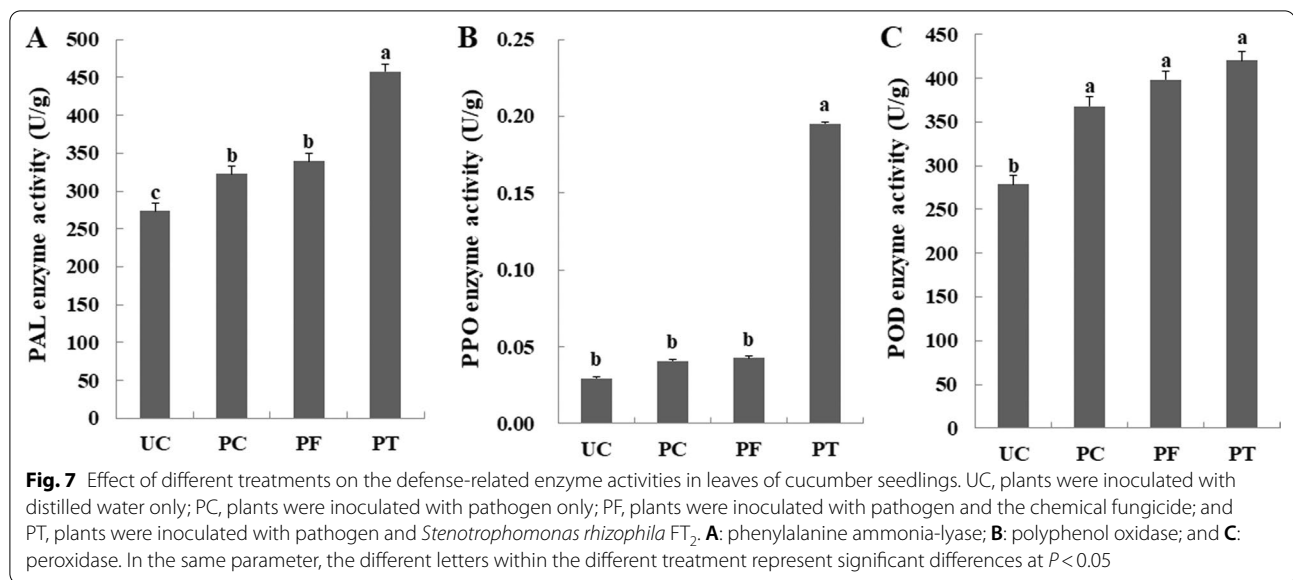


Fig. 6 Wilt symptoms of cucumber seedlings under different treatments. UC, plants were inoculated with distilled water only; PC, plants were inoculated with pathogen only; PF, plants were inoculated with pathogen and the chemical fungicide; and PT, plants were inoculated with pathogen and *Stenotrophomonas rhizophila* FT₂. The experiment was performed with 3 replicates

inoculated with *S. rhizophila* FT₂ were significantly increased compared to the control treatment.

Compared to the control, *S. rhizophila* FT₂ inoculation had a promoting effect on the growth parameters of *G. uralensis* seedling, with the greatest increase in root length and dry weight (Fig. 9).

Compared to the control, it was observed that the activities of phosphatase and catalase in rhizosphere soil were significantly increased by *S. rhizophila* FT₂ inoculation, and the contents of available nitrogen and phosphorus in rhizosphere soil also increased. Regarding the bacterial count in rhizosphere soil, *S. rhizophila* FT₂ inoculation showed a significant increase than the control (Table 6).



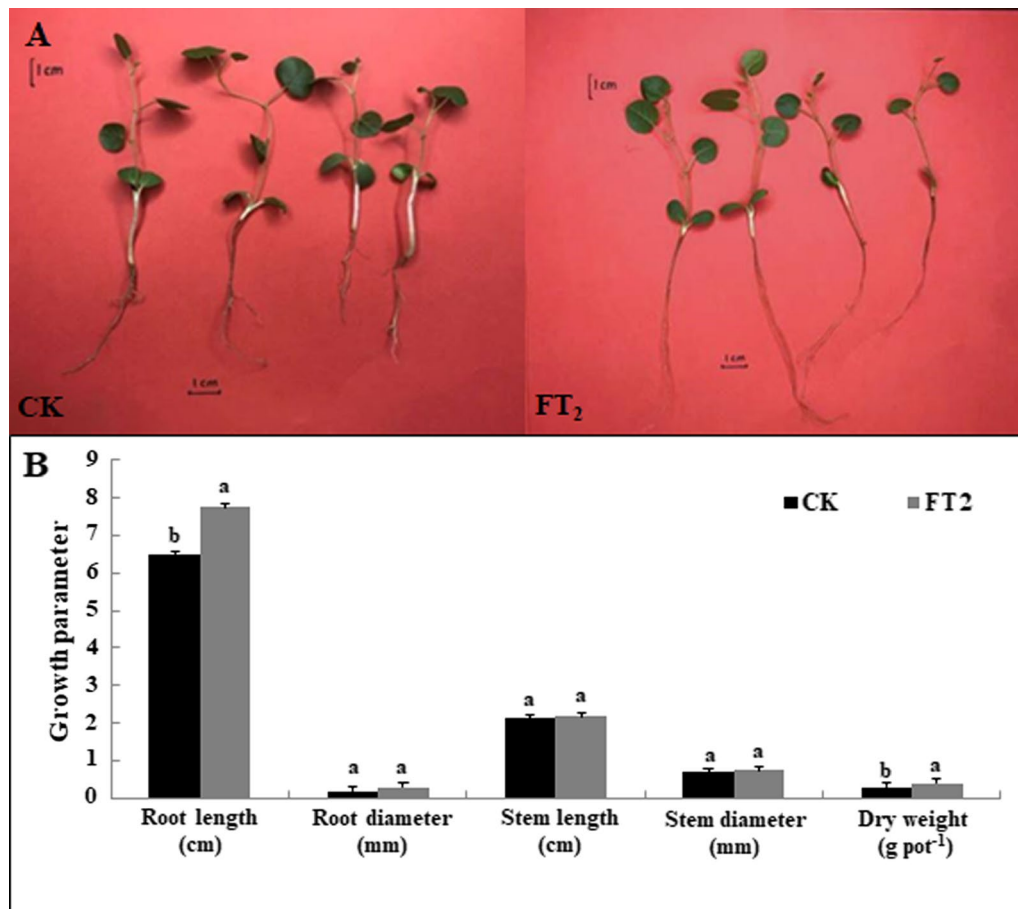


Fig. 9 Effect of *Stenotrophomonas rhizophila* FT₂ on seedling growth of *Glycyrrhiza uralensis*. **A:** Comparison photograph of *G. uralensis* seedling growth under different treatments: control and inoculated with *S. rhizophila* FT₂; **B:** comparison of different growth parameters of *G. uralensis* seedling under different treatments: control and inoculated with *S. rhizophila* FT₂. In the same parameter, the different letters within the different treatment represent significant differences at $P < 0.05$

Table 6 Effect of *Stenotrophomonas rhizophila* FT₂ on enzyme activities, available nutrient contents and bacterial count in *Glycyrrhiza uralensis* rhizosphere soil

| Treatment | Catalase (ml) | Phosphatase (mg/g) | Sucrase (mg/g) | Urease (mg/g) | Available nitrogen (mg/Kg) | Available phosphorus (mg/Kg) | Total bacterial count(10 ⁵) |
|-----------------|---------------|--------------------|----------------|---------------|----------------------------|------------------------------|---|
| CK | 16.7 b | 3.77 b | 3.28 a | 0.113a | 57.17 b | 4.22 b | 5.10 b |
| FT ₂ | 21.7 a | 4.23 a | 4.10 a | 0.113 a | 127.17 a | 5.24 a | 7.95 a |

CK, control; FT₂, *Stenotrophomonas rhizophila* FT₂. Means followed by the different letters within each column are significantly different at $P < 0.05$

Discussion

Isolation and identification of antagonistic activity of endophytic bacteria from *G. uralensis* seeds

There are two main sources of endophytic bacteria in plants: One is the external environment on the plant surface, and the other is seeds (Ferreira et al. 2008). In the present study, one strain antagonistic endophytic bacterium out of 9 strain endophytic bacteria isolated from *G.*

uralensis seeds was screened using 5 fungal phytopathogens (*Phyllosticta* sp., *Fusarium acuminatum*, *Botrytis cinerea* Pers., *F. oxysporum* Sacc, *Scutellariae botrytis*) as targets. This research showed that the strain FT₂ had an inhibitory effect on the mycelial growth of 5 fungal phytopathogens. The result was in accordance with previous report by Khanna et al. (2022). The bacterial isolate FT₂ was applied to the detached root assay, and the result

indicated that FT₂ significantly reduced disease index and rotted area on *A. sinensis* root slices caused by *F. acuminatum*, and the biocontrol effect over 80%. Importantly, this is the first time that antagonistic endophytic bacteria isolated from *G. uralensis* seeds that have been tested against the pathogen *F. acuminatum*. Based on the comparison of 16S RNA gene sequence and phylogenetic analysis, FT₂ was identified as *Stenotrophomonas rhizophila*. Bacteria of the genus, *Stenotrophomonas* are of increasing interest in biotechnology due to their ubiquity and versatility in seeds (Ryan et al. 2009). Plant growth-promoting traits and antagonistic behavior against plant pathogens of the genus, *Stenotrophomonas* have been well documented. The genus, *Stenotrophomonas*, currently comprises 8 species, and the *S. maltophilia* is the most intensively studied species. *S. rhizophila*, a separate species isolated from *S. maltophilia*, is still not well studied.

Plant growth promotion traits of *S. rhizophila* FT₂

The critical factor of effective biocontrol agent is inhibition efficiency, whereas the ability to promote plant growth is an added advantage. *S. rhizophila* FT₂ showed multiple plant growth promotion traits: IAA, NH₃, ACC deaminase, siderophore and extracellular enzymes production, phosphorus and potassium solubilization, nitrogen fixation (Table 3). Many previous studies reported that endophytic bacteria belonging to the genus *Stenotrophomonas* demonstrated plant growth promotion traits (Silambarasan et al. 2020). Similar results were obtained from antagonistic endophytic bacteria of other genera isolated from other sources. For example, *Lysinibacillus fusiformis* strain S4C11, isolated from the roots of an apple plant, had an antifungal effect against 4 phytopathogenic fungal strains and showed ability to produce siderophores and IAA (Passera et al. 2021). *Bacillus* species isolated from the rhizosphere soil of the tomato (*Lycopersicon esculentum*) had the biocontrol activity against *F. oxysporum* and were found to be positive for IAA, siderophore and ammonia production, phosphate and potassium solubilization and nitrogen fixation (Shah et al. 2020).

Biocontrol efficiency of *S. rhizophila* FT₂ against Cucumber *Fusarium* Wilt

As previously reported, endophytic bacteria may indirectly promote plant growth by inhibiting fungal pathogen infection (Wang et al. 2013). Indeed, the cucumber seedlings that treated with *S. rhizophila* FT₂ showed a significant increase in all growth parameters than the pathogen control, while the disease severity index of cucumber seedlings treated with *S. rhizophila* FT₂ significantly dropped. These results could be attributed to that

S. rhizophila FT₂ could produce extracellular enzymes, which are involved in cell wall degradation of fungal phytopathogens in the process of antagonism. The result is consistent with previous reports on various endophytic bacterial genera, such as *Bacillus*, *Micrococcus*, *Microbacterium* and *Pseudomonas* (Bibi et al. 2012). When plants were inoculated with biocontrol bacteria, the levels of defense-related enzyme activities such as POD, PAL and PPO were increased, thereby resisting the invasion and expansion of pathogens (Jiao et al. 2019). In this study, the activities of POD, PAL and PPO in cucumber seedlings treated with *S. rhizophila* FT₂ were increased; this was in agreement with the findings of Chen et al. (2012). When *F. oxysporum* Sacc was applied to the seedlings, the activity of PPO was non-significantly increased in comparison with the untreated control. The result showed that the *F. oxysporum* Sacc could promote self-infection by inhibiting PPO enzyme activity. To our knowledge, *S. rhizophila* has not been reported as endophytic bacteria isolated from *G. uralensis* seeds, and combining beneficial effects on *Fusarium* wilt suppression and plant growth promotion. In future research, these potential effects should be explored under field conditions.

Effect of *S. rhizophila* FT₂ on seed germination and seedling growth of *G. uralensis*

Seed germination experiment can be used to determine whether the strains inoculation has the effect of growth promoting. In the present experiment, seeds germination rate, germination energy, germination index and seedling vigor index had all significant improvement after inoculation with *S. rhizophila* FT₂. The result greatly corroborated with previous result obtained by Zhang et al. (2020a). The significant improvement in germination characteristics of all seeds observed after inoculation with *S. rhizophila* FT₂ may be due to changes that *S. rhizophila* FT₂ was able to exert at the hormone levels such as IAA (Glick et al. 2007). Many previous studies have reported that endophytic bacteria could improve growth and increase biomass of many plants. In the present study, the inoculation of *G. uralensis* plants with *S. rhizophila* FT₂ revealed an important increase in their root diameter, stem diameter, stem length, root length and dry weight, and this may be attributed to the ability of *S. rhizophila* FT₂ to nitrogen fixation, and potassium and phosphate solubilization.

Soil enzymes are produced by plants, animals and microorganisms. As Jyot (2015) stated, soil enzymatic activities measurement could potentially provide an indicator of soil fertility. In this study, soil catalase activity was remarkably increased, which may be attributed to the removal of H₂O₂ by FT₂ to prevent damage to plant membranes and organs. Soil phosphatases activity

was also significant increased, supposedly due to FT₂ improved soil available phosphorus, which was major factor affecting soil phosphatase activity. Hydrolytic enzymes are affected by various soil conditions, such as pH, organic matter and texture, as well as anthropogenic impacts (Baležentienė 2014), and these may be the reason why FT₂ had not significant improvement in the soil urease and saccharide activity. At the same time, the content of soil available phosphorus was significantly increased, which was mainly due to phosphatase promoting the decomposition of organophosphorus compounds (Shen et al. 2020). Soil microbial biomass size is one of the most vital and basic indicators of soil quality. The increase in soil available phosphorus content was closely related to the enhancement of rhizosphere soil bacterial count and activity. Therefore, *S. rhizophila* FT₂ can not only promote *G. uralensis* growth, but also improve soil quality, which was in agreement with reports in various plants (Guo et al. 2020).

Conclusions

An endophytic bacteria strain FT₂ isolated from *G. uralensis* seeds was identified as *S. rhizophila* and showed the strongest inhibitory activity against five fungal phytopathogens by using dual culture method. Detached root assay showed that FT₂ significantly reduced disease index and rotted area on *A. sinensis* root slices caused by *F. acuminatum*. In addition, it also exhibited the potential to promote plant growth and suppress *Fusarium* Wilt, which makes it promising candidate for the selection and development as biopesticides and bio-fertilizers.

Abbreviations

G. uralensis: *Glycyrrhiza uralensis* Fisch.; IAA: Indole acetic acid; ACC: 1-Aminocyclopropane-1-carboxylate; *F. acuminatum*: *Fusarium acuminatum*; *F. oxysporum* Sacc: *Fusarium oxysporum* Sacc; *A. sinensis*: *Angelica sinensis*; NA: Nutrient agar; PDA: Potato dextrose agar; LB: Luria–Bertani; CAS: Chrom Azurol S; DI: Disease index; BE: Biocontrol effect; DSI: Disease severity index; PAL: Phenylalanine ammonia-lyase; PPO: Polyphenol oxidase; POD: Peroxidase.

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Author contributions

LM Wang conducted all the experiment and wrote the manuscript; N Xi conducted part of the experiment and collected the reference; DY Lang performed the growth experiment and modified the details; L Zhou made the tables and figures; YJ Zhang modified the language; and XH Zhang provided the ideas and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and material

The data sets used and/or analyzed during the study can be obtained from the corresponding author on a reasonable request.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the author.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing interests that could have appeared to influence the work reported in this paper.

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References

- Abdel-Kareem MM, Zohri ANA, Nasr SAE (2021) Novel marine yeast strains as plant growth-promoting agents improve defense in wheat (*Triticum aestivum*) against *Fusarium oxysporum*. *J Plant Dis Prot* 128:973–988
- Abo-Elyousr KAM, Abdel-Rahim IR, Almasoudi NM, Alghamdi SA (2021) Native endophytic *Pseudomonas putida* as a biocontrol agent against common bean rust caused by *Uromyces appendiculatus*. *J Fungi* 7:745–758
- Adam E, Bernhart M, Müller H, Winkler J, Berg G (2018) The *Cucurbita pepo* seed microbiome: genotype-specific composition and implications for breeding. *Plant Soil* 422:35–49
- Ahmed W, Zhou GS, Yang J, Munir S, Ahmed A, Liu Q, Zhao ZX, Ji GH (2022) *Bacillus amyloliquefaciens* WS-10 as a potential plant growth-promoter and biocontrol agent for bacterial wilt disease of flue-cured tobacco. *Egypt J Biol Pest Co* 32:25–39
- Avinash TS, Rai RV (2014) Antifungal activity of plant growth promoting rhizobacteria against *Fusarium oxysporum* and *Phoma* sp. of cucurbitaceae. In: Kharwar R, Upadhyay R, Dubey N, Raghuwanshi R (eds) Microbial diversity and biotechnology in food security. Springer, New Delhi, pp 257–264
- Baležentienė L (2014) Indicating soil quality using urease and saccharase activity in abandoned grassland and differently managed crop fields. In: Sokolović D, Huyghe C, Radović J (eds) Quantitative traits breeding for multifunctional grasslands and turf. Springer, Dordrecht, pp 387–394
- Bibi F, Yasir M, Song GC, Lee SY, Chung YR (2012) Diversity and characterization of endophytic bacteria associated with tidal flat plants and their antagonistic effects on Oomycetous plant pathogens. *Plant Pathol J* 28:20–31
- Chen LH, Cui YQ, Yang XM, Zhao DK, Shen QR (2012) An antifungal compound from *Trichoderma harzianum* SQR-T037 effectively controls *Fusarium* wilt of cucumber in continuously cropped soil. *Australasian Plant Pathol* 41:239–245
- Chen MS, Li FN, Chen XH, Huang ZH, Yan XR, Tuo L (2021a) *Jiella mangrove* sp. nov., a novel endophytic bacterium isolated from leaf of *Rhizophora stylosa*. *Antonie Van Leeuwenhoek* 114:1633–1645

- Chen YH, Li S, Liu N, He H, Cao XY, Lv C, Zhang K, Dai JL (2021b) Effects of different types of microbial inoculants on available nitrogen and phosphorus, soil microbial community, and wheat growth in high-P soil. *Environ Sci Pollut Res* 28:23036–23047
- Fang S, Tao Y, Zhang Y, Kong F, Wang Y (2018) Effects of metalaxyl enantiomers stress on root activity and leaf antioxidant enzyme activities in tobacco seedlings. *Chirality* 30:469–474
- Feng X, Li SP, Lu YF, Zhang JJ, Zhu YY, Li Y, Yang HJ, He XH (2021) *Bjerkandera adusta* M1 inhibits the growth of *Fusarium oxysporum* f. sp. *conglutinans* and fusarium wilt incidence in *Brassica napus* L. *J Plant Pathol* 103:483–491
- Ferreira A, Quecine MC, Lacava PT, Oda S, Azevedo JL, Azevedo WL (2008) Diversity of endophytic bacteria from *Eucalyptus* species seeds and colonization of seedlings by *Pantoea agglomerans*. *FEMS Microbiol Lett* 287:8–14
- Glick BR, Cheng ZY, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur J Plant Pathol* 119:329–339
- Guo JX, Bowatte S, Hou FJ (2021) Diversity of endophytic bacteria and fungi in seeds of *Elymus nutans* growing in four locations of Qinghai Tibet Plateau, China. *Plant Soil* 459:49–63
- Gou JY, Suo SZ, Shao KZ, Zhao Q, Yao D, Li HP, Zhang JL, Rensing C (2020) Biofertilizers with beneficial rhizobacteria improved plant growth and yield in chili (*Capsicum annuum* L.). *World J Microbiol Biotechnol* 36:86–98
- Guan SY (1986) Soil enzyme and their methodology. Agricultural Press, Beijing, pp 274–340
- Guenoun K, Chattaoui M, Bouri M, Rhouma A, Naghmouchi K, Raies A (2018) Biological control of growth promoting rhizobacteria against verticillium wilt of pepper plant. *Biologia* 74:237–250
- Holt JG, Krieg NR, Sneath PHA, Staley JT (1994) Bergy's manual of determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore
- Jiao R, Munir S, He PF, Yang HW, Wu YX, Wang JW, Cai YZ, Wang G, He YQ (2019) Biocontrol potential of the endophytic *Bacillus amyloliquefaciens* YN201732 against tobacco powdery mildew and its growth promotion. *Biol Control*. <https://doi.org/10.1016/j.biocontrol.2019.104160>
- Jyot G, Mandal K, Singh B (2015) Effect of dehydrogenase, phosphatase and urease activity in cotton soil after applying thiamethoxam as seed treatment. *Environ Monit Assess* 187:298–305
- Khanna A, Raj K, Kumar P, Wati L (2022) Antagonistic and growth-promoting potential of multifarious bacterial endophytes against *Fusarium* wilt of chickpea. *Egypt J Biol Pest Cont* 32:17–26
- Lin XG (2010) Principles and methods of soil microbiology research. Higher Education Press, Beijing
- Li Q, Zhou SB, Liu N (2021) Diversity of endophytic bacteria in *Cardamine hupingshanensis* and potential of culturable selenium-resistant endophytes to enhance seed germination under selenate stress. *Curr Microbiol* 78:2091–2103
- Liu N, Song F, Zhu X, You J, Yang Z, Li X (2017) Salicylic acid alleviates aluminum toxicity in soybean roots through modulation of reactive oxygen species metabolism. *Front Chem* 5:96–107
- Mi CY, Yang RH, Rao JF, Yang SZ, Wei FG, Li O, Hu XF (2017) Unveiling of dominant fungal pathogens associated with rusty root rot of *Panax notoginseng* based on multiple methods. *Plant Dis* 101:2046–2052
- Mohamed BFF, Sallam NMA, Alamri SAM, Abo-Elyousr KAM, Mostafa YS, Hashem M (2020) Approving the biocontrol method of potato wilt caused by *Ralstonia solanacearum* (Smith) using *Enterobacter cloacae* PS14 and *Trichoderma asperellum* T34. *Egypt J of Biol Pest Cont* 30:61–74
- Prasom P, Sikhao P, Koohakan P (2017) In vitro study of endophytic bacteria isolated from tomato plant against *Fusarium oxysporum*. *Int J Agric Technol* 13:1217–1230
- Passera A, Rossato M, Oliver JS, Battelli G, Shahzad GIR, Cosentino E, Sage JM, Toffolatti SL, Lopatriello G, Davis JR, Kaiser MD, Delledonne M, Casati P (2021) Characterization of *Lysinibacillus fusiformis* strain S4C11: in vitro, in planta, and in silico analyses reveal a plant-beneficial microbe. *Microbiol Res* 244:126665–126682
- Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg G, Daniel VDL, Dow JM (2009) The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nat Rev Microbiol* 7:514–525
- Shah R, Amareesan N, Patel P, Jinal HN, Krishnamurthy R (2020) Isolation and characterization of *Bacillus* spp. endowed with multifarious plant growth-promoting traits and their potential effect on tomato (*Lycopersicon esculentum*) seedlings. *Arab J Sci Eng* 45:4579–4587
- Shahzad R, Khan AL, Bilal S, Asaf S, Lee IJ (2018) What is there in seeds? vertically transmitted endophytic resources for sustainable improvement in plant growth. *Front Plant Sci* 9:24–34
- Shen T, Wang QY, Cui YL, Yan M, Li YX, Tu WG, Zhang F, Wang J, Yu XM (2020) Effects of plant rhizospheric growth-promoting bacteria on copper enrichment in roots of *Pueraria chinensis* and soil physicochemical properties. *J Agro-Environ Sci* 39:572–580
- Silambarasan S, Logeswari P, Ruiz A, Cornejo P, Kannan VR (2020) Influence of plant beneficial *Stenotrophomonas rhizophila* strain CASB3 on the degradation of diuron-contaminated saline soil and improvement of *Lactuca sativa* growth. *Environ Sci Pollut Res* 27:35195–35207
- Tian GL, Bi YM, Jiao XL, Zhang XM, Li JF, Niu FB, Gao WW (2021) Application of vermicompost and biochar suppresses *Fusarium* root rot of replanted American ginseng. *Appl Microbiol Biot* 105:6977–6991
- Wang SL, Wang WT, Jin ZG, Du BH, Ding YQ, Ni T, Jiao FZ (2013) Screening and diversity of plant growth promoting endophytic bacteria from peanut. *Afr J Microbiol Res* 7:875–884
- White JF, Kingsley KI, Kowalski KP, Irizarry I, Micci A, Soares MA, Bergen MS (2018) Disease protection and allelopathic interactions of seed-transmitted endophytic *Pseudomonas* of invasive reed grass (*Phragmites australis*). *Plant Soil* 422:195–208
- Zhang KY, Liu XL, Dong XY, Liu RJ, He LH, Xie ZH (2020a) Isolation of endophytic bacteria from *Sesbania* seeds and its effects on germination. *J Agric Sci Tech* 22:40–48
- Zhang XH, Xie ZC, Lang DY, Chu YK, Jia XX (2020b) *Bacillus pumilus* improved drought tolerance in *Glycyrrhiza uralensis* G5 seedlings through enhancing primary and secondary metabolisms. *Physiol Plantarum* 171:1–12
- Zhang XT, Kong WL, Wu XQ, Ye JR (2021) *Bacillus velezensis* JK-XZ8 prevents and controls crown gall disease on *Prunus subhirtella* by colonizing and inducing resistance. *J for Res*. <https://doi.org/10.1007/s11676-021-01393-x>
- Zhao S, Du CM, Tian CY (2012) Suppression of *Fusarium oxysporum* and induced resistance of plants involved in the biocontrol of *Cucumber Fusarium Wilt* by *Streptomyces bikiniensis* HD-087. *World J Microbiol Biotechnol* 28:2919–2927
- Zhu ZY, Tian ZH, Li JX (2020) A *Streptomyces morookaensis* strain promotes plant growth and suppresses *Fusarium* wilt of banana. *Trop Plant Pathol* 46:175–218

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