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New Observation in Biocontrol of *Penicillium caperatum* against *Fusarium oxysporum* on *Saposhnikovia divaricata* and as a Plant Growth Promoter

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Abstract: *Fusarium oxysporum*, a common fungal pathogen that infects economic crops, causes Fusarium wilt disease to *Saposhnikovia divaricata* at an annual incidence rate of more than 15%. This study aimed to assess the potential of rhizospheric fungi as antifungal agents against Fusarium wilt of *Saposhnikovia divaricata*. In this study, 104 fungi were isolated from *S. divaricata* rhizospheric soil. Twelve rhizospheric strains that showed antagonistic activity against *F. oxysporum*, MR-16, MR-32, MR-38, etc., were screened out. Biocontrol activities of the twelve strains, especially MR-16, were subsequently characterized and evaluated. Strain MR-16 as potential stock for biocontrol had good antibiotic activity against *F. oxysporum* in vitro experiment. Based on the analysis of morphological properties and rDNA internal transcribed spacers (ITS), we identified an isolate MR-16 as *Penicillium caperatum* (GenBank No. OK287146.1), a new record of this species of China. The results of the in vitro antagonistic assay indicated that the conidial germination rate was significantly decreased, and the mycelia morphology of *F. oxysporum* induced change via the culture filtrate of *P. caperatum* MR-16, such as deformation and degradation. In an outdoor pot experiment, inoculation of *S. divaricata* plants with *F. oxysporum* created severe wilting symptoms; however, in inoculation trials, MR-16 effectively suppressed disease lesions, with a strong control efficacy of 60.76%. In addition, strain MR-16 could successfully colonize and form stable populations in the soil, and it showed a continuous positive growth-promoting effect on *S. divaricata* plants.

Keywords: antagonistic activities; identification; soil colonization; biological control

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1. Introduction

As a worldwide pathogenic fungus, *Fusarium oxysporum* is one of the top 10 most important plant-pathogenic fungi that has a wide host range [1]. *F. oxysporum* infects a wide variety of agricultural, horticultural, and medicinal plants as a ubiquitous soil-borne pathogen that produces severe losses in crops [2]. The dry root of *Saposhnikovia divaricata* (Turcz.) Schisck., known as Saposhnikoviae Radix, called "Fang-feng" in China, is one of the most famous Chinese traditional herbs, and it has been commonly used in the clinical compound prescription for the treatment of rheumatism, headache, vertigo, and arthralgia in China and other Asian countries [3–5].

The main source of Chinese herbal material of Saposhnikoviae Radix is the cultivated *S. divaricata* in the Chinese market [6,7], with an increasing cultivated area in northern China due to the over-digging of the wild resource, *S. divaricata*. Due to the geographical environment, Fusarium wilt disease, which is caused by overwintering spores of *F. oxysporum*, is one of the serious diseases that occur in the cultivated area of *S. divaricata*. After a disease outbreak, *F. oxysporum* penetrates the root epidermis, spreads through the vascular bundle, and conquers the xylem vessels of plants, resulting in vessel blockage and severe

water stress. The disease symptoms caused by *S. divaricate,* Fusarium wilt, includes stunting, epinasty, yellowing, and wilting of the leaves; browning, withering, and necrosis of the stems; eventually, progressive wilting, defoliation, metabolic failure of aboveground plants, and, often, plant death [8]. The annual incidence of Fusarium wilt is more than 15%, which seriously affects the yield of *S. divaricata* [8]. Notwithstanding, chemical fungicides can effectively control Fusarium wilt of *S. divaricata* and are the main strategy for control of this disease. However, the soil microbiota have been disrupted due to aggressive fungicide use, which reduces numbers of beneficial soil microorganisms, causing resistant pathogens and other agroecological pollution [9,10]. Consequently, the use of alternative approaches is required, which can suppress the development of pathogens to achieve eco-friendly and sustainable management [11].

The probiotic microorganisms used for the control of plant disease are effective and ecologically safe and provide substantial economic and ecological benefits [12]. Biological control is an ideal solution to challenge pesticide resistance. Fungal pathogens are otherwise beneficial to plants and can be used in accordance with organic farming practices because they could be self-propagating, conferring resistance via multiple strategies. For example, *Bacillus subtilis* [13,14] and *Trichoderma harzianum* [1,15,16] have strong adaptability to all sorts of environmental conditions and can inhibit pathogens propagation, promote plant growth, and have been developed as biological agents. However, biological control of Fusarium wilt of *S. divaricata* has not yet been reported. Therefore, in this study, we screened and isolated several biocontrol strains from rhizosphere soils collected from *S. divaricata* fields, the rhizosphere fungus MR-16, which has an antagonistic effect on Fusarium wilt of *S. divaricata*, was mainly identified, and its ability of soil colonization, growth promotion, and biological control was explored. Our aim was to evaluate the possibility of using rhizosphere fungi as biocontrol agents to control *S. divaricata* wilt disease and provide a good source for the development of biological fungi, which could provide a theoretical foundation for the biological control of plant diseases.

2. Materials and Methods

2.1. Rhizospheric Fungi of S. divaricata

Rhizosphere soil samples of healthy *S. divaricata* were collected from a field of the Jilin Agricultural University (JLAU), Changchun, China; the depth of excavation was 30 cm. We isolated the rhizospheric fungi according to the previously described dilution plate method with some modifications [17]. In brief, ten grams of soil sample was suspended in 90 mL of ddH_2O and vortexed thoroughly for 10 min. The suspension of soil was then diluted and spread on a potato dextrose agar (PDA) medium. Various fungal colonies were selected and purified on the PDA medium, numbered as MR, and then stored at −20 °C for later use.

2.2. In Vitro Inhibition Activity and Inhibition Spectrum

The antagonistic activities of fungi isolates derived from rhizospheric soils against *F. oxysporum* were performed by dual-culture and confrontation culture experiments [18,19]. Nine plant pathogens (provided by the Plant Disease Integrated Management Laboratory, Jilin Agricultural University), including *Botrytis cinerea*, *F. solani*, *Phytophthora cactorum*, *F. equiseti*, *Mycocentrospora acerina*, *Rhizoctonia solani*, *Alternaria tenuissima*, *Cylindrocarpon destructans*, and *A. liriodendron* were used to test a broad spectrum of activities in vitro on the MR-16 isolate using dual culture assays [18] on PDA in Petri dishes (φ = 90 mm) at 25 °C for 7 d in the dark. In parallel, we similarly prepared control plates but used the pathogens without MR-16. We prepared three replicates for each assay. We preserved all the fungal pathogens at the Key Laboratory for Ecological Restoration and Ecosystem Management of Jilin Province at Jilin Agricultural University.

2.3. Culture Characteristics and Phylogenetic Analysis of MR-16

The isolate MR-16 was inoculated on Czapek yeast autolysate agar with 5% NaCl (CYAS), Czapek dox agar (CA), Czapek yeast extract agar (CYA), 25% glycerol nitrate agar base (G25N), malt extract agar (MEA), and PDA plates at 25 °C for 10 d in the dark in a three-point manner under aseptic conditions with three replicates for each experiment. The colony texture, the abundance, texture, and color of mycelia, and the presence and colors of soluble pigments and exudates of the strains were observed in each medium plate, and the colony diameter was recorded [20,21]. The colors of the fungal colonies were determined by comparison with the color charts of the International Society Color Council and the National Bureau of Standards.

For micro-morphological identification, the morphological properties of mycelia, conidia, and sclerotia of strain MR-16 were observed using a ZEISS sigma300 field emission scanning electron microscope (Carl Zeiss Jena, Oberkochen, Germany). For molecular identification, the genomic DNA from the mycelia of the isolate MR-16 was extracted using a TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (Takara Bio, Shiga, Japan). The strain was incubated on a rotary shaker (150 rpm) at 25 °C for 5 days. PCR of ITS rDNA was performed according to a previous report [22]. The PCR products were subjected to agarose gel electrophoresis and sent to Sangon Biotech (Shanghai, China) for sequencing. The obtained sequences were submitted to the NCBI's nucleotide database for comparative analysis using the Basic Local Alignment Search Tool (BLAST). Phylogenetic analysis of the isolated strain was conducted by the Maximum Likelihood method using MEGA X [23], and the phylogenetic tree was constructed by the neighbor-joining (NJ) method.

2.4. Effects of MR-16 on Mycelial Growth of F. oxysporum

We evaluated the antagonistic activity of the culture filtrate of MR-16 on a PDA mixture medium using culture filtrate assays (CFA), as described by [24]. In brief, the PDA mixture medium was obtained in sterile conditions when the culture filtrate of MR-16 and PDA were mixed in a ratio of 1:4 (*v*/*v*). The agar-mycelium discs (8 mm diameter) of *F. oxysporum* were taken from the edge of an actively growing fungal colony for CFA. The agar-mycelium discs of *F. oxysporum* were placed in the center of PDA media containing culture filtrate of MR-16, as well as control of the PDA medium without culture filtrate were prepared, and all assays were prepared three replicates. All treatments were in dark cultures at 25 °C for 5 d, and observed the mycelial morphology of *F. oxysporum* every day. We determined the inhibition rate (%) of MR-16 culture filtrate using the following formula:

$$\text{Inhibition rate } (\%) = \frac{A_c - A_f}{A_c} \times 100$$

where A_c is the diameter of *F. oxysporum* colonies growing in PDA, and A_f is the diameter of *F. oxysporum* colonies growing in PDA with MR-16 culture filtrate.

2.5. Effects of MR-16 on Conidial Germination of F. oxysporum

The spore suspension of the fungal pathogen was prepared as follows: *F. oxysporum* was incubated on PDA at 25 °C for 10 d. Then, the surface of *F. oxysporum* conidia on PDA was eluted with sterile distilled water (sdH_2O), and fungal spores were collected using a spreader and then were filtered through cheesecloth. The spore concentration was adjusted to 10^6 CFU·mL^{-1}, which we then stored at 4 °C until later use.

The spore suspension of *F. oxysporum* and MR-16 culture filtrate were mixed in a ratio of 1:1 (*v*/*v*) with a control mixed sdH_2O, which then had three replicates prepared. All the treatments were incubated at 25 °C. At 6, 12, 24, and 48 h, we observed conidia of *F. oxysporum* using the method described by [25].

2.6. Soil Colonization Assays

We obtained a modified variant of the rifampicin-resistant (Rif) mutant, as described by Darma et al. (2020) [26]. For successive cultures, we inoculated the Rif mutant of MR-16 isolate into PDB containing increasing concentrations of rifampicin (Rif, Shanghai Macklin Biochemical Co., Ltd., Shanghai, China): 50, 100, 200, 300, 350, and 400 $\mu g \cdot mL^{-1}$. We tested the stability of Rif mutants of the MR-16 isolate by subculturing on PDA with 400 $\mu g \cdot mL^{-1}$ of Rif, observing no significant change compared with the MR-16 isolate. We labeled the Rif mutants of MR-16 isolate as MRRif-16 after we tested there was good stability of Rif mutants of the MR-16 isolate and stored them at −20 °C until use.

MRRif-16 strains were used for the colonization of soil from *S. divaricata* field to verify their ability to colonize plant soil. For this experiment, a one-year-old *S. divaricata* seedling was grown in a polypropylene pot (d = 200 mm, h = 180 mm) filled with soil previously mixed with the spore suspension of MRRif-16 at a ratio of 1:10 (*v*/*v*) under greenhouse conditions (16 h-sunlight at 28 °C and 8 h- darkness at 16 °C). We prepared 20 replicates for each treatment in a randomized complete block design (RCBD). After 7 to 35 days of inoculation, we recovered strain MRRif-16 and isolated it from the soil, and we determined and recorded the amount of soil colonization.

2.7. Biocontrol Activity of MR-16 Isolate on S. divaricata Wilt

We performed a modified version of the antifungal assay described by [27,28]. As previously described, the spore suspension of isolate MR-16 was prepared and adjusted to 1×10^7 CFU·mL^{-1}. We pre-infected the soil substrate (soil from *S. divaricata* field, vermiculite; 2:1, *v*/*v*) with *F. oxysporum*. The assays were performed based on five different treatments as follows: untreated control (water), carbendazim 50% WP (5.0 g L^{-1}) fungicide treatment, bacterial suspension of *B. subtilis* (10^8 CFU·mL^{-1}), spore suspension of *T. harzianum* (10^7 CFU·mL^{-1}), and spore suspension of MR-16 (10^7 CFU·mL^{-1}). One-year-old *S. divaricata* plants inoculated with *F. oxysporum* (cultivated in the Medicinal Botanical Garden of JLAU) were transplanted and grown for 70 d with 20 replicates for each treatment in a CRBD. We calculated the disease index of *S. divaricata* Fusarium wilt and control efficacy of Fusarium wilt disease as previously described by [29].

The disease rating scale (0–9) for root rot was as follows: 0 = asymptomatic healthy plants, healthy; 1 = onset of symptoms, leaves displaying yellowing and wilting, lesions covering < 10% of the leaves; 3 = leaves displaying wilting and yellowing, lesions covering 11% to 25% of the leaves; 5 = indicating wilt symptoms, lesions covering 26% to 50% of the leaves; 7 = leaves displaying wilting, yellowing, and browning lesions, lesions covering 51% to 75% of the leaves; 9 = leaves show infection, complete dying, and drying of the plant, lesions covering 76% to 100% of the leaves.

$$\text{Disease index (DI)} = \frac{0N_0 + 1N_1 + 3N_3 + 5N_5 + 7N_7 + 9N_9}{9N} \times 100$$

$$\text{Control efficacy (CE, \%)} = \frac{DI_{\text{CK}} - DI_{treatment}}{DI_{\text{CK}}} \times 100$$

where N_0 to N_9 represent the number of plants with each corresponding disease scale, and N represents the total number of plants assessed.

2.8. Plant Growth Promotion of MR-16 Isolate

To evaluate the effects of strain MR-16 on the growth of *S. divaricata*, we performed virulence assays of the spore suspension of MR-16 by inoculating *S. divaricata* plants. The plants were dipped in a suspension of 1×10^7 CFU mL^{-1} fungal spores of MR-16. We maintained the inoculated plants in a phytotron at 25~28 °C with 70% relative humidity, which we then monitored daily for plant growth.

The assays were performed based on four different treatments: nontreated control (water), bacterial suspension of *B. subtilis* (10^8 CFU·mL^{-1}), spore suspension of *T. harzianum*

(1×10^7 CFU·mL^{-1}), and spore suspension of MR-16 (1×10^7 CFU·mL^{-1}) in order to evaluate the effect of the spore suspension of MR-16 on the yield of *S. divaricata*. One seedling of *S. divaricata* was transplanted into a polypropylene pot (28 cm diameter and 20 cm height) filled with soil and vermiculite (2:1). We then performed 20 replications for each treatment in an RCBD. After 60 d with conventional agricultural management, we randomly selected nine *S. divaricata* plants. Subsequently, we measured and recorded the *S. divaricata* growth characteristics, including plant height, root length, the plant's fresh biomass and dry biomass, and the root's fresh biomass and dry biomass.

2.9. Statistical Analysis

The results were tested using analysis of variance (ANOVA) with 95% confidence intervals for Duncan's DMRT in SPSS Statistics 13.0 software and graphed it with OriginPro 9.5.

3. Results

3.1. Antagonistic Activities of Fungal Isolate against F. oxysporum

In this study, we evaluated the antagonistic activities in vitro of 104 fungal isolates using *F. oxysporum* causing Fusarium wilt of *S. divaricata*. Among them, 12 fungal isolates (11.54%) acted as antagonists and displayed significant growth inhibition against *F. oxysporum* on PDA, which showed antagonistic rates of more than 56%. Compared with the other 11 strains, such as MR-82, strain MR-16 showed a significantly antagonistic effect against *F. oxysporum* ($p < 0.05$), which the inhibition rate and inhibition zone were 66.67% and 10.05 mm, respectively, and the spread of the pathogenic fungus was effectively controlled (Table 1). According to several experimental verifications, we found that the inhibitory activity of the MR-16 strain was stable, so the strain can be considered as a candidate for further studies.

Table 1. Antifungal activities of selected rhizospheric fungi against *F. oxysporum*.

Strain	Inhibition Rate (%)	Inhibition Zone (mm)
MR-16	66.67 ± 2.22^{a}	10.05 ± 0.19^{a}
MR-82	63.70 ± 1.28^{b}	9.00 ± 0.19^{bc}
MR-87	62.59 ± 1.70^{bc}	4.71 ± 0.32^{h}
MR-32	61.35 ± 0.92^{bcd}	8.53 ± 0.31^{c}
MR-52	61.10 ± 0.78^{bcde}	6.10 ± 0.40^{f}
MR-73	60.12 ± 0.82^{cdef}	6.46 ± 0.26^{ef}
MR-43	59.63 ± 1.73^{cdef}	7.42 ± 0.18^{d}
MR-39	58.89 ± 2.94^{defg}	6.69 ± 0.23^{e}
MR-59	58.15 ± 0.64^{efg}	8.95 ± 0.28^{bc}
MR-84	57.83 ± 0.30^{fg}	9.20 ± 0.13^{b}
MR-96	57.41 ± 1.28^{fg}	4.29 ± 0.32^{h}
MR-38	56.30 ± 2.31^{g}	5.36 ± 0.35^{g}

Mycelia of *F. oxysporum* cultured on a PDA media during dual culture assays and confrontation culture assays with 104 fungal isolates from rhizospheric soil at 25 °C, respectively. Data are presented as the means ± SD of three replications. Different letters in the same column indicate the results of Duncan's DMRT ($p < 0.05$).

3.2. Antifungal Spectrum of MR-16 Isolate

The fungal isolate MR-16 showed the ability to inhibit the growth of nine plant pathogenic fungi, with inhibition rates ranging from 54.44% to 80.00%. Of the nine inhibited fungi, the growth of six pathogenic fungi, such as *P. cactorum*, *C. destructans*, *M. acerina*, etc., were significantly inhibited (greater than 71% inhibition rate). Moreover, the suppression rates of strain MR-16 against *R. solani* were as high as 54% (Table 2). The results showed that isolate MR-16 has broad-spectrum inhibition and could be developed as a potential biological control agent for fungal diseases of *S. divaricata* and other plants.

Table 2. Antibiotic activities of strain MR-16 against fungal pathogens.

Plant Pathogen	Inhibition Rate (%) [1]
Phytophthora cactorum	80.00 ± 1.28 [a]
Cylindrocarpon destructans	78.88 ± 0.64 [ab]
Mycocentrospora acerina	77.11 ± 0.56 [b]
Alternaria liriodendron	73.77 ± 0.93 [c]
Alternaria tenuissima	72.22 ± 0.64 [c]
Fusarium solani	71.88 ± 0.21 [c]
Fusarium equiseti	68.22 ± 0.42 [d]
Botrytis cinerea	68.14 ± 1.28 [d]
Rhizoctonia solani	54.44 ± 1.11 [e]

Antagonistic activity test of the MR-16 isolate against nine plant pathogenic fungi cultivated as dual culture assays on PDA. [1] Different letters in the same column indicate the results of Duncan's DMRT ($p < 0.05$).

3.3. Identification of Antagonistic Strain MR-16

3.3.1. Identification of Culture Characteristics

The colony morphologies of strain MR-16 on CA, CYA, CYAS, G25N, MEA, and PDA medium are shown in Figure 1. On CA, the colony diameter was 45.3 mm on average; the middle of the colony was fluffy; the mycelia were spread and sparse; the front of the colony was yellowish; exudate was absent, as was soluble pigment absent. For colonies growing on CYA, their diameter was 43.5 mm on average, and the colony surface was flat and velutinous, and the middle of colony was brilliant yellow; the mycelia were tangerine in the margins of the colony; exudate and soluble pigment were absent. On CYAS, the colony diameter was 15.6 mm on average; the mycelium texture was velutinous, slowly growing; the margins of the colony were regular; the colony surface was brilliant yellow; exudate and soluble pigment were absent. For colonies growing on G25N, their diameter was 10.2 mm on average; the colony texture was fluffy; the reverse color of central colonies was red-brown, and the margins were white and irregular, barely growing; mycelia were sparse; exudate and soluble pigment were absent. On MEA, the colony diameter was 46.3 mm on average; colony texture was granular, fluffy, and plain; the middle of the colony was blackish green at the center; the mycelia of margins were white; exudate and soluble pigment were absent. Colonies growing on PDA were, on average, 45.7 mm in diameter; colony texture was flat and fluffy; and colony edge was chartreuse, which was covered with grayish-green to white mycelium and showed heavy sporulation. These morphological characteristics of MR-16 on the media are similar to those of the genus *Penicillium*, as described by [30,31].

3.3.2. Identification of Microscopic Features

Our observed microscopic characteristics of isolate MR-16 are similar to those of the genus *Penicillium*, as described by Kong [32]. The conidiophores of isolate MR-16 were born from hyphae with a smooth wall. Stipe was 30–55 × 2–3.5 µm with no enlargement. Branching patterns of penicillus were terminal and monoverticillate with 2–5 obviously lanceolate phialides (7–12 × 3–3.5 µm). MR-16 had ellipsoidal-to-subspheroidal and 2–3 × 1.5–2 µm conidia with rough walls. The ascus of MR-16 was 110–160 × 90–140 µm, and ascospores were ellipsoidal with rough walls, 2–2.5 × 1.5–2 µm (Figure 2).

3.3.3. Molecular Identification

The ITS gene sequence of strain MR-16 was amplified and sequenced to obtain a base sequence of 565 bp with the GenBank accession number OK287146.1. We found that MR-16 showed high sequence homology with *Penicillium caperatum* (MK450677.1) and *P. javanicum* (MH865296.1) of over 99%, *P. meloforme* (MT529271.1), and *P. setosum* (MK450718.1) by comparison of the 5.8S rRNA gene fragment using NCBI's BLAST. Moreover, MR-16 showed similarity to *P. pulvillorum* (MH865335.1), *P. janthinellum* (KP992936.1), and *P. tanzanicum* (KT887863.1) of close to 98%. Based on a phylogenetic tree construction, the results

indicated that strain MR-16 and *P. caperatum* (NR_138333.1) have high homology and are in the same clade (Figure 3).

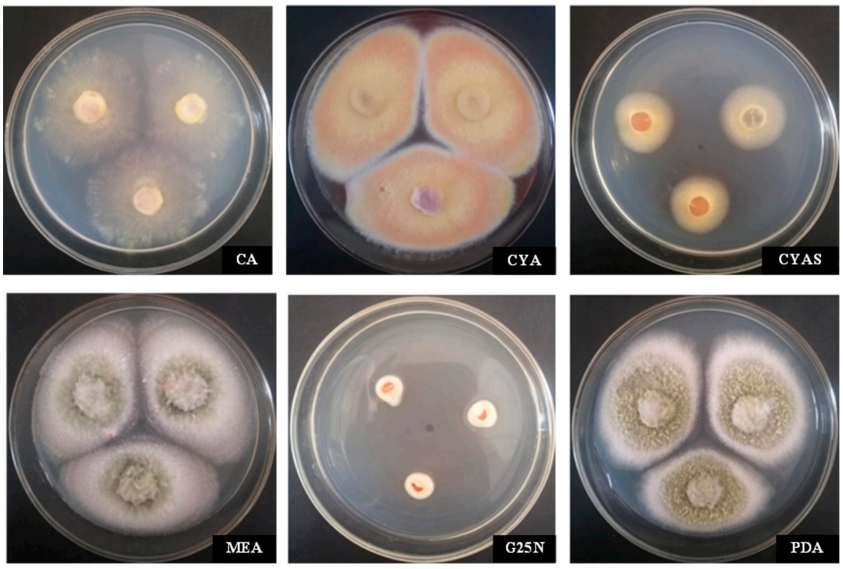

Figure 1. Morphological characteristics of the cultures of MR-16 on six differential media. The front side of the colony was cultured on CA, CYA, CYAS, MEA, G25N, and PDA at 25 °C in the dark for 10 days.

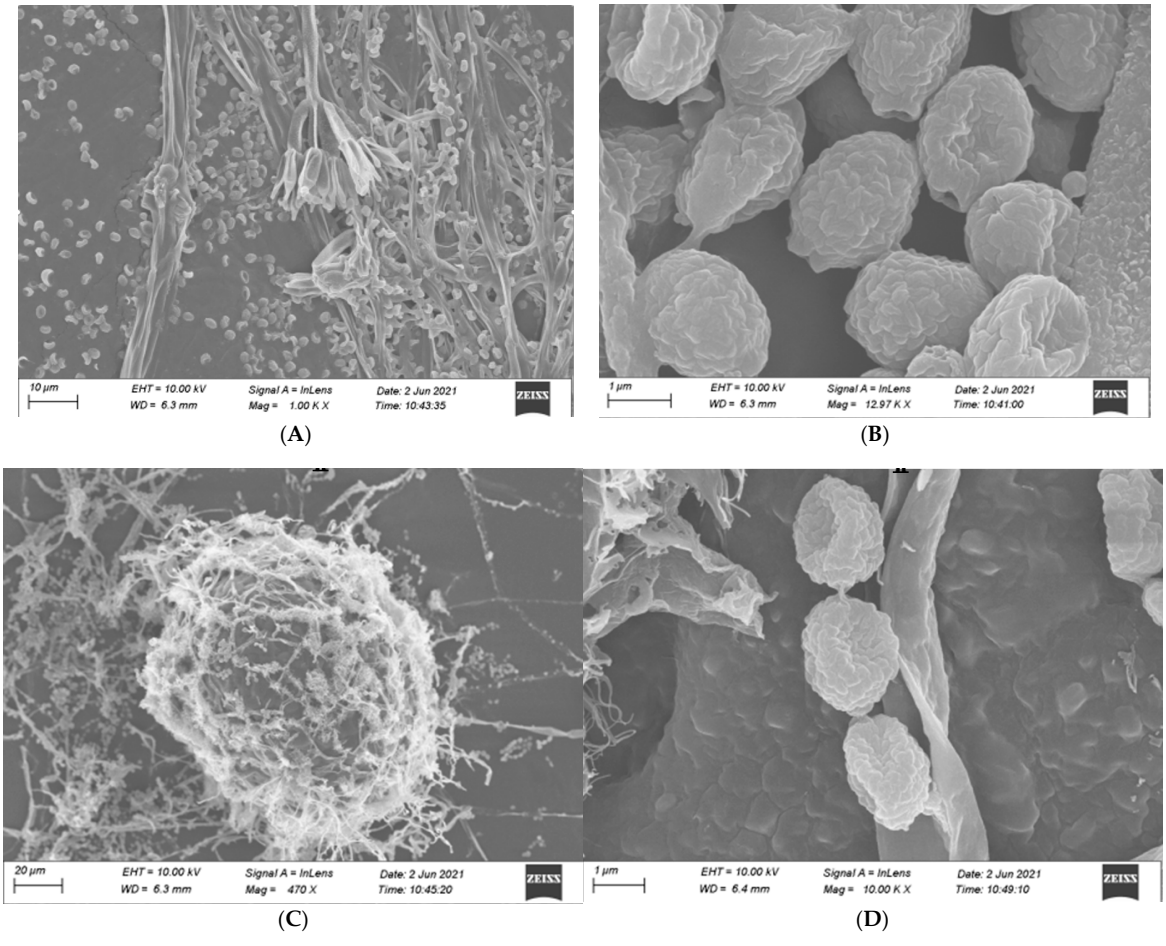

Figure 2. Morphology of reproductive structures of MR-16. (**A**): Phialide, metulae and Stipe, (**B**): Conidia, (**C**): Ascomata, (**D**): Ascospores. Scale bars: (**A**) = 10 µm; (**B**) = 1 µm; (**C**) = 20 µm; and (**D**) = 1 µm.

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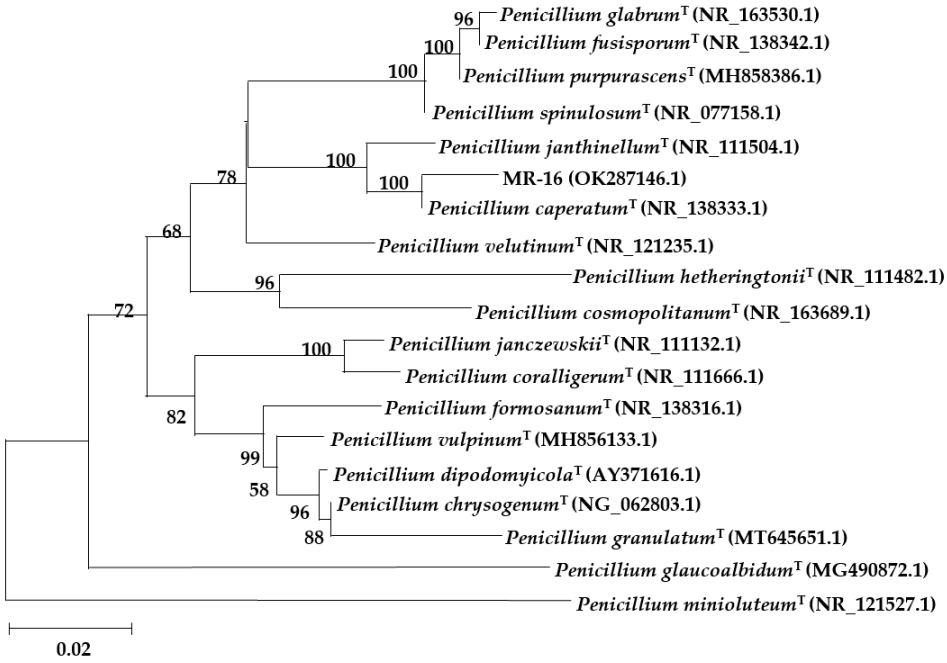

Figure 3. Phylogenetic analysis of MR-16 and its relatives based on the nucleotide sequences of ITS sequences by using the neighbor-joining method (NJ). The values of significant bootstrap (>58%) and the scale (0.02) of the phylogenetic tree are exhibited.

Based on the above identification results, the MR-16 strain was identified as *P. caperatum*, which is the first time that the strain MR-16 has been discovered and reported in China [31,32].

3.4. MR-16 Culture Filtrate Inhibition of F. oxysporum Mycelium

The MR-16 culture filtrate substantially inhibited the mycelial growth of *F. oxysporum*. The healthy mycelia of *F. oxysporum* had a smooth exterior surface (Figure 4A,B). The inhibited pathogenic colonies thinned, with clear and irregular edges, sparse aerial mycelium, and a slow growth trend, and the mycelia dissolved obviously (Figure 4C).

In the co-culture assay, compared to single culture treatments, the mycelia of *F. oxysporum* at the edge of the colony showed an irregular extension with thickened cell walls; the branching ends were enlarged, mycelia became coarsened, and its surfaces were rough and uneven; including shrunken protoplasts and uneven distribution (Figure 4D). The inhibited mycelia of *F. oxysporum* were distorted, shortened, constricted, and dilative, among other deformities (Figure 4E,F). The results indicated that *P. caperatum* MR-16 isolate could inhibit the growth of *F. oxysporum* by producing certain substances.

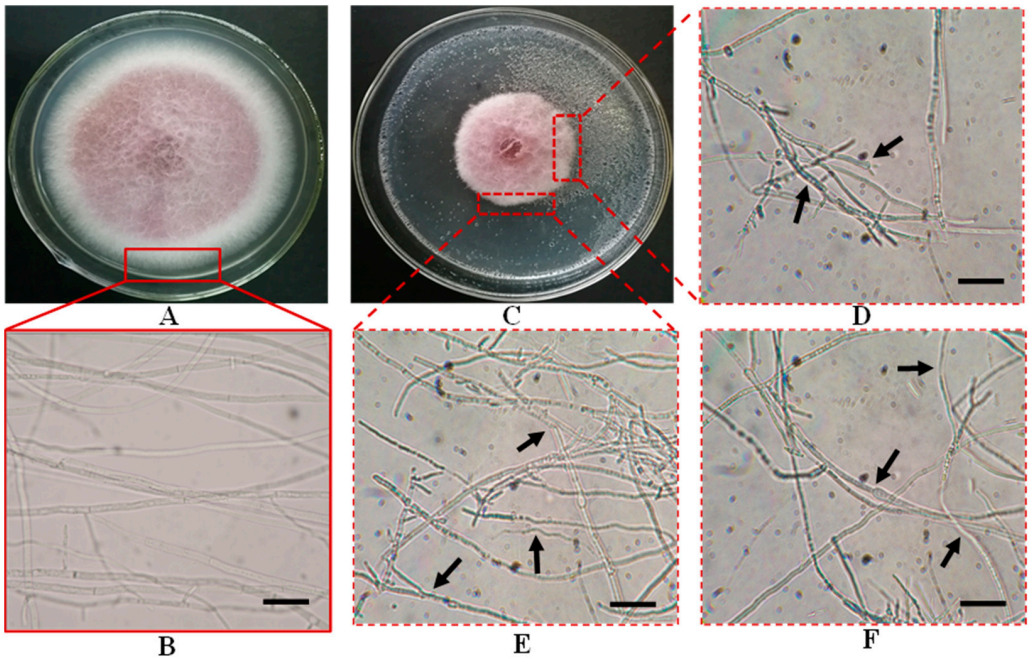

Figure 4. In vitro inhibition of mycelial growth of *F. oxysporum* in co-culture with *P. caperatum* MR-16 on PDA medium. (**A**,**B**): *F. oxysporum* in single culture. (**C**–**F**): Mycelia of *F. oxysporum* in co-culture with culture filtrate of MR-16. Scale bars = 20 μm.

3.5. Culture Filtrate of MR-16 on Spore Germination of F. oxysporum in Co-Culture

The effect of the co-culture of MR-16 culture filtrate and spore suspension of *F. oxysporum* was shown in Table 3. The result demonstrated that the spore germination of the pathogen was significantly inhibited by the strain MR-16 (Figure S1), in which the spore germination rate of *F. oxysporum* in co-culture for 48 h and the inhibitory rates were 9.04% and 84.88%, respectively (Table 3).

Table 3. Effects of culture filtrate of MR-16 on the spore germination of *F. oxysporum*.

Treatment	Spore Germination Rate (%) [1]				Inhibition Rate at 48 h (%)
	6 h	12 h	24 h	48 h	
CK	2.70 ± 0.35 [a]	9.56 ± 3.34 [a]	22.83 ± 3.01 [a]	59.7 ± 5.97 [a]	—
MR-16	1.1 ± 0.4 [b]	1.69 ± 0.23 [b]	7.4 ± 0.37 [b]	9.04 ± 2.68 [b]	84.88 ± 3.94

Conidial in vitro germination rate of *F. oxysporum* (Mean ± SD) at 6, 12, 24, and 48 h after inoculation with the addition of culture filtrate of MR-16 isolate at 25 °C. [1] Different letters indicate significant differences for the same column at $p < 0.05$ according to Duncan's DMRT.

3.6. Soil Colonization Ability of MR-16 Isolate

The strain stably grew on PDA plates containing rifampicin (400 μg·mL^{-1}) after 10 generations of subculture, in which the morphology of MRRif-16 had no noticeable change. MR-16 and MRRif-16 had a similar effect against *F. oxysporum*; the inhibition rate of SWRif-34 was up to 65%. The results indicated that strain MRRif-16 was genetically stable and still maintained high inhibition activity against *F. oxysporum*. The ability of MRRif-16 to colonize in soil was tested for 35 d. In the soil, the population density of MRRif-16 first increased and then decreased. The MRRif-16 population was 6.05 × 10^6 CFU per gram of soil at seven days of inoculation. It peaked, and the cell counts increased from 6.05 × 10^6 to 7.90 × 10^6 CFU per gram soil at twenty-eight days of inoculation in soil. Subsequently, it began to decrease in the soil. Then, the amount of colonization of the MRRif-16 population was still 5.56 × 10^6 CFU per gram of soil thirty-five days after inoculation (Figure 5).

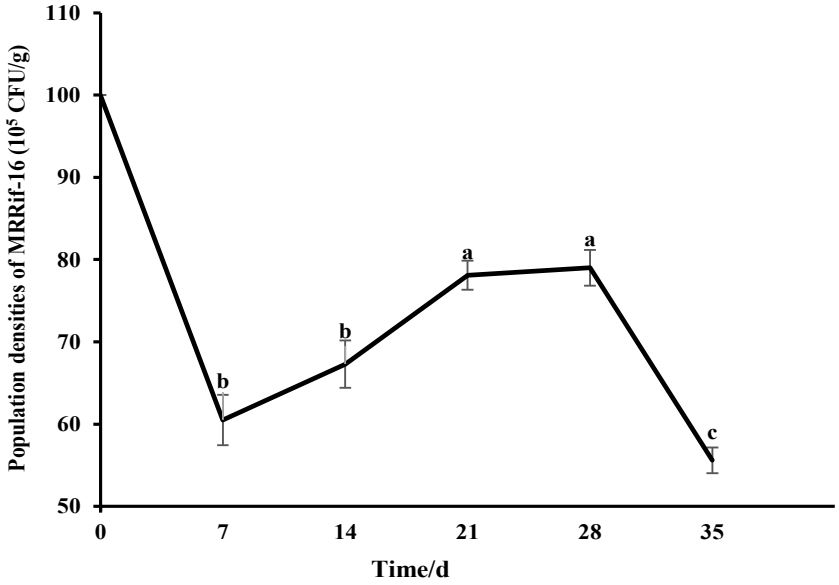

Figure 5. Colonization of MRRif-16 in the soil in which *S. divaricata* was grown. Soil samples were treated with a spore suspension of MRRif-16 at a final density of 10^7 CFU per gram of soil. After 7 d, 14 d, 21 d, 28 d, and 35 d of inoculation, the population densities of the MRRif-16 were determined. The different letters indicate significant differences in Duncan's DMRT ($p < 0.05$).

3.7. Control Efficiency of MR-16 against Fusarium wilt of S. divaricata

There was a reduced number of disease symptoms in plants of *S. divaricata* treated with a spore suspension of MR-16 (Table 4). *S. divaricata* plants in the nontreated control treatment inoculated with *F. oxysporum* showed mass disease spots on their leaves, which was up to 50.59 on the disease severity index, whereas Fusarium wilt of *S. divaricata* was strongly inhibited in the treatment with the spore suspension of MR-16 and the antifungal efficacy was 60.76% ten weeks after the treatment. Moreover, there was no significant difference in antifungal efficacy of the spore suspension of MR-16 compared with that of carbendazim 50% WP (68.38%), a bacterial suspension of *Bacillus subtilis* (56.15%), or spore suspension of *Trichoderma harzianum* (61.28%).

Table 4. Effect of the MR-16 isolate on Fusarium wilt of *S. divaricate*.

Treatment [2]	Disease Severity [1]	Disease Control (%) [1]
A	19.85 ± 2.06 [b]	60.76 ± 4.08 [a]
B	16.00 ± 4.23 [b]	68.38 ± 8.36 [a]
C	22.18 ± 3.72 [b]	56.16 ± 7.35 [a]
D	19.59 ± 2.28 [b]	61.28 ± 4.51 [a]
CK	50.59 ± 4.29 [a]	—

[1] Duncan's DMRT analysis was performed ($p < 0.05$). [2] Different treatments are as follows, A: MR-16, B: Carbendazim 50% WP, C: *B. subtilis*, D: *T. harzianum*, CK: untreated control (clear water). The same letters represent no significant differences ($p > 0.05$), with the same letter indicators used in all tables.

3.8. The Growth-Promoting Effect of MR-16 on S. divaricata

The spore suspension of MR-16 showed no pathogenicity on *S. divaricata* plants at 10 days post-inoculation, and we observed no disease symptoms in *S. divaricata*. The physiological traits of *S. divaricata* plants were noticeably affected by the MR-16 isolate, *T. harzianum*, and *B. subtilis* (Table 5). The plant height, fresh biomass, total dry plant biomass, fresh root biomass, and dry root biomass of the specie treated with strain MR-16 were significantly higher than those of plants in other treatment groups ($p < 0.05$). The average increase of plant height, fresh biomass, etc., of *S. divaricata* in the MR-16 treatment group was 42.09%, compared with those of plants in the CK treatment. The growth of

specie, including fresh plant biomass, dry plant biomass, fresh root biomass, and dry root biomass, was promoted by the application of the spore suspension of MR-16, which increased by more than 43% compared with those of the CK. The results demonstrated that the MR-16 isolate could stimulate the growth of *S. divaricata* as a plant-growth-promoting fungus. MR-16 isolate has the potential to be used as a beneficial microorganism for yield increase.

Table 5. Effects of MR-16 on promoting the growth of *S. divaricate*.

Treatment [1]	Plant Height/cm	Root Length/cm	Plant Fresh Biomass/g	Root Fresh Biomass/g	Plan Dry Biomass/g	Root Dry Biomass/g
A	56.30 ± 4.47 [a]	29.87 ± 3.34 [a]	9.85 ± 0.49 [a]	3.98 ± 1.03 [a]	2.70 ± 0.67 [a]	1.42 ± 0.28 [a]
B	51.63 ± 3.58 [b]	29.34 ± 2.15 [a]	7.66 ± 1.62 [b]	3.04 ± 0.47 [b]	2.49 ± 0.97 [a]	1.04 ± 0.30 [b]
C	51.35 ± 4.14 [b]	28.63 ± 2.84 [a]	7.89 ± 1.98 [b]	3.10 ± 0.39 [b]	2.54 ± 1.01 [a]	1.06 ± 0.25 [b]
CK	50.99 ± 4.57 [b]	28.25 ± 2.43 [a]	6.62 ± 1.73 [c]	2.33 ± 0.79 [c]	1.88 ± 0.57 [b]	0.82 ± 0.19 [c]

For each column, means labeled with the same letter did not differ significantly at $p > 0.05$ (Duncan's DMRT).
[1] Different treatments are as follows, A: MR-16, B: *B. subtilis*, C: *T. harzianum*, CK: clear water.

4. Discussion

The genus *Penicillium* is a member of the soil saprophytic group of fungi that are environmentally adaptable, comprising approximately 480 accepted species to date [33]. Although some members of the *Penicillium* can cause food-borne contamination and fungal diseases in plants and animals [34,35], *Penicillium*, being one of the first fungal species, has important research and economic value in the field of biotechnology [36]. In addition, some *Penicillium* strains have strong ecological competitiveness and have shown outstanding applications, including insecticides [37,38], plant growth promotion [39], heavy metal biosorption [40], industrial wastewater treatment [41], etc. They have received considerable attention, have been widely studied by countries worldwide, and are considered to be a suitable source of fungi biocontrol with development and application value [42].

A rhizospheric fungus, strain MR-16 was screened in this study, which showed a strong antagonistic effect on *F. oxysporum*, which causes Fusarium wilt disease of *S. divaricata*. This strain has obvious antagonistic effects on nine common pathogenic fungi. Based on our findings of culture characteristics, microscopic features, and molecular identification, strain MR-16 was identified as *Penicillium caperatum*. MR-16, tested in this study, was first discovered and reported in China, and we isolated this fungal species. *P. caperatum* is a species in the *Lanata-Divaricata* section of *Penicillium*. As a first report, this species was isolated from the soil of Murrumbidgee Irrigation Area in NSW, Australia [33,43].

The antifungal mechanisms of antagonistic fungi mainly include competition, lysis, induced plant resistance, and hyperparasitism [44]. Fungi of the *Penicillium* genus could effectively antagonistically affect the growth of pathogenic fungi by penicierythritols, calbistrins, and other active substances [45,46]. Moreover, the *Penicillium* genus can induce resistance in plant hosts, thus protecting them from plant pathogens [47]. The mycelium and spore germination of *F. oxysporum* was inhibited by *P. caperatum* aseptic fermentation filtrate, which caused the mycelium to be malformed and the spore germination rate was decreased. It was speculated that *P. caperatum* could destroy the cell wall or membrane structure of *F. oxysporum* mycelium by secreting extracellular antibacterial substances. Meanwhile, the respiration of pathogen spores was disturbed, which resulted in the inhibition of the growth of pathogenic fungi, suggesting that the extracellular secondary metabolites of *P. caperatum* could be developed as potential biocontrol agents for plant diseases. However, the specific substances responsible for the inhibitory effect of *P. caperatum* remain unknown, which is worth further study.

The effectiveness and stability of biocontrol effects could be directly affected by the ability of antagonistic microorganisms to colonize the soil, which is an important indicator for assessing potential biocontrol sources [48]. In our study, we found that *P. caperatum* MR-16 possesses soil colonization capacity through antibiotic labeling, which could grow

and stably propagate in the soil. We will evaluate the colonial effect of *P. caperatum* on *S. divaricata* in future research.

The *Penicillium* genus can prevent and control plant diseases [49]. In this present study, we isolated *Penicillium caperatum* from the rhizospheric soil from *S. divaricata* field, and it was first discovered in soil in China, suggesting that *P. caperatum* may be a soil saprophytic group of fungi. In addition, we found that Fusarium wilt disease of *S. divaricata* was effectively prevented and controlled by *P. caperatum* MR-16, and the effect of MR -16 on the growth-promoting nature of *S. divaricata* plants was stronger than *B. subtilis* and *T. harzianum*. To the best of our knowledge, this is the first report on the bio-control activities of *P. caperatum* against plant fungal pathogens of a medicinal plant and its plant growth promotion effects. The dominant microorganisms in the habitat or other environmental factors limit the growth and reproduction resulting from the competition of many exogenous microorganisms introduced into the soil, resulting in unsatisfactory biocontrol effects. In addition, the original microecological balance of the soil is affected by non-indigenous microorganisms that may have a substitution effect on the dominant microorganisms in the soil microenvironment [50]. The diversity and structure of indigenous communities play an important role in the functioning of indigenous communities. Excessive interference by exogenous microorganisms may directly or indirectly affect the interaction between host plants and soil microorganisms [51]. *T. harzianum* and *B. subtilis*, as exogenous microorganisms not originating from inherent soil from *S. divaricata* field, may compete with indigenous microorganisms for ecological niches when they are introduced into the soil, which the biological activity weakened, and disease control and plant-growth-promotion capacity decreased [52]. In conclusion, the *P. caperatum* MR-16, which was isolated and screened from the rhizospheric soil from the *S. divaricata* field, may be used for eco-friendly biological control of Fusarium wilt disease on *S. divaricata* and other plants.

5. Conclusions

In the present study, we isolated and identified a potential biocontrol agent for *S. divaricata*, *Penicillium caperatum* MR-16. A new record of this species of China, it exhibited biocontrol activity against the Fusarium wilt disease of *S. divaricata*. This is the first report of MR-16 showing broad-spectrum antibiotic capacities, which displayed good antifungal efficacy in vitro as a beneficial microorganism. In addition, *P. caperatum* MR-16 could successfully colonize and form a stable population in the soil.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9040361/s1, Figure S1: Effects of culture filtrate of MR-16 on the spore germination of *F. oxysporum*.

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