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ORIGINAL ARTICLE

Screening of endophytic fungi from *Cremastra appendiculata* and their potential for plant growth promotion and biological control

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Abstract

Biocontrol fungi are widely used to promote plant growth and pest control. Four fungi were isolated from *Cremastra appendiculata* tubers and screened for plant growth-promoting and antagonistic effects. Based on the morphological characterization and ITS, 18S rRNA and 28S rRNA gene sequencing analysis, the fungi were identified to be related to *Colletotrichum gloeosporioides* (DJL-6), *Trichoderma tomentosum* (DJL-9), *Colletotrichum godetiae* (DJL-10) and *Talaromyces amestolkiae* (DJL-15). The growth inhibition tests showed that the four isolates had different inhibitory effects on *Colletotrichum fructicola*, *Alternaria alternata* and *Alternaria longipes*, among which DJL-9 showed the highest inhibitory activity. Their culture filtrates (especially that of DJL-15) can also inhibit pathogens. Four isolates were positive for the production of indole-3-acid (IAA) and β-1,3-glucanase and possessed proteolytic activity but were negative for the production of iron siderophore complexes. The four fungi showed strong nitrogen fixation and potassium dissolution abilities. In addition to DJL-9 being able to solubilize phosphate, DJL-10 was able to produce chitinase and cellulase. Pot experiments indicated that the four fungi increased the germination rate of *C. appendiculata* and soybean seeds and increased soybean radicle growth and plant biomass. Among them, DJL-6 had a better growth-promoting effect. Therefore, we successfully screened the biocontrol potential of endophytes from *C. appendiculata*, with a focus on preventing fungal diseases and promoting plant growth, and selected strains that could provide nutrients and hormones for plant growth.

Keywords Endophyte · Fungistasis · Phytopathogen · Growth-promoting fungi

Introduction

Cremastra appendiculata (D. Don) Makino (*C. appendiculata*) is a valuable herbal with a medicinal application history of more than a thousand years (Liu et al. 2021). It has become an endangered species and a scarce traditional Chinese medicine in Orchidaceae in China and is listed as a national secondary protected plant. Many scholars have studied the chemical constituents and pharmacological effects of *C. appendiculata* and found that it has antitumor (Zhang et al. 2021), hypotension (Ikeda et al. 2005), immune regulation (Zhao et al. 2017) and anti-inflammatory (Hur et al. 2010) effects. In recent years, the price of *C. appendiculata* has skyrocketed due to its scare resource. The main reasons are the low seed germination rate, slow growth rate under natural conditions and easy invasion by pathogens during artificial cultivation, resulting as a high mortality. Therefore, we attempted the rapid tissue propagation of *C. appendiculata* tubers. However, it was supering that a large number of plant tissues were contaminated by microorganisms despite a strict aseptic operation throughout the whole process. Based on this, we found that a large number of endophytes colonized *C. appendiculata* tubers.

Plant endophytes are microorganisms living in healthy plant organs but do not cause disease symptoms, and endophytic lifestyles play crucial roles in plant development, growth, fitness and diversification (Hardoim et al. 2015). They have the same or similar anabolic pathway as the host plants, which can promote plant growth and reproduction to

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a certain extent (Wani et al. 2015). Beneficial microbes also exert pressure on the survival of plant pathogens, thereby reducing their pathogenic effects on plants (such as the production of antibiotics and extracellular hydrolases) (Afzal et al. 2019).

Currently, the control of plant diseases and insect pests and the promotion of crop growth mostly rely on pesticides, chemical fertilizers and other chemical agents, most of which are artificially synthesized and have a slow degradation rate (Wang et al. 2018). Long-term and large-scale use of these agents has led to pesticide residues and increased the number of resistant weeds. In the process of killing bacteria, these agents also threaten beneficial microorganisms, cause water and soil pollution and even affect the ecological balance (Wang et al. 2020a). On the other hand, these agents induce pathogens to become drug-resistant and aggravate plant diseases, which are difficult to cure. To solve this problem, biocontrol preparations have attracted attentions, and endophytes are of the greatest interest. Biological control refers to the means of controlling plant diseases by living organisms such as animals, plants and microorganisms or their secondary metabolites (Zhang et al. 2020). It has the characteristics of low toxicity, safety and high efficiency, which meet the needs of environmental protection and sustainable agricultural development, and is an important strategy as an alternative to chemical control. Thus, it has gradually become an ideal method of disease prevention and control (Tarek et al. 2020; Cook 1993).

At present, many biocontrol isolates, such as *Bacillus*, *Pseudomonas*, *Streptomyces* sp. and *Trichoderma* sp. have been studied, developed and successfully commercialized (Wang et al. 2020b; Maes et al. 2020; Vurukonda et al. 2018; Vos et al. 2015). Among the plant growth-promoting rhizobacterias (PGPRs), strains from *Bacillus* and *Pseudomonas* are the most studied and exploited. The 7 strains isolated from barley roots by Yang (2019) had antagonistic activity against *Fusarium oxysporum* and effectively inhibited the occurrence of watermelon *Fusarium* wilt. Wang et al. (2020a) found that *Pseudomonas aeruginosa* CQ-40 was able to control tomato grey mould and promote tomato seed germination, seedling growth and fruit preservation. *P. fluorescens* is an inhibitory agent in the soil, which stifles the viability of *F. oxysporum* (Jain et al. 2021). However, due to the impact of cost and the stability of antibacterial activity, whether biocontrol fungi can be isolated from *C. appendiculata* tubers remains to be further studied.

C. appendiculata is often vulnerable to pathogen in artificial cultivation stage and is easy to has leaf spot, anthracnose, grey mould, leaf blight and other diseases (Wu et al. 2020). Anthracnose is caused by *Colletotrichum* sp., which mainly damages the leaves of plants and even lead to the death of *C. appendiculata* (Zhu 2006). *Cercospora* sp., *Phyllosticta citricarpa*, *Tolypocladium cylindrosporum* and other phytopatogenic fungi can cause leaf spot, which frequently occurs in the middle and lower parts of the leaf, and cause the death of leaf segment above the disease spot (Qin et al. 2017). The occurrence of these diseases will directly affect the survival of *C. appendiculata*. In summary, it is important to evaluate the antagonistic effect of *C. appendiculata* endophytes on pathogens and their abilities to promote plant seed germination and plant growth. Hence, we focused on beneficial microbial strains from *C. appendiculata* tubers with plant growth-promoting activity and antagonistic activity. We isolated and screened endophytic fungi from *C. appendiculata* tubers that can promote plant growth and inhibit pathogens to provide and develop potential biocontrol strains to control plant fungal diseases and promote crop growth. At the same time, the plant utilization rate of rare orchids was improved, and a good theoretical guidance and technical basis for the development of a new biocontrol agent instead of chemical pesticides were provided.

Materials and methods

Separation, purification and screening of endophytic fungi

C. appendiculata were obtained from Mianyang City, Sichuan Province in China on October 21, 2020, which were identified by Prof. Zhishan Ding. A voucher sample (No. Ding 0005) was kept in the laboratory of School of Medical Technology and Information Engineering, Zhejiang Chinese Medical University. Thirty-five healthy *C. appendiculata* tubers were selected and added to 2% sodium hypochlorite (v/v) for 15 min, 75% alcohol for 30 s and then sterile water for three washes, followed by cutting into pieces. The tubers were placed on potato dextrose agar (PDA) (Hangzhou Microbial Reagent Co., Ltd., Zhejiang, China) plates. After incubation at 28 °C (Ren et al. 2021) for 10 days, the mycelium at the tip of the fungal colonies was selected and transplanted to new PDA plates to separate the cultures (Fig. S1). This was repeated several times, after which purified colonies were obtained.

Antagonic effect of fungal endophytes on phytopatogenic fungi

Three pathogens that cause anthracnose and leaf spot in various plants including *Colletotrichum fructicola*, *Alternaria alternata*, and *Alternaria longipes* were provided by Dr. Fangmei Zhou (Zhejiang Chinese Medicine University, China). The endophytic fungi from *C. appendiculata* tubers and pathogens were incubated in 20 mL of solidified PDA plates at 28 °C. A 7-day-old fungal pathogen mycelial disc (5 mm) was placed in the centre of 9-cm Petri dish PDA

plates. The endophytes from *C. appendiculata* tubers were inoculated 15 mm from the central pathogen block. The PDA plates inoculated with pathogens alone were served as the control. After 7 days for incubation at 28 °C, the colony diameters of fungal pathogens were measured and compared to the control plate.

The fungistatic rate was calculated using the equation:

$$\text{the fungistatic rate } (\%) = \left[\left(D_C - D_E\right)/D_C\right] \times 100$$

where D_C represents the colony diameter of three pathogens on a control plate and D_E indicates the colony diameter of three pathogens on a plate that co-culture with endophytic fungi from *C. appendiculata* tubers (Bilański and Kowalski 2022). This experiment was repeated three times.

Identification of the fungi

Morphological identification: Four strains with fungistatic activity were inoculated on PDA medium plates and placed at 28 °C for 5 days to allow the colony area to cover 2/3 of the plate. Colony characteristics, such as morphology, size, colour and shape, were observed and recorded.

Molecular identification: Three genes, ITS, 18S rRNA and 28S rRNA were amplified for identification to identify the endophytes (Liu et al. 2015). The PCR system was composed of template DNA (1.0 µL), 1 × TSE101 mix (45 µL), upstream primer (2.0 µL) and downstream primer (2.0 µL). The thermocycling conditions were 1 cycle of 2 min at 98 °C, 35 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 10 s, extension at 72 °C for 10 s and followed by 72 °C for 5 min. The PCR products were then recovered from the gel, and the recovered products were sent to Beijing Qingke Biotechnology Co., Ltd. (Beijing, China) for sequencing analysis. The sequencing results were compared by fungal sequences in NCBI GenBank using BLAST software (http://www.ncbi.nlm.nih.gov/). The phylogenetic tree was constructed with MEGA version 7.0. The nucleotide sequences generated in this study have been deposited in the GenBank database under the accession numbers shown in Table 2.

Determination of the antifungal activity of the culture filtrates of endophytic fungi

DJL-6, DJL-9, DJL-10 and DJL-15 were inoculated in 100 mL of potato dextrose broth (PDB) medium in 250-mL conical flasks in shake culture (160 rpm) at 28 °C for 5 days. The culture filtrates (CFs) were acquired by centrifuging the culture at 12000 g for 10 min and then filtering through 0.22 µm MS® PES syringe filters. Then, the CFs were added to PDA at a proportion of 1:4 (v/v) with a final CF concentration of 20%. Sterile water was added to PDA as control (Jin et al. 2021). Mycelial discs of *C. fructicola*, *A. alternata* and *A. longipes* were inoculated on the CF-amended and control PDA. The colony diameters of three pathogens were measured after culturing for 7 days at 28 °C.

The growth inhibition rate was calculated by the following equation:

$$\text{the inhibition rate } (\%) = \left[\left(D_C - D_{CF}\right)/D_C\right] \times 100$$

where D_{CF} represents the colony diameter in the treatment amending with the CFs and D_C represents the colony diameter in the control PDA.

Determination of the effects of endophytic fungi on plant growth and disease resistance

The ability of the endophytic fungi to secrete extracellular enzymes was determined. Four isolates were inoculated on carboxymethylcellulose sodium (CMC) medium, chitinase medium and skim milk agar medium (Brzezinska and Jankiewicz 2012; Lin et al. 2019; Muniroh et al. 2019), cultured at 28 °C for 5 days and then observed for a transparent zone. The secretion of cellulase, chitinase and protease was evaluated and measured by the presence of the transparent zone. The production of β-1,3-glucanase was determined by dinitrosalicylate (DNS) (Ueki et al. 2019). Glucose was used as a standard. Four isolated cultures were inserted into PDB and oscillated for 5 days at 160 rpm and 28 °C. Then, the cultures were centrifuged at 12,000 g for 10 min. The supernatant was used as the crude extract of the enzyme. Then, 1 mL dextran substrate (100 mg laminarin in 100 mL 0.2 mol/L pH 5 sodium acetate-acetic acid buffer) and 1 mL of crude enzyme were mixed, and the mixture was incubated at 40 °C for 30 min. DNS solution (2 mL) was added to stop the reaction at 100 °C, and the absorbance was measured at 540 nm (model Epoch2, BioTek, America). The production of β-1,3-glucanase was calculated according to the glucose standard curve.

For the determination of nitrogen fixation ability, four fungi were inoculated on Ashby nitrogen-free medium (Jing et al. 2020), cultured at 28 °C for 5 days and observed for whether the strains grew in the medium; the experiment was repeated three times.

For determination of the ability to dissolve phosphate and potassium, phosphate solubilization and potassium dissolution tests were conducted using Pikovskaya agar (PKO) (Castro et al. 2018) and potassium fungi screening agar plate (Kushwaha et al. 2020) assays. Endophytic fungi were cultured at 28 °C for 5 days. The PKO plate was observed to determine whether there was a clear circle and whether the colony on the potassium fungi screening agar plate grew towards potassium to determine whether the strain had the ability to dissolve phosphorus and potassium.

To test for the ability to produce siderophores, a chrome azurol S (CAS) assay was used to detect the production of

siderophores (Jamali et al. 2020). The strains were inoculated on the medium and then placed at 28 °C for 5 days, and the presence of an orange halo around the colony was observed. The experiment was repeated three times.

Quantitative estimation of IAA production was carried out using the Salkowski reagent (1 mL 0.5 M $FeCl_3$, 50 mL 35% $HClO_4$) (Gang et al. 2019). To determine the amount of IAA produced by the four strains, a standard curve was prepared using a series of IAA dilutions at 0, 5, 10, 15, 20, and 25 mg/L. Then, 1 mL centrifuged fungal fluid was added to 1 mL Salkowski reagent and incubated for 30 min in darkness. The colour densities of the mixtures were read using a microplate reader at 530 nm absorbance. The experiment was repeated three times.

Effects of endophytic fungi on seeds germination and seedlings growth

Pot experiments were conducted to examine the germination promotion effect of endophytic fungi on *C. appendiculata* seeds. Endophytic fungi were cultured in 250-mL conical flasks containing 150 mL PDB on an orbital shaker at 160 rpm and 28 °C for 5 days. The mycelium was filtered and washed with sterile water three times, mixed with 500 g sterile soil and put into a 20 cm × 15 cm × 11 cm pot dish. The soil used for the pot experiment was collected from a greenhouse at Zhejiang Chinese Medical University, Hangzhou, China. The soil was steam-sterilized twice at 121 °C for 1.5 h continuously. *C. appendiculata* seeds were surface-disinfected and sown in a mixture of mycelium and sterile soil at the same density, after which a 5 cm × 4 cm area was selected from each group to determine the quantity sown. The treatments with only sterile soil (without endophytic fungi) were used as controls.

After various treatments were applied, pot experiments were carried out at 23–25 °C and 65–85% relative humidity with 13 pots (each strain was subjected to three replicates). The germination rate of *C. appendiculata* seeds in the potted soil of each group was calculated and analysed in the same 5 cm × 4 cm area at 20 days, 40 days, 60 days and 80 days after inoculation with the endophytic fungi. This experiment was replicated three times.

Same-size grain soybean seeds were selected and added to 2% sodium hypochlorite (v/v) for 15 min and then 75% alcohol for 30 s, followed by thorough washing three times in sterile water. Endophytic fungi were cultured in 250-mL conical flasks containing 150 mL PDB on an orbital shaker at 160 rpm and 28 °C for 7 days. The cultures were centrifuged at 8000 r/mim for 20 min, washed and resuspended in sterile water. The number of spores in suspension of endophytic fungi was counted by hemocytometer (Yang 2019). Then the spore suspension of four isolates was diluted in the order of 0 times, 10 times, 10^2 times, 10^3 times and 10^4 times. Eighteen soybean seeds were soaked in each dilution of the gradient for 3 h, while sterile water was used as a negative control. The seeds were placed in sterile petri dishes containing moist filter gauze at 25 °C in the dark. The soybean seedlings were harvested after 6 days of the endophytic fungi inoculation treatment, the germination rate of soybean seeds in each group was calculated, and the radicle length, fresh weight and dry weight were determined. The optimum concentrations of the spore suspension of the 4 strains were selected. The average values of these parameters were calculated and replicated three times.

Statistical analysis

All the above screening tests adopted a completely random design (CRD) with 3 replicates per experiment to maintain the consistency of the experiment. The results were subjected to analysis of variance (ANOVA), while means were assessed using least significant difference (LSD) at $P < 0.05$. Statistical analyses were performed with R software (GraphPad Prism 8.2).

Results

Screening of endophytic fungi with fungistatic activity

A total of twenty-one fungi were isolated and purified from *C. appendiculata* tubers. Dual culture tests were used to evaluate the inhibition of plant pathogens of the isolates. Table 1 shows that only 4 endophytes of *C. appendiculata* had inhibitory effects on three kinds of pathogens. DJL-9 showed the strongest inhibition activity, and the fungistatic effects on *C. fructicola*, *A. alternata* and *A. longipes* were 72.15 ± 1.07%, 63.42 ± 2.38% and 68.21 ± 1.27%, respectively. DJL-6, DJL-10 and DJL-15 could also inhibit the growth of the three pathogens to a certain extent, but the effect was not as obvious as that of DJL-9. The most frequent type of interaction between pathogens vs. endophyte was from physical contact of mycelia (Fig. 1a2-a4, b2, b4, c1, c2, c4), while a1, b1, b3 and c3 showed an inhibition zone between pathogens and endophyte with the width of the inhibition zone less than 2 mm (Fig. 1a1, b1, b3, c3).

Identification of endophytic fungi

Morphology assessments were conducted. DJL-6 colonies were yellow, flat and opaque, with rough edges and irregular shapes. The DJL-9 colonies were white and transparent, and the hyphae radiated outwards with irregular shapes on

Table 1 Antagonistic activity of our collected fungal isolates from *C. appendiculata* tubers on 3 pathogenic fungal isolates

Isolates	Fungal pathogens		
	C. fructicola	*A. alternata*	*A. longipes*
DJL-1	-	-	+
DJL-2	-	-	+
DJL-3	-	-	+
DJL-4	-	-	-
DJL-5	-	-	+
DJL-6	++	+	+
DJL-7	-	-	+
DJL-8	-	-	+
DJL-9	+++	+++	+++
DJL-10	+	+	+
DJL-11	-	-	-
DJL-12	-	+	-
DJL-13	-	-	-
DJL-14	-	-	-
DJL-15	++	++	++
DJL-16	-	+	-
DJL-17	-	-	-
DJL-18	+	-	-
DJL-19	-	-	-
DJL-20	-	-	-
DJL-21	-	-	-

"-" means no inhibition; "+" means fungistatic rate: < 25%; "++" means fungistatic rate: 20–50%; "+++" means fungistatic rate: > 50%

PDA medium plates. The DJL-10 colonies were round, dry and convex, with a black centre and a white edge that was opaque. DJL-15 colonies were brown, dry and convex, and the hyphae are flocculent, opaque and irregular in shape on PDA medium (Fig. 2).

For molecular identification, the sequences of four endophytic fungi were obtained by sequencing (showed in supplementary information 2) and compared with the GenBank database and reference strains. Here, the use of 18S rRNA and 28S rRNA gene sequence analysis allowed a phylogenetic differentiation down to the levels of families or genera, and ITS sequence further identified 4 endophytes to species. The endophytes were assigned as particular species only if minimum threshold similarity was ≥ 99% compared to the most closely related strain (Yuan et al. 2010). The phylogenetic tree generated using the phylogenetic analysis of sequences in the GenBank database and reference strains is presented in Fig. 3. DJL-6 was identified as *Colletotrichum gloeosporioides*, DJL-9 as *Trichoderma tomentosum*, DJL-10 as *Colletotrichum godetiae*, and DJL-15 as *Talaromyces amestolkiae*. The maximum identities for each isolate exceeded 99% with an E-value of 0 and the gene sequences of 4 strains were submitted to the GenBank database, and the accession numbers were obtained (Table 2).

Inhibition of pathogens by the CFs of endophytic fungi

The 4 endophytic fungi could produce antifungal substances inhibitory to *C. fructicola*, *A. alternata* and *A. longipes*. Figure 4 shows that the diameter of pathogens in the control group was significantly larger than that in the culture medium with CFs. Among them, the CFs of DJL-15 showed the strongest inhibition activity, and the inhibition rate against *C. fructicola*, *A. alternata* and *A. longipes* were 38.89 ± 1.81%, 52.73 ± 3.26% and 62.07 ± 2.45%, respectively. The CFs of DJL-6, DJL-9 and DJL-10 can also inhibit the growth of pathogens.

Characteristics of endophytic fungi related to growth promotion and disease resistance

All strains tested were able to produce IAA in PDB medium with and without L-tryptophan, and the production of IAA was significantly increased in the medium with L-tryptophan, as shown in Table 3. In PDB medium supplemented with L-tryptophan, the highest amount of IAA (7.60 ± 0.32 mg/L) was produced by DJL-15, followed by DJL-6 and DJL-10, while DJL-9 produced the lowest amount of IAA (3.17 ± 0.16 mg/L). In the PDB medium with no L-tryptophan supplement, DJL-15 produced the highest amount of IAA (2.85 ± 0.14 mg/L), while DJL-9 produced the lowest amount of IAA (1.58 ± 0.11 mg/L).

The results showed that all the four endophytic fungi could produce β-1,3-glucanase, and DJL-10 had the highest value (12.76 ± 0.18 mg/L). DJL-6 and DJL-10 could produce chitinase, and DJL-10 also showed cellulase activity.

Four isolates were tested for phosphate solubilizing activity in PKO media plates, and a clear transparent circle appeared around the colony, showing that the ability to solubilize phosphate was positively exhibited by the four endophytic fungi, except for DJL-9. All the strains showed proteolytic activity, as tested by the ability to produce a transparent zone in a skim milk medium plate assay. All the strains were able to fix nitrogen and resolve potassium, as indicated by the strains growing in Ashby nitrogen-free medium and on potassium fungi screening agar plates. However, none of the fungal strains were positive for iron siderophore complexes.

Endophytic fungi promoted seed germination and seedling growth

The endophytic fungi were assessed for their ability to promote germination of *C. appendiculata* seeds. In the

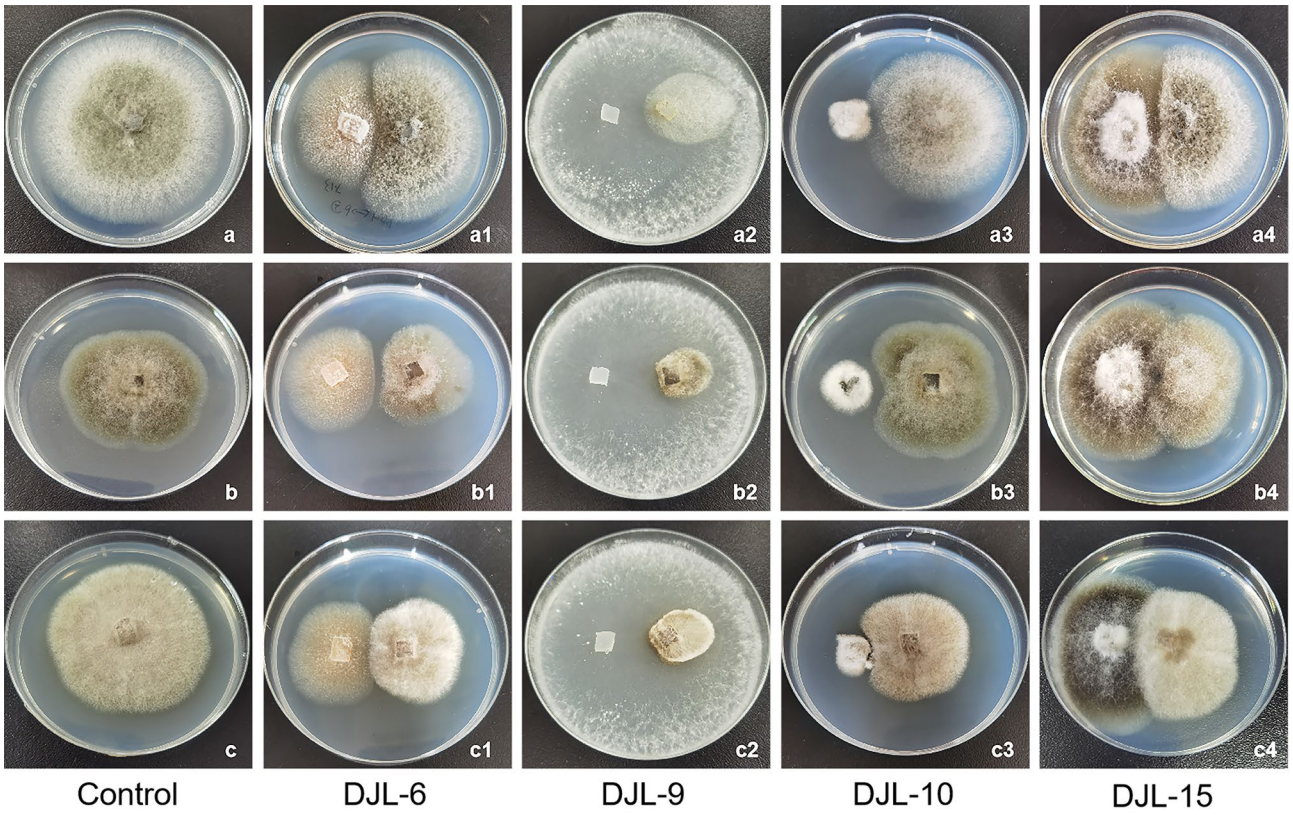

Fig. 1 Inhibition of growth of pathogens by selected endophytic fungi strains. **a** CK *C. fructicola*. **b** CK *A. alternata*. **c** CK *A. longipes*. **a1, b1**, **c1** Inhibition of three pathogens growth with DJL-6. **a2**, **b2**, **c2** Inhibition of three pathogens growth with DJL-9. **a3**, **b3**, **c3** Inhibition of three pathogens growth with DJL-10. **a4**, **b4**, **c4** Inhibition of three pathogens growth with DJL-15. Notes: "CK" means without any endophytic fungi isolated from *C. appendiculata* tubers. In the picture, endophytes are on the left side of the medium and pathogens are on the right

pot experiment under greenhouse conditions, the growth of *C. appendiculata* seeds was slow without inoculation of endophytic fungi, and less than 5% of the seeds showed germination trend after 80 days. After inoculation with the endophytic fungi, all the isolates were able to significantly increase the germination rate of *C. appendiculata* seeds, and the seeds of all experimental groups showed a favourable growth trend. Among them, the mycelia of DJL-9 promoted seed germination of *C. appendiculata* most obviously. In the sterile soil mixed with DJL-9 mycelia, the germination rate was 15.06 ± 0.28% (Fig. 5).

The endophytic fungi were also assessed for their ability to promote the germination and growth of soybean. The optimal spore suspension concentrations of DJL-6, DJL-9,

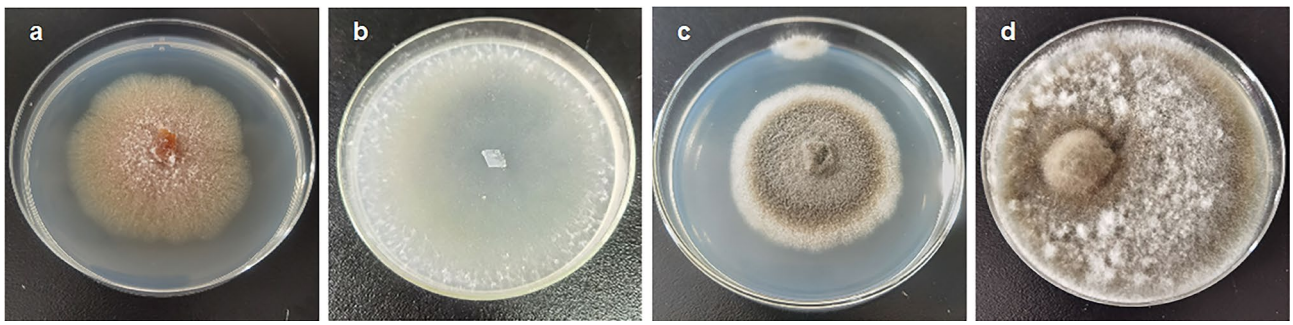

Fig. 2 Morphological characteristics of endophytic fungi on PDA medium culture. **a** Colony morphology of DJL-6. **b** Colony morphology of DJL-9. **c** Colony morphology of DJL-10. **d** Colony morphology of DJL-15

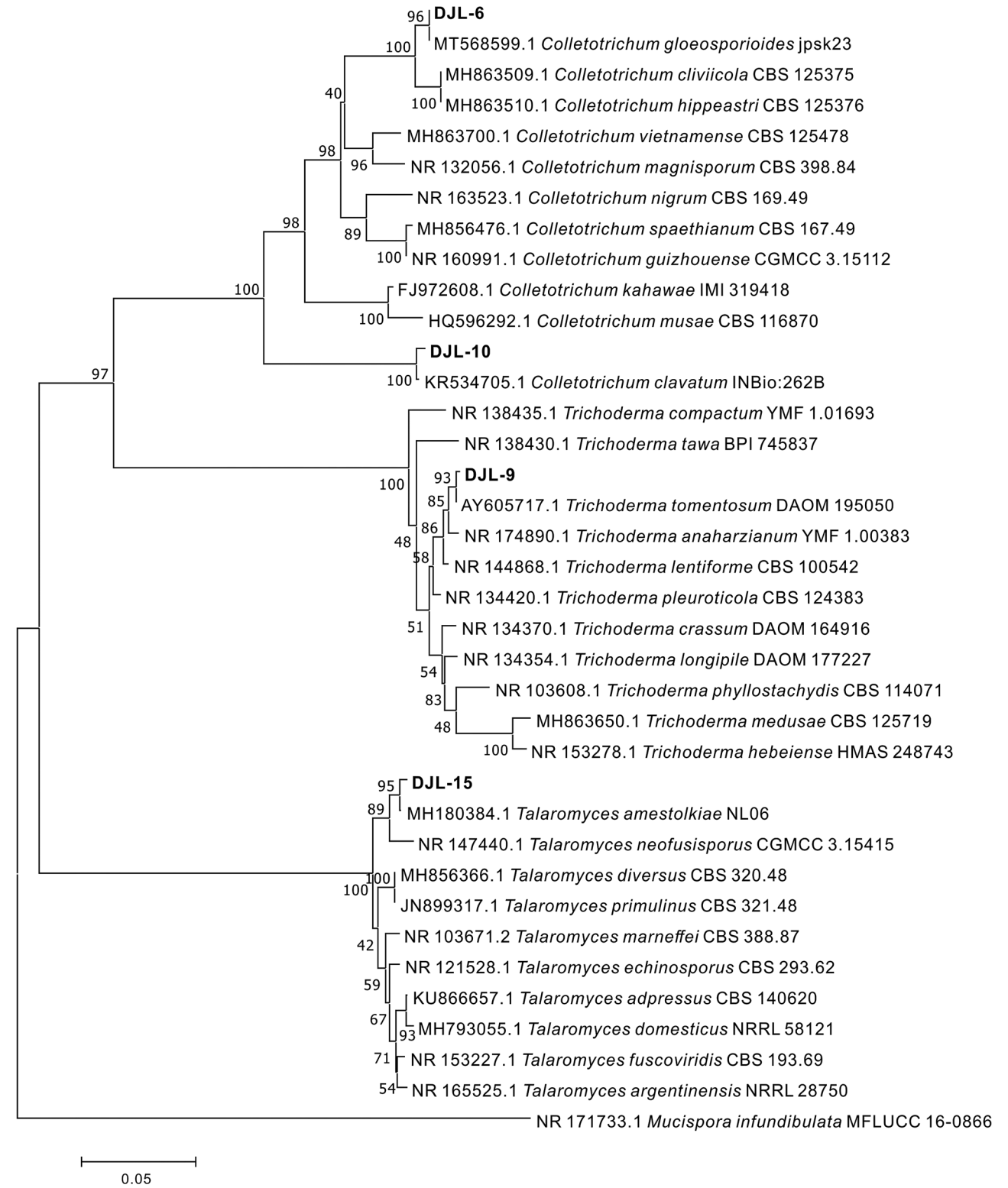

Fig. 3 rDNA ITS, 18S and 28S rRNA sequence phylogenetic tree of four endophytic fungi isolated from *C. appendiculata* tubers. The numbers at branch points were the significant bootstrap values (expressed as percentages based on 1000 replicates). The scale bar represents 0.05 substitutions per nucleotide position

Table 2 Identification of fungus isolates obtained from *C. appendiculata* tubers by rDNA ITS, 18S rRNA and 28S rRNA gene sequence analysis

Isolates	Accession numbers	Closest match of 18S rRNA sequence	Closest match of 28S rRNA sequence	Closest match of ITS sequence	Max identity (%)
DJL-6	ON238110	*Colletotrichum* sp. (AB076801)	*Colletotrichum* sp. (KT282891)	*Colletotrichum gloeosporioides* (MT568599)	99.64
DJL-9	ON238111	*Trichoderma* sp. (MT889737)	*Trichoderma tomentosum* (NG_069831)	*Trichoderma tomentosum* (AY605717)	99.88
DJL-10	ON238112	*Colletotrichum* sp. (AJ301969)	*Colletotrichum godetiae* (MH875599)	*Colletotrichum godetiae* (KR534705)	99.32
DJL-15	ON238113	*Talaromyces* sp. (MK368459)	*Talaromyces amestolkiae* (LT558955)	*Talaromyces amestolkiae* (MH180384)	99.81

DJL-10 and DJL-15 for promoting soybean seed germination and growth were, respectively, 1.0×10^7, 1.0×10^8, 3.5×10^8 and 2.0×10^9 cfu/mL (Fig. 6). Compared to noninoculated soybean seeds, those inoculated with endophytic fungi showed a significantly enhanced germination rate (Table 4). These four endophytic fungi significantly increased the length of the soybean radicle. All the isolates, except for DJL-9, significantly increased dry weight compared to uninoculated soybean, and only DJL-6 and DJL-15 significantly increased fresh weight of soybeans. DJL-6 had the most obvious growth-promoting effect on soybean seeds; the radicle length of soybean reached 18.83 ± 1.18 cm, and the fresh weight and dry weight increased most significantly, at 0.956 ± 0.11 g and 0.168 ± 0.02 g, respectively.

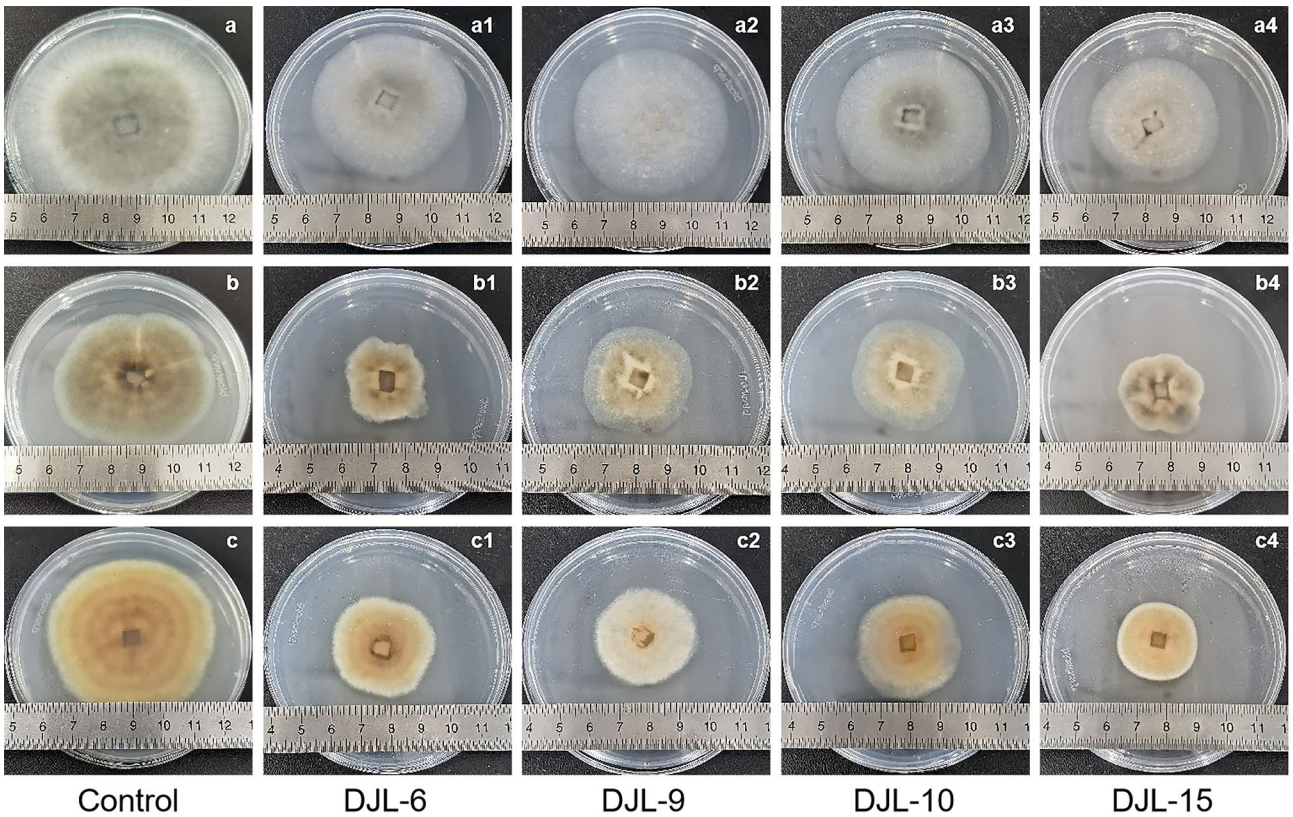

Fig. 4 Inhibition of growth of pathogens by the culture filtrates of the endophytic fungi. **a** CK *C. fructicola*. **b** CK *A. alternata*. **c** CK *A. longipes*. **a1**, **b1**, **c1** Inhibition of three pathogens growth with the culture filtrates of DJL-6. **a2**, **b2**, **c2** Inhibition of three pathogens growth with the culture filtrates of DJL-9. **a3**, **b3**, **c3** Inhibition of three pathogens growth with the culture filtrates of DJL-10. **a4**, **b4**, **c4** Inhibition of three pathogens growth with the culture filtrates of DJL-15. Notes: "CK" means the pathogens grow without any culture filtrates of endophytic fungi

Table 3 Characteristics of endophytic fungi related to growth promotion and disease resistance

Identification index		DJL-6	DJL-9	DJL-10	DJL-15
IAA production (mg/L)	With L-Try	4.82 ± 0.13	3.17 ± 0.16	4.57 ± 0.08	7.60 ± 0.32
	Without L-Try	1.94 ± 0.06	1.58 ± 0.11	2.37 ± 0.03	2.85 ± 0.14
β-1,3-Glucanase (U/mL)		11.23 ± 0.09	8.93 ± 0.03	12.76 ± 0.18	9.96 ± 0.04
Chitinase		+	−	+	−
Cellulase		−	−	+	−
Protease		+	+	+	+
Nitrogen fixation		+	+	+	+
Potassium removal		+	+	+	+
Phosphate solubilization		+	−	+	+
Siderophore		−	−	−	−

"+" means positive production, "−" means negative production

Discussion

In the present study, four strains with antagonistic activity against a variety of plant pathogens were isolated from *C. appendiculata* tubers. In vitro and in vivo experiments showed that the strain was beneficial to plants in a variety of ways, including inhibiting pathogens and promoting seed germination and plant growth.

Results have shown that biocontrol strains have disease resistance and growth-promoting effects on several vegetables, fruits and Chinese herbal medicines (Olanrewaju et al. 2017). *C. fructicola*, *A. alternata* and *A. longipes* are common plant pathogens in agricultural production, and they cause anthracnose, leaf spot and leaf blight diseases on various plants, such as carrots (Vintal et al. 2002), potatoes (Shoaib et al. 2014), apples (Li et al. 2019) and peaches (Usman et al. 2021). *C. appendiculata* grows in humid conditions and is susceptible to fungal infection. Infection of these three pathogens causes anthracnose, leaf spot disease and even the death of the plant, which poses a great threat to the survival of *C. appendiculata* (Jain et al. 2021).

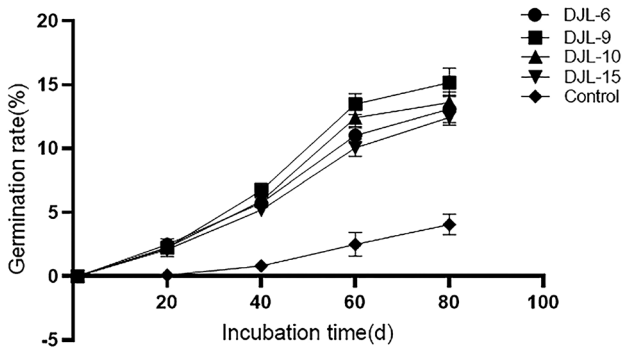

Fig. 5 Effects of endophytic fungi from *C. appendiculata* tubers on germination of *C. appendiculata* seeds. Results are means ± SE, statistically significant differences between treatments, $p < 0.05$

If endophytic fungi of *C. appendiculata* tubers can inhibit the growth of these three pathogens, it can be potentially developed into biological agents and applied in agricultural production.

Endophytes can act as biocontrol agents to directly or indirectly antagonize pathogens through various ways, including antibiosis, mycoparasitism, growth enhancement (endophytes promote plant growth by microbial hormones and reducing the effects of the disease) and competition (Thambugala et al. 2020). In this experiment, 4 strains isolated from *C. appendiculata* tubers differently demonstrated the effects of promoting plant growth and resisting pathogens. In the dual culture test, they mainly showed in vitro antagonistic activity against plant pathogens through physical contact of mycelia, and they can inhibit the growth of pathogens by competing for living space and nutrients in the medium. Meanwhile, their CFs also inhibited the growth of three plant pathogens, which shows the four endophytic fungi can produce fungistatic metabolites. They all promoted the germination of *C. appendiculata* seeds and the germination and growth of soybean seeds. These indicate the biocontrol potential of the four endophytic fungi.

In our experiment, we used three genes to identify four endophytes, which were 18S rRNA, 28S rRNA and ITS. Both the ITS region and 18S rRNA genes are broadly applied in molecular studies of fungi (Liu et al. 2015). Research showed that the ITS region could reveal higher richness, diversity and more dynamic than 18S rRNA. It could be concluded that ITS region is more rapid and precise for fungal community analysis than 18S rRNA (Weber et al. 2009). Also, the 28S rRNA gene beyond the D1-D2 region is useful for designing broad-range fungal PCR assays with a good ability to distinguish between species (Khot et al. 2009). *Trichoderma* plays an effective role in inhibiting pathogens and promoting plant growth. Around 90% of fungal biocontrol agents against pathogenic microorganisms belong to different strains of *Trichoderma* (Sood et al. 2020). Khruengsai et al. (2021) found that the volatile compounds produced

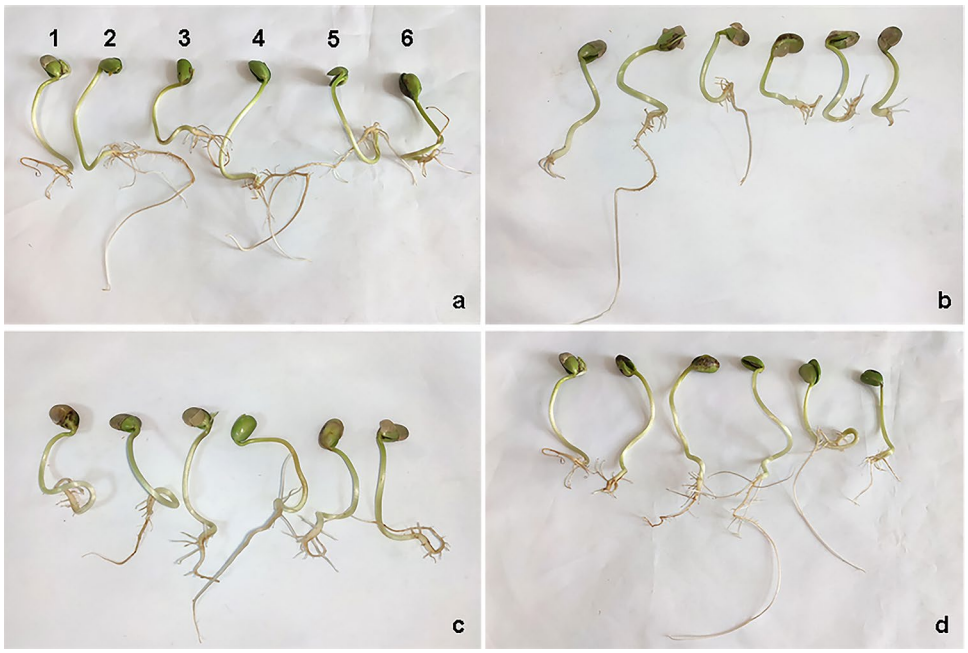

Fig. 6 Effects of endophytic fungi spore suspension with different dilution ratios on soybean seedling morphology. **a** DJL-6. **b** DJL-9. **c** DJL-10. **d** DJL-15. Sterile water control (1), spore suspension (2), tenfold diluted spore suspension (3), 10^2-fold diluted spore suspension (4), 10^3-fold diluted spore suspension (5), 10^4-fold diluted spore suspension (6)

by two *T. afroharzianum* strains inhibited the growth of *F. oxysporum* and *F. proliferatum* and significantly reduced the post-harvest decay of chili fruits. Appropriate doses of *T. asperellum* spore can increase the germination and radical length of vegetable crops, including tomato, brinjal, chilli, okra, ridge gourd and guar (Singh et al. 2016). In this study, DJL-9 was identified as *T. tomentosum*, which has a strong inhibitory effect on pathogens and promotion effects on plant growth. However, at present, there are relatively few studies on *T. tomentosum* and few reports on its biocontrol effect. Further studies on *T. tomentosum* will be carried out in the future.

In a study by Prieto et al. (2021), *T. amestolkiae* showed strong hydrolytic activity. Some β-glycosidases produced by *T. amestolkiae* catalyse the transfer of sugar molecules to specific glycosides through glycosylation and degrade plant biomass by secreting complex extracellular enzyme mixtures, which play an important role in destroying the cell structure of plant pathogens. In line with this result, in this study, strain DJL-15 produced a variety of hydrolytic enzymes and showed different antifungal activities against three plant pathogens in vitro.

DJL-6 and DJL-10 were identified as *C. gloeosporioides* and *C. godetiae*. In the existing literature reports, *Colletotrichum* is considered as the pathogenic fungus, which has a wide host range, often damaging trees, vegetables, medicinal plants and the roots, stems, leaves and flowers of field crops, fruits and their seedlings. It causes plant withering, fruit rot and seedling death without overt symptoms, and even results in severe economic losses (Cannon et al. 2012; Boufleur et al. 2021). However, in this study, we found that *Colletotrichum* (DJL-6 and DJL-10) can play a biocontrol role. Jin et al. (2017) also found that the *Fusarium* strain YCEF005 could promote the growth of tobacco seedlings, which significantly increased the number of leaves, fresh weight and dry weight of roots and shoots. A strain of *C. gloeosporioides* isolated from *Justicia gendarussa* is a novel endogenetic Taxol-producing fungus (Chen et al. 2020). These results indicate that some pathogenic fungi can appear as endophytes under specific conditions and produce

Table 4 Effect of spore suspension of endophytic fungi on morphological indexes of soybean seeds

Treatment	Germination rate (%)	Radicle length (cm)	Fresh weight (g)	Dry weight (g)
DJL-6	91.67 ± 2.78^{ab}	18.83 ± 1.18^{a}	0.956 ± 0.11^{a}	0.168 ± 0.02^{a}
DJL-9	95.83 ± 2.41^{a}	15.45 ± 1.44^{b}	0.854 ± 0.09^{ab}	0.153 ± 0.01^{ab}
DJL-10	93.06 ± 4.61^{ab}	9.65 ± 0.92^{c}	0.809 ± 0.09^{ab}	0.163 ± 0.02^{a}
DJL-15	88.89 ± 3.93^{b}	16.87 ± 1.53^{ab}	0.939 ± 0.13^{a}	0.159 ± 0.01^{a}
Control	79.17 ± 2.40^{c}	7.67 ± 1.50^{d}	0.752 ± 0.07^{b}	0.135 ± 0.02^{b}

All values represent the mean of three replicates ± standard error. Data with the different lowercase letters in the same column indicated significant difference at 0.05 level

compounds similar to the host. This conclusion needs further study and discussion.

The fungal cell walls are mainly composed of chitin and β-1,3-glucan (Fesel and Zuccaro 2016). Studies have found that some PGPR secrete chitinase, cellulase, protease and other hydrolases to decompose peptidoglycan from the pathogenic bacterial cell wall and extracellular virulence factors to achieve disease resistance (Abdelshafy et al. 2020). In our experiment, all four isolates had proteolytic activity and β-1,3-glucanase production capacity, and DJL-10 could also produce chitinase and cellulase. These isolated strains exhibited different antifungal activities.

In addition, endophytes can directly promote plant growth by improving the utilization rate of phosphorus and potassium, nitrogen fixation and production of IAA and other plant growth hormones (Lata et al. 2018). The capacity of IAA production, phosphate solubilization capacity, potassium solubilization capacity, nitrogen fixation capacity and production capacity of siderophores have been intensively studied. Most microorganisms isolated from plant roots can secrete IAA (Patten and Glick 1996). IAA is a plant growth regulator that promotes root growth and the absorption of certain nutrients. In the present study, the 4 endophytic fungi isolated from *C. appendiculata* tubers all had the ability to produce IAA, and the IAA yields of the 4 isolates were significantly increased after the addition of L-tryptophan. Regarding phosphate solubilization, phosphorus-containing inorganic salts were able to promote root formation, growth, seedling development and flower bloom, enabling an earlier mature of fruits and seeds and improving the quality and yield of the crops (Matos et al. 2017). Strain DJL-9 in the present study lacked the ability to solubilize inorganic phosphate, while the other three strains had strong phosphate solubilizing ability. Potassium ions can improve the activity of many enzymes involved in photosynthesis, thus enhancing plant photosynthesis and increasing plant resistance to stress (Liu et al. 2019). All the strains selected in our study showed potassium solubilization activity. Nitrogen fixation is the process by which organisms reduce nitrogen in the atmosphere to ammonia. Nitrogen is a synthetic element of plant proteins, nucleic acids, chlorophyll and many enzymes that can promote photosynthesis and other biological activities, thus promoting plant growth and development (Lindstrom and Mousavi 2020). All the strains in the present study could play a great role in nitrogen fixation. Therefore, a variety of mechanisms suggest that the strains in this study can promote plant growth and control diseases. Many biocontrol fungi, such as *T. asperellum* and *Penicillium chrysogenum*, can produce iron siderophore complexes (Qi and Zhao 2013; Chowdappa et al. 2020). However, none of the strains in this study exhibited iron siderophore complex production capacity.

Orchids have a low seed germination rate and slow plant growth in the natural environment and are susceptible to fungal infection, which leads to their scarce quantity. In current studies, natural control is a method for disease management. Tissue culture and the rapid propagation of *C. appendiculata* have been successfully studied (Mao et al. 2007), but there are few reports on improving the seed germination rate. In this study, by mixing soil with the mycelium, we found that compared with the blank control group, the mycelium of endophytic fungi could significantly improve the germination rate and growth rate of *C. appendiculata* seeds and soybean seeds. These results indicated that four endophytic strains of *C. appendiculata* tubers had growth-promoting effect on plants and are expected to be used in the cultivation of *C. appendiculata*, alleviating its resources shortage and generating social and economic benefits. In addition, the fungistatic activity of the four endophytic strains was significant, which lays a practical and theoretical foundation for the development of new biological pesticides, reduces the use of chemical fertilizers, pesticides and the possibility of environmental pollution and contributes to the cause of environmental protection.

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Author contribution ZD and FZ designed the research and wrote the manuscript. SW, SC, QL, JZ and BW performed the experiments. SW and QL analysed the data. All the authors read and approved the manuscript.

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Data availability Data available in a publicly accessible repository.

Declarations

Ethics approval This article does not contain any studies related to human participants or animals.

Conflict of interest The authors declare no competing interests.

References

Abdelshafy MO, Ma JB, Liu YH, Zhang D, Hua S, Bhute S, Hedlund BP, Li WJ, Li L (2020) Beneficial endophytic bacterial populations associated with medicinal plant *Thymus vulgaris* alleviate salt stress and confer resistance to *Fusarium oxysporum*. Front Plant Sci 11:47. https://doi.org/10.3389/fpls.2020.00047

Afzal I, Shinwari ZK, Sikandar S, Shahzad S (2019) Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. Microbiol Res 221:36–49. https://doi.org/10.1016/j.micres.2019.02.001

Bilański P, Kowalski T (2022) Fungal endophytes in *Fraxinus excelsior* petioles and their *in vitro* antagonistic potential against the ash dieback pathogen *Hymenoscyphus fraxineus*. Microbiol Res 257:126961. https://doi.org/10.1016/j.micres.2022.126961

Boufleur TR, Ciampi-Guillardi M, Tikami I, Rogerio F, Thon MR, Sukno SA, Massola JN, Baroncelli R (2021) Soybean anthracnose caused by *Colletotrichum* species: current status and future prospects. Mol Plant Pathol 22:393–409. https://doi.org/10.1111/mpp.13036

Brzezinska MS, Jankiewicz U (2012) Production of antifungal chitinase by *Aspergillus niger* LOCK 62 and its potential role in the biological control. Curr Microbiol 65:666–672. https://doi.org/10.1007/s00284-012-0208-2

Cannon PF, Damm U, Johnston PR, Weir BS (2012) *Colletotrichum* - current status and future directions. Stud Mycol 73:181–213. https://doi.org/10.3114/sim0014

Castro RA, Dourado MN, Almeida JR, Lacava PT, Nave A, Melo IS, Azevedo JL, Quecine MC (2018) Mangrove endophyte promotes reforestation tree (*Acacia polyphylla*) growth. Braz J Microbiol 49:59–66. https://doi.org/10.1016/j.bjm.2017.04.002

Chen FL, Liu SS, Zhao ZY, Gao WB, Ma YB, Wang XX, Yan SM, Luo DQ (2020) Ultrasound pre-treatment combined with microwave-assisted hydrodistillation of essential oils from *Perilla frutescens* (L.) Britt. leaves and its chemical composition and biological activity. Ind Crops Prod 143:111908. https://doi.org/10.1016/j.indcrop.2019.111908

Chowdappa S, Jagannath S, Konappa N, Udayashankar AC, Jogaiah S (2020) Detection and characterization of antibacterial siderophores secreted by endophytic fungi from *Cymbidium aloifolium*. Biomolecules 10:1412. https://doi.org/10.3390/biom10101412

Cook RJ (1993) Making greater use of introduced microorganisms for biological control of plant pathogens. Annu Rev Phytopathol 31:53–80. https://doi.org/10.1146/annurev.py.31.090193.000413

Fesel PH, Zuccaro A (2016) β-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants. Fungal Genet Biol 90:53–60. https://doi.org/10.1016/j.fgb.2015.12.004

Gang S, Sharma S, Saraf M, Buck M, Schumacher J (2019) Analysis of indole-3-acetic Acid (IAA) production in klebsiellaby LC-MS/MS and the Salkowski method. Bio Protoc 9:e3230. https://doi.org/10.21769/BioProtoc.3230

Hardoim PR, van Overbeek LS, Berg G, Pirttila AM, Compant S, Campisano A, Doring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293–320. https://doi.org/10.1128/MMBR.00050-14

Hur S, Lee YS, Yoo H, Yang JH, Kim TY (2010) Homoisoflavanone inhibits UVB-induced skin inflammation through reduced cyclooxygenase-2 expression and NF-kappaB nuclear localization. J Dermatol Sci 59:163–169. https://doi.org/10.1016/j.jdermsci.2010.07.001

Ikeda Y, Nonaka H, Furumai T, Igarashi Y (2005) Cremastrine, a pyrrolizidine alkaloid from *Cremastra appendiculata*. J Nat Prod 68:572–573. https://doi.org/10.1021/np049650x

Jain A, Sarsaiya S, Chen J, Wu Q, Lu Y, Shi J (2021) Changes in global Orchidaceae disease geographical research trends: recent incidences, distributions, treatment, and challenges. Bioengineered 12:13–29. https://doi.org/10.1080/21655979.2020.1853447

Jamali H, Sharma A, Roohi SAK (2020) Biocontrol potential of *Bacillus subtilis* RH5 against sheath blight of rice caused by *Rhizoctonia solani*. J Basic Microbiol 60:268–280. https://doi.org/10.1002/jobm.201900347

Jin HQ, Cheng CH, Xu QQ, Li XJ, Liu HY, Mao LJ, Liu N, Feng XX, Lin FC, Zhang CL (2017) Effects of endophytic fungi on tobacco growth and heavy metal content in leaves. Mycosystema 36:186–192. https://doi.org/10.13346/j.mycosystema.150280

Jin L, Yang L, Li W, Xu D, Yang N, Li G, Wan P (2021) Diversity and biocontrol potential of culturable endophytic fungi in cotton. Front Microbiol 12:698930. https://doi.org/10.3389/fmicb.2021.698930

Jing X, Cui Q, Li X, Yin J, Ravichandran V, Pan D, Fu J, Tu Q, Wang H, Bian X, Zhang Y (2020) Engineering *Pseudomonas protegens* Pf-5 to improve its antifungal activity and nitrogen fixation. Microb Biotechnol 13:118–133. https://doi.org/10.1111/1751-7915.13335

Khot PD, Ko DL, Fredricks DN (2009) Sequencing and analysis of fungal rRNA operons for development of broad-range fungal PCR assays. Appl Environ Microbiol 75:1559–1565. https://doi.org/10.1128/AEM.02383-08

Khruengsai S, Pripdeevech P, D'Souza PE, Panuwet P (2021) Biofumigation activities of volatile compounds from two *Trichoderma afroharzianum* strains against *Fusarium* infections in fresh chilies. J Sci Food Agric 101:5861–5871. https://doi.org/10.1002/jsfa.11238

Kushwaha P, Kashyap PL, Srivastava AK, Tiwari RK (2020) Plant growth promoting and antifungal activity in endophytic *Bacillus* strains from pearl millet (*Pennisetum glaucum*). Braz J Microbiol 51:229–241. https://doi.org/10.1007/s42770-019-00172-5

Lata R, Chowdhury S, Gond SK, White JF Jr (2018) Induction of abiotic stress tolerance in plants by endophytic microbes. Lett Appl Microbiol 66:268–276. https://doi.org/10.1111/lam.12855

Li H, Bian R, Liu Q, Yang L, Pang T, Salaipeth L, Andika IB, Kondo H, Sun L (2019) Identification of a novel hypovirulence-inducing hypovirus from *Alternaria alternata*. Front Microbiol 10:1076. https://doi.org/10.3389/fmicb.2019.01076

Lin LC, Ye YS, Lin WR (2019) Characteristics of root-cultivable endophytic fungi from *Rhododendron ovatum* Planch. Braz J Microbiol 50:185–193. https://doi.org/10.1007/s42770-018-0011-8

Lindstrom K, Mousavi SA (2020) Effectiveness of nitrogen fixation in rhizobia. Microb Biotechnol 13:1314–1335. https://doi.org/10.1111/1751-7915.13517

Liu J, He C, Tang Y, Liu W, Xu Y, Li Z, Qin X, Jin S (2021) A review of *Cremastra appendiculata* (D.Don) Makino as a traditional herbal medicine and its main components. J Ethnopharmacol 279:114357. https://doi.org/10.1016/j.jep.2021.114357

Liu J, Yu Y, Cai Z, Bartlam M, Wang Y (2015) Comparison of ITS and 18S rDNA for estimating fungal diversity using PCR-DGGE. World J Microbiol Biotechnol 31:1387–1395. https://doi.org/10.1007/s11274-015-1890-6

Liu JJ, Liu JL, Liu JH, Cui MM, Huang YJ, Tian Y, Chen AQ, Xu GH (2019) The potassium transporter SlHAK10 is involved in mycorrhizal potassium uptake. Plant Physiol 180:465–479. https://doi.org/10.1104/pp.18.01533

Maes S, De Reu K, Van Weyenberg S, Lories B, Heyndrickx M, Steenackers H (2020) *Pseudomonas putida* as a potential biocontrol agent against *Salmonella* Java biofilm formation in the drinking water system of broiler houses. Bmc Microbiol 20:373. https://doi.org/10.1186/s12866-020-02046-5

Mao TF, Liu T, Liu ZY, Zhu GS, Huang YH (2007) Rapid propagation of *Cremastra appendiculata* in vitro. Zhong Yao Cai 30:1057–1059

Matos A, Gomes I, Nietsche S, Xavier AA, Gomes WS, Dos SJN, Pereira M (2017) Phosphate solubilization by endophytic bacteria isolated from banana trees. An Acad Bras Cienc 89:2945–2954. https://doi.org/10.1590/0001-3765201720160111

Muniroh MS, Nusaibah SA, Vadamalai G, Siddique Y (2019) Proficiency of biocontrol agents as plant growth promoters and hydrolytic enzyme producers in *Ganoderma boninense* infected oil palm seedlings. Curr Plant Biol 20:100116. https://doi.org/10.1016/j.cpb.2019.100116

Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol 33:197. https://doi.org/10.1007/s11274-017-2364-9

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220. https://doi.org/10.1139/m96-032

Prieto A, de Eugenio L, Méndez-Líter JA, Nieto-Domínguez M, Murgiondo C, Barriuso J, Bejarano-Muñoz L, Martínez MJ (2021) Fungal glycosyl hydrolases for sustainable plant biomass valorization: *Talaromyces amestolkiae* as a model fungus. Int Microbiol 24:545–558. https://doi.org/10.1007/s10123-021-00202-z

Qi W, Zhao L (2013) Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress. J Basic Microbiol 53:355–364. https://doi.org/10.1002/jobm.201200031

Qin Q, Yu YP, Huang XY, Lu ZZ, Xie ZX, Luo Q (2017) Advances in research of orchid fungal leaf spot. Agric Res Appl 02:61–65

Ren W, Yang R, Zhang YX, Tang M (2021) Diversity of endophytic fungi in the roots of *Cremastra appendiculata* (Orchidaceae) at different altitudes in Taibaishan Nature Reserve, Central China. Mycosystema 40:992–1007

Shoaib A, Akhtar N, Akhtar S, Hafeez R (2014) First report of *Alternaria longipes* causing leaf spot of potato cultivar sante in Pakistan. Plant Dis 98:1742 (PDIS-05-14-0539-PDN)

Singh V, Upadhyay RS, Sarma BK, Singh HB (2016) *Trichoderma asperellum* spore dose depended modulation of plant growth in vegetable crops. Microbiol Res 193:74–86. https://doi.org/10.1016/j.micres.2016.09.002

Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M, Araniti F, Sharma A (2020) *Trichoderma*: the "secrets" of a multitalented biocontrol agent. Plants 9:762. https://doi.org/10.3390/plants9060762

Tarek GA, Maurice SM, Ahmed IA, Hamdy ISE, Hassan AE (2020) Field application of bio-control agents and aqueous plant extracts for controlling bacterial soft rot and enhancement yield quality of *Solanum tuberosum* L. cv. Diamond. Bull Natl Res Cent 44:1–16. https://doi.org/10.1186/s42269-020-00338-4

Thambugala KM, Daranagama DA, Phillips AJL, Kannangara SD, Promputtha I (2020) Fungi vs. fungi in biocontrol: an overview of fungal antagonists applied against fungal plant pathogens. Front Cell Infect Microbiol 10:604923. https://doi.org/10.3389/fcimb.2020.604923

Ueki A, Takehara T, Ishioka G, Kaku N, Ueki K (2019) Production of beta-1,3-glucanase and chitosanase from clostridial strains isolated from the soil subjected to biological disinfestation. AMB Express 9:114. https://doi.org/10.1186/s13568-019-0842-1

Usman HM, Tan Q, Karim MM, Adnan M, Yin WX, Zhu FX, Luo CX (2021) Sensitivity of *Colletotrichum fructicola* and *Colletotrichum siamense* of peach in China to multiple classes of fungicides and characterization of pyraclostrobin-resistant isolates. Plant Dis 105:3459–3465. https://doi.org/10.1094/PDIS-04-21-0693-RE

Vintal H, Shtienberg D, Shlevin E, Ben-Noon E (2002) First report of leaf blight caused by *Alternaria longipes* on carrots in Israel. Plant Dis 86:186. https://doi.org/10.1094/PDIS.2002.86.2.186B

Vos CM, De Cremer K, Cammue BP, De Coninck B (2015) The toolbox of *Trichoderma* spp. in the biocontrol of *Botrytis cinerea* disease. Mol Plant Pathol 16:400–412. https://doi.org/10.1111/mpp.12189

Vurukonda S, Giovanardi D, Stefani E (2018) Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. Int J Mol Sci 19:952. https://doi.org/10.3390/ijms19040952

Wang GF, Meng JF, Tian T, Xiao XQ, Zhang B, Xiao YN (2020a) Endophytic *Bacillus velezensis* strain B-36 is a potential biocontrol agent against lotus rot caused by *Fusarium oxysporum*. J Appl Microbiol 128:1153–1162. https://doi.org/10.1111/jam.14542

Wang H, Shi Y, Wang D, Yao Z, Wang Y, Liu J, Zhang S, Wang A (2018) A biocontrol strain of *Bacillus subtilis* WXCDD105 used to control tomato *Botrytis cinerea* and *Cladosporium fulvum* Cooke and promote the growth of seedlings. Int J Mol Sci 19:1371. https://doi.org/10.3390/ijms19051371

Wang X, Zhou X, Cai Z, Guo L, Chen X, Chen X, Liu J, Feng M, Qiu Y, Zhang Y, Wang A (2020b) A biocontrol strain of *Pseudomonas aeruginosa* CQ-40 promote growth and control *Botrytis cinerea* in tomato. Pathogens 10:22. https://doi.org/10.3390/pathogens10010022

Wani ZA, Ashraf N, Mohiuddin T, Riyaz-Ul-Hassan S (2015) Plant-endophyte symbiosis, an ecological perspective. Appl Microbiol Biotechnol 99:2955–2965. https://doi.org/10.1007/s00253-015-6487-3

Weber SD, Hofmann A, Pilhofer M, Wanner G, Agerer R, Ludwig W, Schleifer KH, Fried J (2009) The diversity of fungi in aerobic sewage granules assessed by 18S rRNA gene and ITS sequence analyses. FEMS Microbiol Ecol 68:246–254. https://doi.org/10.1111/j.1574-6941.2009.00660.x

Wu SS, Dai ZW, Ling R, Dong N, Meng XX, Zhai JW, Ding CC (2020) Planting system research on pollution-free cultivation of *Cremastra appendiculata*. World Chin Med 15:2249–2254. https://doi.org/10.3969/j.issn.1673-7202.2020.15.010

Yang W (2019) Components of rhizospheric bacterial communities of barley and their potential for plant growth promotion and biocontrol of *Fusarium* wilt of watermelon. Braz J Microbiol 50:749–757. https://doi.org/10.1007/s42770-019-00089-z

Yuan ZL, Zhang CL, Lin FC, Kubicek CP (2010) Identity, diversity, and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (*Oryza granulate*) from a nature reserve in Yunnan, China. Appl Environ Microbiol 76:1642–1652. https://doi.org/10.1128/AEM.01911-09

Zhang H, Godana EA, Sui Y, Yang Q, Zhang X, Zhao L (2020) Biological control as an alternative to synthetic fungicides for the management of grey and blue mould diseases of table grapes: a review. Crit Rev Microbiol 46:450–462. https://doi.org/10.1080/1040841X.2020.1794793

Zhang L, Yang K, Wang M, Zeng L, Sun E, Zhang F, Cao Z, Zhang X, Zhang H, Guo Z (2021) Exploring the mechanism of *Cremastra appendiculata* (SUANPANQI) against breast cancer by network pharmacology and molecular docking. Comput Biol Chem 94:107396. https://doi.org/10.1016/j.compbiolchem.2020.107396

Zhao XM, Zhao J, Hu RJ, Yao Q, Zhang GX, Shen HS, Yague E, Hu YH (2017) Ruanjian Sanjie decoction exhibits antitumor activity by inducing cell apoptosis in breast cancer. Oncol Lett 13:3071–3079. https://doi.org/10.3892/ol.2017.5832

Zhu XH (2006) Exploration on prevention and control of orchid disease. Sci Technol West China 09:50–52