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Identification, Antimicrobial and Plant Growth Promoting Activities of Endophytic Fungi Associated with *Cynomorium songaricum* Rupr., a Traditional Medicinal Plant in Mongolia

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Abstract: Endophytic fungi colonize the inner tissues and provide direct and indirect benefits to plants. Although Mongolia is rich in medicinal plants, due to climatic and anthropogenic reasons, the resources are being depleted, and many species are under threat of gradual extinction, while the endophytic fungi of Mongolian plants are largely unknown. In this study, a total of 24 culturable endophytic fungal strains were isolated from *Cynomorium songaricum* (Rupr.), a medicinal and vulnerable plant species of Mongolia. Based on the morphological characteristics and the sequences of the rDNA internal transcribed spacer (ITS) region, the isolates were identified into six genera: *Fusarium* (8), *Clonostachys* (7), *Penicillium* (6), *Alternaria* (1), *Aspergillus* (1), and *Madurella* (1). The antimicrobial activity was assessed by the agar-diffusion method, revealing that 15 strains were able to inhibit the growth of at least one of the test organisms. Among them, 1 strain showed inhibitory activity against *Escherichia coli*, 12 against *Bacillus subtilis*, 13 against *Staphylococcus aureus*, and 8 against *Aspergillus niger*, respectively. The ability to solubilize complex phosphorus and zinc minerals was observed in 3 and 21 strains, respectively, and the production of indole-3-acetic acid (IAA) was detected in nine strains in the presence of tryptophan. Our study provides the first insight into the cultivable endophytic fungal composition of *C. songaricum*, parasitizing the roots of *Nitraria sibirica* growing in the Gobi Desert of Mongolia. The resulting fungi, which have antimicrobial and plant growth-promoting properties, were preserved in the national culture collection and can be used to further exploit their biotechnological potential, as well as for the propagation of endangered and vulnerable medicinal plants.

Keywords: culture collection; *Cynomorium songaricum*; fungal diversity; indole acetic acid; ITS region; phosphate solubilization; Ulaan goyo; zinc solubilization

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1. Introduction

To implement the Convention on Biological Diversity [1] and the Law on Genetic Resources of Mongolia, which entered into force on 30 December 2021 [2], the establishment and maintenance of facilities for *ex situ* conservation of biological diversity, research, and management of the collection of biological resources, especially microorganisms, are in demand in Mongolia. Mongolia is one of the countries most affected by climate change, with a temperature increase of 2.14 °C confirmed between 1940 and 2008 [3], which can lead to the loss of biodiversity. In addition to climatic reasons, overexploitation makes medicinal plants more vulnerable. Therefore, we focus on endophytic microorganisms, isolate the key culturable representatives residing in the medicinal, endangered, and vulnerable plants in Mongolia, preserve them in the culture collection, and explore their biotechnological potential for further sustainable use.

Endophytic fungi are microorganisms that live in internal plant tissue for at least a certain period of their life cycle without causing harm to the host plant under any circumstance [4]. They are well known to provide direct (nutrient acquisition and phytohormone

production) and indirect (activation of systemic resistance, production of secondary metabolites, and protection for abiotic and biotic stresses) benefits to the host plant [5]. The direct benefits of endophytic fungi result in enhanced root development, increased plant height, biomass production, and overall yield; hence, they can be referred to as biofertilizers [6]. Phosphorus is the second most important nutrient for overall plant development and productivity. However, its structural and chemical characteristics make it a limiting nutrient for plant growth by reducing its free availability [7]. Zinc is one of the essential micronutrients for plants, and a deficiency of this element leads to a decrease in the quality of the crop. Zinc-solubilizing microorganisms can solubilize the inaccessible form of zinc by secreting organic acids, siderophores, and other chelating compounds, but this property is well known to bacteria and, to a lesser extent, fungi [8]. Endophytic fungi produce phytohormones such as auxins, gibberellins (GAs), and cytokinins, and indole-3-acetic acid (IAA) is the main auxin produced by endophytes [5].

Over the past several years, endophytic fungi have attracted attention due to their ability to produce novel bioactive secondary metabolites and have become known as a treasure house of bioactive compounds of medicinal importance [9]. Their metabolites are progressively being studied, and metabolites are categorized into various functional groups: alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, saponins, tannins, terpenoids, tetralones, xanthones, and many others that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties [10–13]. However, it is estimated that only 1–2% of approximately 300,000 plant species have been studied, meaning that the vast majority of endophytic fungal symbiotic relationships remain unexplored [14].

Cynomorium songaricum Rupr., called Ulaan goyo or Zuungariin goyo in Mongolia, is a medicinal, parasitic, and rare plant species distributed in southern Mongolia and northwest Inner Mongolia in China [15]. This plant usually parasitizes the roots of *Nitraria tangutorum* Bobr. and *Nitraria sibirica* Pall. located in dry sandy regions, and it is widely used as a functional food and medicine in traditional Mongolian medicine and traditional Chinese medicine [16]. In traditional Mongolian medicine, it has been used to treat kidney diseases, high blood pressure, liver and bile dysfunction, diabetes, weakness, dropsy, nervousness, and constipation [17]. Numerous studies on the bioactive compounds and functions of *C. songaricum* have been conducted worldwide, and Cui et al. summarized them and reported that at least 76 biologically active compounds had been isolated and identified from this amazing plant species, including flavonoids, terpenoids, steroids, organic acids, saccharides, glycosides, and phloroglucinol adducts. These compounds have pharmacological functions, such as anti-aging, anti-oxidation, anti-fatigue, and anti-HIV effects, as well as effects on the immune system, nervous system, reproductive system, and other biological activities [16]. Despite extensive research on the bioactive compounds and pharmacological actions of the plant, a study on the distribution and dynamics of endophytic fungi in *C. songaricum* and its host *N. tangutorum* was first reported in 2018, suggesting a possible exchange of endophytic fungi between them. Also, some of the isolates, such as *Fusarium* spp., exhibited the ability to promote seed germination of *C. songaricum* [18]. Further investigations revealed significant correlations between differential secondary metabolites and endophytic fungi in *C. songaricum* distributed across different locations [19], and moreover, plant species and lifestyle, as well as the local environment, strongly influenced the abundance and diversity of the endophytic fungal species in *C. songaricum* and its host *N. tangutorum* [20].

Therefore, the diversity of endophytic fungi in *C. songaricum* that parasitizes other host plant species growing in geographically distant locations and under different environmental conditions is of great interest. *N. sibirica* Pall. has a higher salt tolerance than *N. tangutorum* Bobr. [21], and its fruits have the highest total content of flavonoids and crude protein among the three species (*N. sibirica* Pall., *N. tangutorum* Bobr. and *Nitraria roborowskii* Kom.) [22].

In this study, culturable endophytic fungal strains were isolated and identified from *Cynomorium songaricum* Rupr. parasitizing on the roots of *Nitraria sibirica* Pall., and their antimicrobial activity, as well as their plant growth-promoting properties, such as phosphate solubilization, zinc solubilization, and IAA production, were determined.

2. Materials and Methods

2.1. Collection of Plant Samples

Samples of the medicinal and vulnerable plant *Cynomorium songaricum* Rupr. were collected in July 2021 at a site (45°34′43″ N, 98°13′12″ E) located at an altitude of 1730 m above sea level in a desert area in the Govi-Altai province of Mongolia. The Gobi Desert is a cold desert with average temperature fluctuations from below – 20 °C in winter to over 33 °C in summer. Precipitation is over 200 mm in the Gobi-Altai mountains (sampling area) compared to the extreme arid areas of the Gobi Desert, where it is less than 40 mm [23]. The aboveground and underground parts, as well as fresh and old rhizomes on the root of the host plant *Nitraria sibirica* Pall. were collected (Figure 1).

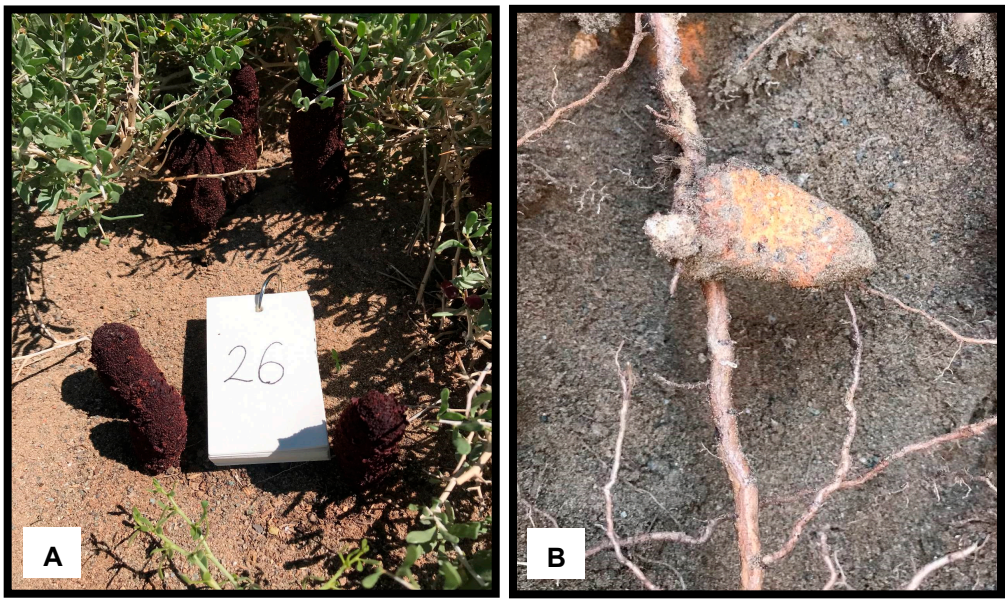

Figure 1. Habitat of the collected plant samples. *Cynomorium songaricum* (**A**) growing in sandy soil, and rhizome of *Cynomorium songaricum* on the root of the host plant *Nitraria sibirica* Pall. (**B**).

2.2. Isolation of Endophytic Fungi

Plant samples were subjected to a three-step surface sterilization procedure according to the method described by Thi Minh Le et al. [24] but with minor modifications. Portions of healthy parts and rhizomes were washed thoroughly under running tap water to remove adhered debris, and then each sample was sterilized sequentially by washing with 70% ethanol for 1 min, 3% sodium hypochlorite for 4 min, and 70% ethanol for 1 min, rinsed three times in sterile distilled water. After drying on sterile filter paper, each sample was cut into small pieces of less than 1 cm in size with a sterile scalpel and placed on a potato dextrose agar (PDA, Biolab Diagnostics Laboratory Inc., Budapest, Hungary) supplemented with 50 mg/L chloramphenicol to suppress bacterial growth. All plates were incubated at 28 °C, and the growth of endophytic fungal hyphae emerging from the segments was monitored daily for up to 3 weeks. Emerging fungi were transferred to fresh PDA plates, incubated for 1–2 weeks, and periodically checked for purity. The effectiveness of the surface sterilization procedure was ascertained by spreading 200 mL of the last wash water on the agar plates and incubating at 28 °C for 1 week to check for microbial growth. The pure cultures were preserved in glycerol suspensions (20%, *v*/*v*) at −80 °C.

All isolated fungi were deposited into the Mongolian National Culture Collection of Microorganisms (MNCCM), Institute of Biology, Mongolian Academy of Sciences.

2.3. Identification of the Isolates

Fungal isolates were cultured on a low carbon agar (LCA) medium composed of glucose 1 g/L, potassium dihydrogen phosphate 1 g/L, magnesium sulfate 0.2 g/L, potassium chloride 0.2 g/L, sodium nitrate 2 g/L, yeast extract 0.2 g/L, and agar 15 g/L at 28 °C for 7 days. The purity and monosporic cultures were confirmed by observing the isolates under an Olympus CX41 microscope (Olympus, Japan) at magnifications of 40–500×, and spore-forming fungi were preliminarily identified by morphological features, such as conidia, conidiophores, and hyphae [25].

Molecular identification was carried out using fungal isolates grown on a PDA at 28 °C for 7 days [26]. Total genomic DNA was extracted using the PrepMan™ Ultra Sample Preparation Reagent (Thermo Fisher Scientific Inc., Foster, CA, USA) according to the manufacturer's instructions.

The internal transcribed spacer (ITS) region, 5.8S gene and the D1/D2 domain of the large subunit (LSU) ribosomal RNA (rRNA) gene were amplified using the primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) as described previously [27]. The amplification was performed with a FastStart Taq DNA polymerase (Roche, China) in the GeneAmp® PCR System 9700 (Thermo Fisher Scientific Inc., USA) according to the following conditions: initial denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 30 s, and extension at 68 °C for 90 s, and a final extension step at 68 °C for 5 min. The PCR products were visualized by electrophoresis on 1% agarose gel and subsequently purified using an *AccuPrep*® PCR/Gel Purification Kit (Bioneer, Daejeon, Republic of Korea) and sent to Macrogen, Korea, for commercial sequencing. The sequences were analyzed by BLAST similarity search on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST) based on their identity values on 29 September 2023.

The obtained sequences were submitted to GenBank, and the accession numbers are LC663161- LC663164 and LC769415- LC769442.

2.4. Antimicrobial Activity Test

The endophytic fungal isolates were screened using the modified agar plug diffusion method for antimicrobial activity against potentially pathogenic bacteria [*Escherichia coli* (NBRC 102203^T), *Bacillus subtilis* (NBRC 13719^T), and *Staphylococcus aureus* (NBRC 100910^T)] and two fungi [*Candida albicans* (NBRC 1385^T) and *Aspergillus niger* (NBRC 33023^T)] [28–30]. Inoculums of the test bacteria (approximately 1×10^8 CFU/mL) and yeast (approximately 1×10^6 CFU/mL) were prepared by comparison with 0.5 McFarland standards, whereas inoculums of test fungi were prepared as 1×10^6 spores/mL. The test bacterial inoculums were seeded on nutrient agar (Biolab Diagnostics Laboratory Inc., Hungary), and yeast and fungal inoculums were seeded on a PDA, respectively. For the preparation of the agar plug, the fungal isolates were precultured on PDA plates at 28 °C for 7 days, and then the cultures were cut into plugs 6 mm in diameter; the plugs were placed on the agar medium seeded with test microorganisms. The plates were incubated at either 37 °C (bacteria and yeast) or 30 °C (fungi). Ampicillin sodium (50 µg/disk) and cycloheximide (25 µg/disk) were taken as positive controls for bacteria and fungi, respectively. Antimicrobial activity was assessed by the size (diameter in mm) of sterile zones formed around the fungal agar plugs.

2.5. Phosphate and Zinc Oxide Solubilization Assay

Screening of fungal isolates for the ability to solubilize phosphate and zinc was carried out on Pikovskaya's (PKV) agar medium and on mineral salts agar medium amended with 0.1% of insoluble ZnO, respectively, as described previously [31]. Agar plugs (6 mm), cut from a 14-day-old culture of endophytic fungi, were placed on the respective plates in triplicate and incubated at 28 °C. The clear zones formed around the colonies were

measured after the incubation period, 4 days and 14 days after inoculation, for the efficiency of solubilization of phosphorus and zinc, respectively.

2.6. IAA Production Assay

The production of indole acetic acid (IAA) in endophytic fungi was determined using the following colorimetric assay. Fungal isolates were incubated in 5 mL of PDB supplemented with 5 mM of L-tryptophan at 28 °C for 5 days. After cultivation, each culture was centrifuged at 5000 rpm for 10 min, and 1 mL of the clear supernatant was mixed with 2 mL of Salkowski reagent (1 mL 0.5 M $FeCl_3$, 50 mL 35% perchloric acid), and the mixture was incubated in the dark at room temperature for 20 min. A mixture of PDB medium with 5 mM of L-tryptophan and Salkowski reagent was used as a control. The development of a pink color indicated IAA production and the pink-to-red color produced by the isolates was categorized into low, medium, and high [32]. The absorbance of a positive reaction was determined with a UV–VIS spectrophotometer at 530 nm, and the amount of IAA product was calculated from a standard graph prepared using known quantities of pure IAA [31].

3. Results

3.1. Isolation and Identification of Endophytic Fungi

In the present study, a total of 24 purified isolates of endophytic fungi were obtained from aboveground parts (4), underground parts (4), fresh rhizomes (8), and old rhizomes (8) of *Cynomorium songaricum* Rupr. parasitizing on the roots of *Nitraria sibirica* Pall., growing in sandy soil in the Govi-Altai province territory of Mongolia. They were morphologically highly diverse and mainly pigmented on a PDA medium (Figure 2).

Molecular identification of fungal endophytes was performed using ITS rDNA sequences (ITS1-5.8S-ITS2) as a marker. ITS rDNA sequences from the 24 isolates were compared with sequences of organisms represented in the GenBank database. BLAST results showed that the 24 isolates belonged to six genera: *Fusarium*, *Clonostachys*, *Penicillium*, *Alternaria*, *Aspergillus*, and *Madurella* (Table 1). The genus *Fusarium* had the highest number of isolates (eight), followed by *Clonostachys* with seven isolates, *Penicillium* with six isolates, while the genera *Alternaria*, *Aspergillus*, and *Madurella* all had one isolate each.

3.2. Antimicrobial Activity

All isolates were evaluated in vitro for antimicrobial activity, and among the 24 isolates, 15 (62.5%) exhibited antimicrobial activity against at least one test microorganism, whereas the rest yielded no activity. There was 1 strain that had antimicrobial activity against *Escherichia coli*, 12 strains against *Bacillus subtilis*, 13 strains against *Staphylococcus aureus*, and 8 strains against *Aspergillus niger* (Table 2). Among the 15 antagonistic strains, 13 (86.6%) belonged to the genera *Fusarium* and *Clonostachys*. All seven strains belonging to *Clonostachys* and six out of eight strains belonging to *Fusarium* had antimicrobial activity. A strain (P26-ZN1-2) belonging to *Madurella* inhibited the growth of *E. coli* along with *B. subtilis* and *S. aureus* with inhibitory zones of 7.5 ± 0.7, 9.5 ± 2.1 and 15.5 ± 0.7, respectively. Moreover, eight strains (P26-H1-3, P26-H2-2, P26-ZN1-2, P26-ZO1-3, P26-ZO1-4, P26-ZO1-5, P26-ZO1-6, P26-ZO2-3) showed a broader spectrum of antimicrobial activity (inhibition zone, or against test bacteria and fungi, both), and two strains (P26-H2-2, P26-R2-1) displayed strong inhibition to the pathogenic fungus (i.e., *Aspergillus niger*). None of the strains inhibited the *Candida albicans* test (Table 2).

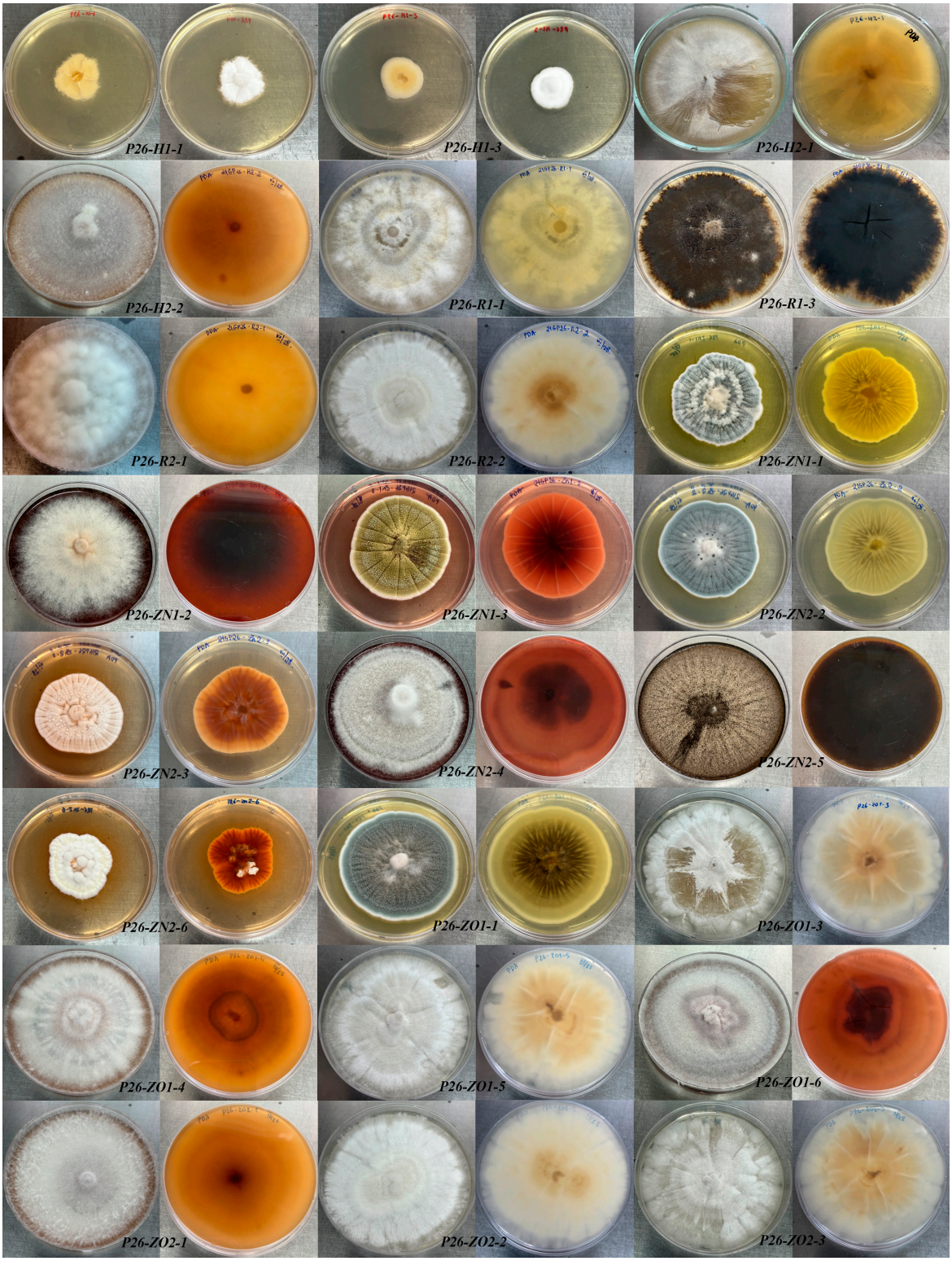

Figure 2. The morphological diversity of endophytic fungi isolated from *Cynomorium songaricum*.

Table 1. Closest relatives of endophytic fungal strains Based on ITS sequence BLAST analyses.

Strain	Accession No	Plant Part	Species with Most Homologous Sequence (Accession No)	Similarity %
P26-H1-1	LC769420	Aboveground	*Fusarium equiseti* CB33-4 (MT558601)	99.81
P26-H1-3	LC769421	Aboveground	*Clonostachys rosea* MR44 (KY320599)	99.65
P26-H2-1	LC769422	Aboveground	*Fusarium solani* GBC-Fungus 27 (MN077430)	100
P26-H2-2	LC769423	Aboveground	*Fusarium solani* N-49-1 (MT560378)	100
P26-R1-1	LC769424	Underground	*Fusarium equiseti* NL-374-D (OQ561206)	99.63
P26-R1-3	LC769425	Underground	*Alternaria* sp. INM5 (KY781740)	99.30
P26-R2-1	LC769426	Underground	*Fusarium solani* N-13-2 (MT560338)	100
P26-R2-2	LC769427	Underground	*Clonostachys rosea* MR44 (KY320599)	99.13
P26-ZN1-1	LC769428	Fresh rhizome	*Penicillium chrysogenum* MZC-0 (MN069559)	99.66
P26-ZN1-2	LC769429	Fresh rhizome	*Madurella fahalii* 332- pus (OQ421454)	98.96
P26-ZN1-3	LC769430	Fresh rhizome	*Aspergillus tabacinus* fung8 (MT635280)	100
P26-ZN2-2	LC769431	Fresh rhizome	*Penicillium chrysogenum* MZC-0 (MN069559)	99.83
P26-ZN2-3	LC769432	Fresh rhizome	*Penicillium roseopurpureum* IHEM:28005 (OU989457)	99.83
P26-ZN2-4	LC769433	Fresh rhizome	*Fusarium* sp. GFR18 (MT447523)	100
P26-ZN2-5	LC769434	Fresh rhizome	*Penicillium vinaceum* 533 (DQ681340)	100
P26-ZN2-6	LC769435	Fresh rhizome	*Penicillium roseopurpureum* G5-2 (MN206951)	100
P26-ZO1-1	LC769436	Old rhizome	*Penicillium* sp. FP-027-A7 (MH102087)	99.49
P26-ZO1-3	LC769437	Old rhizome	*Clonostachys rosea* daef27 (MH550497)	99.12
P26-ZO1-4	LC663164	Old rhizome	*Clonostachys rosea* Potato root (MT448899)	100
P26-ZO1-5	LC769438	Old rhizome	*Clonostachys* sp. 1R1D (OR365747)	99.82
P26-ZO1-6	LC769439	Old rhizome	*Fusarium* sp. GFR18 (MT447523)	99.82
P26-ZO2-1	LC769440	Old rhizome	*Fusarium proliferatum* CBB-6 (MT560216)	100
P26-ZO2-2	LC769441	Old rhizome	*Clonostachys rosea* MR44 (KY320599)	99.83
P26-ZO2-3	LC769442	Old rhizome	*Clonostachys rosea* N25 (MH259861)	100

Table 2. Antimicrobial activity of endophytic fungi.

Strain	Taxa	Diameter of the Inhibitory Zone (mm)				
		Escherichia coli	*Bacillus subtilis*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus niger*
P26-H1-1	*Fusarium equiseti*	-	15.5 ± 0.7	12 ± 0	-	-
P26-H1-3	*Clonostachys rosea*	-	10.5 ± 0.7	-	-	15.5 ± 0.7
P26-H2-1	*Fusarium proliferatum*	-	11.5 ± 1.4	13 ± 0.7	-	-
P26-H2-2	*Fusarium solani*	-	-	10.5 ± 0.7	-	23 ± 2.8
P26-R1-1	*Fusarium equiseti*	-	15.5 ± 0.7	13.5 ± 0.7	-	-
P26-R2-1	*Fusarium solani*	-	-	-	-	22.5 ± 0.7
P26-R2-2	*Clonostachys rosea*	-	-	7.5 ± 2.1	-	-
P26-ZN1-2	*Madurella fahalii*	7.5 ± 0.7	9.5 ± 2.1	15.5 ± 0.7	-	-
P26-ZN1-3	*Aspergillus amoenus*	-	8 ± 1.4	7 ± 0	-	-
P26-ZO1-3	*Clonostachys rosea*	-	15 ± 0	18.5 ± 0.7	-	13.5 ± 2.1
P26-ZO1-4	*Clonostachys rosea*	-	9 ± 4.2	9.5 ± 0.7	-	12.5 ± 2.1
P26-ZO1-5	*Clonostachys rosea*	-	13.5 ± 0.7	16.5 ± 0.7	-	14 ± 1.4
P26-ZO1-6	*Fusarium tonkinense*	-	11.5 ± 0.7	17.5 ± 0.7	-	12 ± 0
P26-ZO2-2	*Clonostachys rosea*	-	12 ± 0	15.5 ± 2.1	-	-
P26-ZO2-3	*Clonostachys rosea*	-	13.5 ± 0.7	16 ± 0	-	13 ± 1.4

Note: -: no inhibitory activity.

3.3. In Vitro Test for Plant Growth Promoting Traits of Endophytes

The endophytic isolates were further studied for their plant growth-promoting traits, including IAA production, phosphate solubilization, and zinc oxide solubilization (Table S2). All 28 strains exhibited positive results for one or more traits; IAA production was seen in nine strains in PDB medium supplemented with 5 mM L-tryptophan (Figure 3), whereas phosphate solubilization and zinc oxide solubilization activity was noticed in 3 and 21 strains, respectively (Figure 4). Three *Penicillium* strains, P26-ZN2-3, P26-ZN2-5, and P26-ZO1-1, were positive for both phosphate and zinc oxide solubilization traits; however, no production of IAA was detected in the presence and absence of L-tryptophan. *Penicillium* strain P26-ZN2-5 showed the most significant phosphate solubilizing activity on a solid PVK medium with a solubilization index (SI) of 2.16 ± 0.02 cm. This strain also showed the most significant zinc solubilizing activity on mineral salt agar with an SI of 3.4 ± 0.1 cm.

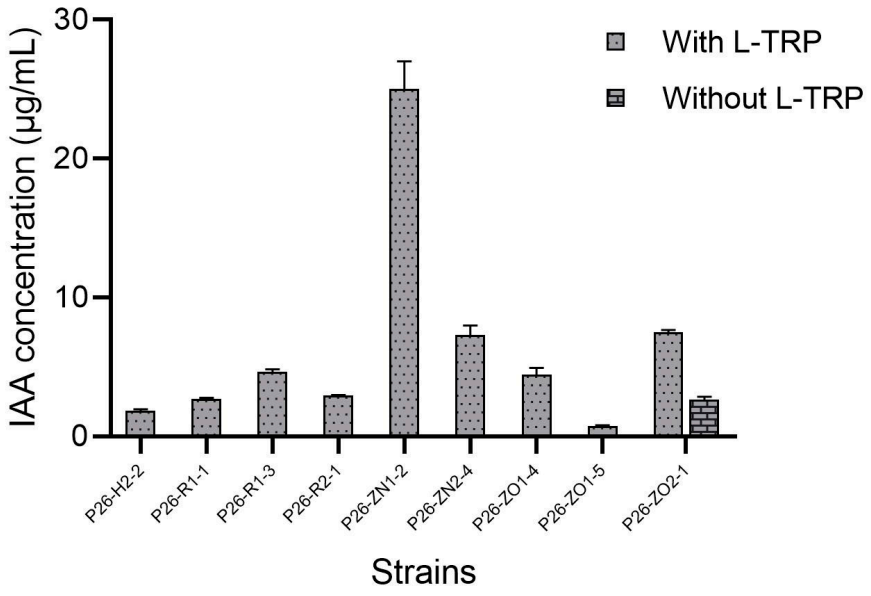

Figure 3. Production of IAA by endophytic fungal strains.

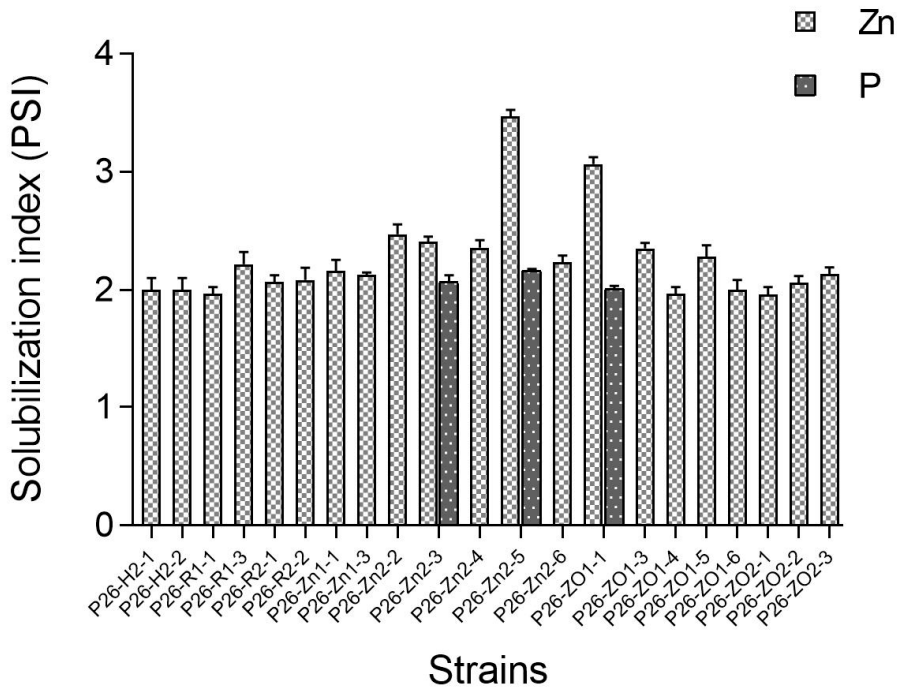

Figure 4. Phosphate and zinc solubilization by endophytic fungal strains.

4. Discussion

It has been shown that the diversity of endophytic fungi is influenced by the plant genotype [33,34] or both the host genotype and geography combined [35]. A recent study by Miao et al. on speciation and genetic diversity of endophytic fungi from their host plants, *C. songaricum*, parasitized *N. tangutorum*, and non-parasitized *N. tangutorum* at three geographic locations, found that only 0.41% to 4.48% of endophytic fungal species were shared between their host plants, consistent with previous studies indicating that the plant genotype strongly affects the endophytic fungal composition [20].

A possible exchange of endophytic fungi between *C. songaricum* and its host *N. tangutorum* was previously suggested [18], so the endophytic fungal composition of *C. songaricum* parasitizing another host, *N. sibirica* Pall, growing in the Gobi Desert was of great interest. It is known that culture-based methods do not reflect the real diversity of fungi in a niche due to artificial selection pressure, and some of these microorganisms cannot be cultivated under laboratory conditions [18,36,37]. However, culturable endophytes are potential sources for applications in biotechnology, medicine, agriculture, and beyond.

In our study, representatives of the taxa *Fusarium*, *Clonostachys*, and *Penicillium* were predominant; representatives of *Alternaria*, *Aspergillus*, and *Madurella* were less prominent. Indeed, *Fusarium* spp., *Penicillium* spp., and *Aspergillus* spp. were the most abundant fungi isolated from *C. songaricum* parasitizing the roots of *N. tangutorum* [18] and among the fungal genera from plants reported in general [10,38,39]. More specifically, *Fusarium* dominated the underground tissues of *Aristolochia chilensis* growing in an arid ecosystem [39], *C. songaricum* parasitizing the roots of *N. tangutorum*, in general, and notably at the tubercle stage, and the fermentation broth of *F. redolens* (KY379544) promoted the germination of host plant seeds [18]. Strains of this genus were isolated in the greatest numbers and were also ubiquitously isolated from all tissue types of *C. songaricum* parasitizing the roots of *N. sibirica* and demonstrated antimicrobial and zinc-solubilizing activity, as well as IAA production. Further studies on these strains are awaited, considering the enormous potential of endophytic *Fusarium* in providing plant host defense and survival strategies reported so far [40].

The second prevalent genus was *Clonostachys*, with seven isolates having 99.13–100% similarity to *Clonostachys rosea* based on ITS sequences. This genus was less prominent

in *C. songaricum,* parasitizing the roots of *N. tangutorum*; only 2 isolates were found out of 111 [18]. *Clonostachys rosea* is a well-recognized mycoparasite whose hyphae penetrate and destroy those of many host fungi, and there are several commercial products based on *C. rosea* available for biocontrol applications worldwide [41,42]. Moreover, *Clonostachys* fungi produce at least 229 secondary metabolites, such as nitrogen-containing metabolites, polyketides, and terpenoids, many of which exhibit biological activities, such as cytotoxic, antimicrobial, antileishmanial, antimalarial activity [43]. Most of our isolates assigned to the genus *Clonostachys* were found in old rhizomes, absent from fresh rhizomes, and exhibited both antibacterial and antifungal activity. Recently, Kapeua-Ndacnou et al. reported that certain *Clonostachys* endophytes from healthy tissues of *Coffea* species and mycoparasites of *Hemileia,* the coffee leaf rust (CLR), significantly reduced the severity of CLR [44].

Representatives of the genus *Penicillium* were isolated exclusively from fresh rhizomes. Interestingly, this genus also appears to be a prevalent endophyte in plant species native to the arid environments of the Atacama Desert, including *Chenopodium quinoa*, *Prosopis chilensis*, and *Aristolochia chilensis* [39,45,46]. *Penicillium* endophytes enhanced the growth of host plant *P. chilensis* by increasing PSII efficiency, nitrogen, and carbohydrate content in leaves [46] and helping *C. quinoa* respond better to drought stress [47]. Our *Penicillium* strains solubilized both phosphate and zinc but did not show antimicrobial activities. *Penicillium* spp. are known to be excellent solubilizers of phosphate [48–50] and zinc solubilizing efficiency to a lesser extent [51]. For example, *P. bilaiae* RS7B-SD1, associated with wheat roots, had the ability to solubilize significant amounts of rock phosphate [49], and another fungal strain, *P. guanacastense* JP-NJ2, solubilized phosphate by producing organic acids, extracellular acidic phosphatase and phytase, and promoted the growth of seedlings of pine *Pinus massoniana* [48]. In addition, there is a commercial inoculant of phosphate solubilizing *Penicillium* sold by NovoZymes (JumpStart®WP; NovoZymes). During the present study, *Penicillium* spp. solubilizing minerals found in fresh rhizomes suggest that they may play a role in promoting plant growth.

One isolate, P26-ZN1-2, was classified into the genus of *Madurella*. Species of *Madurella* are the most common agents of black-grain mycetoma [52]. However, in recent years, several *Madurella* strains have been isolated from different plant species as endophytes [53–55]. *Madurella* strain P26-ZN1-2 had antibacterial activity against Gram-negative and Gram-positive test bacteria and produced the highest amount of IAA.

Our study provides the first insight into the cultivable endophytic fungal composition of *C. songaricum*, a rare medicinal plant parasitizing the roots of *N. sibirica* growing in the Gobi Desert of Mongolia. The resulting fungi, which have antimicrobial and plant growth-promoting properties, can be used to further exploit their biotechnological potential and be applied to propagate endangered and vulnerable medicinal plants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16020122/s1, Table S1: Concentration of IAA produced by endophytic fungi; Table S2: Phosphate and zinc solubilization index.

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References

1. CBD Convention on Biological Diversity. United Nations. 1992. Available online: https://www.cbd.int/doc/legal/cbd-en.pdf (accessed on 11 March 2020).
2. Law on Genetic Resources of Mongolia. 2021. Available online: https://legalinfo.mn/mn/detail?lawId=16390399395691 (accessed on 10 November 2023).
3. Climate Change in Mongolia. Outputs from GCM. M. Available online: https://www.env.go.jp/content/900448010.pdf (accessed on 10 November 2023).
4. Le Cocq, K.; Gurr, S.J.; Hirsch, P.R.; Mauchline, T.H. Exploitation of endophytes for sustainable agricultural intensification. *Mol. Plant Pathol.* **2017**, *18*, 469–473. [CrossRef]
5. Baron, N.C.; Rigobelo, E.C. Endophytic fungi: A tool for plant growth promotion and sustainable agriculture. *Mycology* **2022**, *13*, 39–55. [CrossRef] [PubMed]
6. Bamisile, B.S.; Dash, C.K.; Akutse, K.S.; Keppanan, R.; Wang, L. Fungal Endophytes: Beyond Herbivore Management. *Front. Microbiol.* **2018**, *9*, 544. [CrossRef] [PubMed]
7. Khan, M.S.; Zaidi, A.; Ahemad, M.; Oves, M.; Wani, P.A. Plant growth promotion by phosphate solubilizing fungi—Current perspective. *Arch. Agron. Soil Sci.* **2010**, *56*, 73–98. [CrossRef]
8. Upadhayay, V.K.; Singh, A.V.; Khan, A.; Sharma, A. Contemplating the role of zinc-solubilizing bacteria in crop biofortification: An approach for sustainable bioeconomy. *Front. Agron.* **2022**, *4*, 903321. [CrossRef]
9. Gouda, S.; Das, G.; Sen, S.K.; Shin, H.-S.; Patra, J.K. Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance. *Front. Microbiol.* **2016**, *7*, 1538. [CrossRef]
10. Strobel, G.; Daisy, B. Bioprospecting for Microbial Endophytes and Their Natural Products. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 491–502. [CrossRef]
11. Alam, B.; Lǐ, J.; Gě, Q.; Khan, M.A.; Gōng, J.; Mehmood, S.; Yuán, Y.; Gǒng, W. Endophytic fungi: From symbiosis to secondary metabolite communications or vice versa? *Front. Plant Sci.* **2021**, *12*, 791033. [CrossRef]
12. Caruso, D.J.; Palombo, E.A.; Moulton, S.E.; Zaferanloo, B. Exploring the Promise of Endophytic Fungi: A Review of Novel Antimicrobial Compounds. *Microorganisms* **2022**, *10*, 1990. [CrossRef]
13. Jha, P.; Kaur, T.; Chhabra, I.; Panja, A.; Paul, S.; Kumar, V.; Malik, T. Endophytic fungi: Hidden treasure chest of antimicrobial metabolites interrelationship of endophytes and metabolites. *Front. Microbiol.* **2023**, *14*, 1227830. [CrossRef]
14. Strobel, G. The Emergence of Endophytic Microbes and Their Biological Promise. *J. Fungi* **2018**, *4*, 57. [CrossRef]
15. Tuvaanjav, S.; Shuqin, H.; Komata, M.; Ma, C.; Kanamoto, T.; Nakashima, H.; Yoshida, T. Isolation and antiviral activity of water-soluble *Cynomorium songaricum* Rupr. polysaccharides. *J. Asian Nat. Prod. Res.* **2016**, *18*, 159–171. [CrossRef]
16. Cui, J.-L.; Gong, Y.; Xue, X.-Z.; Zhang, Y.-Y.; Wang, M.-L.; Wang, J.-H. A Phytochemical and Pharmacological Review on *Cynomorium songaricum* as Functional and Medicinal Food. *Nat. Prod. Commun.* **2018**, *13*, 501–510. [CrossRef]
17. Ligaa, U.; Ninjil, N.; Davaadorj, T.; Lkhagvadorj, B.; Erdenetuya, N. *Medicinal Plants of Mongolia Used in Western and Eastern Medicine*, 2nd ed.; JKC Printing: Ulaanbaatar, Mongolia, 2015; pp. 135–136. (In Mongolian)
18. Cui, J.-L.; Vijayakumar, V.; Zhang, G. Partitioning of Fungal Endophyte Assemblages in Root-Parasitic Plant *Cynomorium songaricum* and Its Host *Nitraria tangutorum*. *Front. Microbiol.* **2018**, *9*, 666. [CrossRef] [PubMed]
19. Cui, J.-L.; Gong, Y.; Vijayakumar, V.; Zhang, G.; Wang, M.-L.; Wang, J.-H.; Xue, X.-Z. Correlation in Chemical Metabolome and Endophytic Mycobiome in *Cynomorium songaricum* from Different Desert Locations in China. *J. Agric. Food Chem.* **2019**, *67*, 3554–3564. [CrossRef] [PubMed]
20. Miao, S.-M.; Zhang, Y.-Y.; Cui, J.-L.; Zhang, G. Species and geographic specificity between endophytic fungi and host supported by parasitic *Cynomorium songaricum* and its host *Nitraria tangutorum* distributed in desert. *Arch. Microbiol.* **2021**, *203*, 2511–2519. [CrossRef]
21. Shi, Y.Y.; Yang, J.H.; Liu, T.; Zhang, Z.W.; Mu, K.; Liang, F.H.; Liu, Y. The effect of salt stress on the growth of 3 species of Nitraria seedlings. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *346*, 012025. [CrossRef]
22. Yuan, Y.-Y.; Sun, J.; Zhou, Y.-B.; Wang, J.; Deng, J.; Ye, R.-R.; Peng, M.; Lu, X.-F. Chemical Composition of Three Nitraria Species Fruits. *Asian J. Chem.* **2018**, *30*, 529–532. [CrossRef]
23. Heiner, M.; Galbadrakh, D.; Kiesecker, J. Shifting winds in the Mongolian Gobi Desert: Nature and traditions face the modern era. In *Encyclopedia of the World's Biomes*; Goldstein, M.I., DellaSala, D.A., Eds.; Elsevier: Oxford, UK, 2020; pp. 78–84.
24. Le, T.T.M.; Hoang, A.T.H.; Le, T.T.B.; Vo, T.T.B.; Van Quyen, D.; Chu, H.H. Isolation of endophytic fungi and screening of Huperzine A–producing fungus from *Huperzia serrata* in Vietnam. *Sci. Rep.* **2019**, *9*, 16152. [CrossRef]
25. Barman, A.; Nath, A.; Thakur, D. Identification and characterization of fungi associated with blister blight lesions of tea (*Camellia sinensis* L. Kuntze) isolated from Meghalaya, India. *Microbiol. Res.* **2020**, *240*, 126561. [CrossRef]

26. Hechmi, N.; Bosso, L.; El-Bassi, L.; Scelza, R.; Testa, A.; Jedidi, N.; Rao, M.A. Depletion of pentachlorophenol in soil microcosms with *Byssochlamys nivea* and *Scopulariopsis brumptii* as detoxification agents. *Chemosphere* **2016**, *165*, 547–554. [CrossRef]
27. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: New York, NY, USA, 1990; pp. 315–322. ISBN 978-0-12-372180-8.
28. Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* **2016**, *6*, 71–79. [CrossRef]
29. Ibrahim, D.; Lee, C.C.; Sheh-Hong, L. Antimicrobial Activity of Endophytic Fungi Isolated from *Swietenia macrophylla* Leaves. *Nat. Prod. Commun.* **2014**, *9*, 247–250. [CrossRef]
30. Bosso, L.; Scelza, R.; Varlese, R.; Meca, G.; Testa, A.; Rao, M.A.; Cristinzio, G. Assessing the effectiveness of *Byssochlamys nivea* and *Scopulariopsis brumptii* in pentachlorophenol removal and biological control of two Phytophthora species. *Fungal Biol.* **2016**, *120*, 645–653. [CrossRef]
31. Kumar, C.S.; Jacob, T.; Devasahayam, S.; Thomas, S.; Geethu, C. Multifarious plant growth promotion by an entomopathogenic fungus *Lecanicillium psalliotae*. *Microbiol. Res.* **2018**, *207*, 153–160. [CrossRef]
32. Rajini, S.B.; Nandhini, M.; Udayashankar, A.C.; Niranjana, S.R.; Lund, O.S.; Prakash, H.S. Diversity, plant growth-promoting traits, and biocontrol potential of fungal endophytes of *Sorghum bicolor*. *Plant Pathol.* **2020**, *69*, 642–654. [CrossRef]
33. Saikkonen, K. Forest structure and fungal endophytes. *Fungal Biol. Rev.* **2007**, *21*, 67–74. [CrossRef]
34. Dastogeer, K.M.G.; Oshita, Y.; Yasuda, M.; Kanasugi, M.; Matsuura, E.; Xu, Q.; Okazaki, S. Host Specificity of Endophytic Fungi from Stem Tissue of Nature Farming Tomato (*Solanum lycopersicum* Mill.) in Japan. *Agronomy* **2020**, *10*, 1019. [CrossRef]
35. Dastogeer, K.M.G.; Li, H.; Sivasithamparam, K.; Jones, M.G.K.; Wylie, S.J. Host Specificity of Endophytic Mycobiota of Wild Nicotiana Plants from Arid Regions of Northern Australia. *Microb. Ecol.* **2018**, *75*, 74–87. [CrossRef] [PubMed]
36. Fan, S.; Miao, L.; Li, H.; Lin, A.; Song, F.; Zhang, P. Illumina-based analysis yields new insights into the diversity and composition of endophytic fungi in cultivated *Huperzia serrata*. *PLoS ONE* **2020**, *15*, e0242258. [CrossRef] [PubMed]
37. Lücking, R.; Aime, M.C.; Robbertse, B.; Miller, A.N.; Ariyawansa, H.A.; Aoki, T.; Cardinali, G.; Crous, P.W.; Druzhinina, I.S.; Geiser, D.M.; et al. Unambiguous identification of fungi: Where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus* **2020**, *11*, 14. [CrossRef] [PubMed]
38. Paul, N.C.; Yu, S.H. *Endophytic Fungi from Medicinal Plants in Korea*; Lap Lambert Academic Publishing: Saarbrücken, Germany, 2011.
39. Guevara-Araya, M.J.; Vilo, C.; Urzúa, A.; González-Teuber, M. Differences in community composition of endophytic fungi between above- and below-ground tissues of Aristolochia chilensis in an arid ecosystem. *Rev. Chil. Hist. Nat.* **2020**, *93*, 3. [CrossRef]
40. Toghueo, R.M.K. Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites. *Mycology* **2020**, *11*, 1–21. [CrossRef]
41. Gams, W.; Diederich, P.; Põldmaa, K. Fungicolos Fungi. In *Biodiversity of Fungi: Inventory and Monitoring Methods*; Muller, G.M., Bills, G.F., Foster, M.S., Eds.; Academic Press: Burlington, NJ, USA, 2004; Volume 465, pp. 343–392. [CrossRef]
42. Jensen, D.F.; Dubey, M.; Jensen, B.; Karlsson, M. Clonostachys rosea to control plant diseases. In *Microbial Bioprotectants for Plant Disease Management*; Köhl, J., Ravensberg, W., Eds.; Burleigh Dodds Science Publishing: Cambridge, UK, 2021; pp. 429–472. [CrossRef]
43. Han, P.; Zhang, X.; Xu, D.; Zhang, B.; Lai, D.; Zhou, L. Metabolites from *Clonostachys* Fungi and Their Biological Activities. *J. Fungi* **2020**, *6*, 229. [CrossRef]
44. Kapeua-Ndacnou, M.; de Abreu, L.M.; de Macedo, D.M.; da Nóbrega, T.F.; Pereira, C.M.; Evans, H.C.; Barreto, R.W. Assessing the Biocontrol Potential of *Clonostachys* Species Isolated as Endophytes from *Coffea* Species and as Mycoparasites of *Hemileia* Rusts of Coffee in Africa. *J. Fungi* **2023**, *9*, 248. [CrossRef] [PubMed]
45. González-Teuber, M.; Vilo, C.; Bascuñán-Godoy, L. Molecular characterization of endophytic fungi associated with the roots of *Chenopodium quinoa* inhabiting the Atacama Desert. *Genom. Data* **2017**, *11*, 109–112. [CrossRef]
46. González-Teuber, M.; Urzúa, A.; Morales, A.; Ibáñez, C.; Bascuñán-Godoy, L. Benefits of a root fungal endophyte on physiological processes and growth of the vulnerable legume tree *Prosopis chilensis* (Fabaceae). *J. Plant Ecol.* **2019**, *12*, 264–271. [CrossRef]
47. González-Teuber, M.; Urzua, A.; Plaza, P.; Bascuñán-Godoy, L. Effects of root endophytic fungi on response of *Chenopodium quinoa* to drought stress. *Plant Ecol.* **2018**, *219*, 231–240. [CrossRef]
48. Qiao, H.; Sun, X.-R.; Wu, X.-Q.; Li, G.-E.; Wang, Z.; Li, D.-W. The phosphate-solubilising ability of *Penicilium guanacastense* and its effects on the growth of *Pinus massoniana* in phosphate limiting conditions. *Biol. Open* **2019**, *8*, bio046797. [CrossRef]
49. Wakelin, S.A.; Warren, R.A.; Harvey, P.R.; Ryder, M.H. Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. *Biol. Fertil. Soils* **2004**, *40*, 36–43. [CrossRef]
50. Doilom, M.; Guo, J.-W.; Phookamsak, R.; Mortimer, P.E.; Karunarathna, S.C.; Dong, W.; Liao, C.-F.; Yan, K.; Pem, D.; Suwannarach, N.; et al. Screening of Phosphate-Solubilizing Fungi from Air and Soil in Yunnan, China: Four Novel Species in *Aspergillus*, *Gongronella*, *Penicillium*, and *Talaromyces*. *Front. Microbiol.* **2020**, *11*, 585215. [CrossRef]
51. Devi, D.; Gupta, S.B.; Mishra, B.K.; Verma, N.P. Isolation and identification of zinc solubilizing fungal isolates from cumin of semi-arid region of Rajasthan. *Pharma Innov.* **2022**, *11*, 1036–1040.

52. de Hoog, G.S.; van Diepeningen, A.D.; Mahgoub, E.-S.; van de Sande, W.W.J. New Species of *Madurella*, Causative Agents of Black-Grain Mycetoma. *J. Clin. Microbiol.* **2012**, *50*, 988–994. [CrossRef] [PubMed]
53. Pushpa, H.; Kavya, S.; Pooja, K.; Sneha, L.; Arer, V.O. An isolation, identification and diversity of endophytic fungi from *Catharanthus roseus* and screening for their L-asparaginase activity. *Int. J. Environ. Ecol. Fam. Urban Stud.* **2018**, *8*, 7–18.
54. Hapida, Y.; Elfita, E.; Widjajanti, H.; Salni, S. Biodiversity and antibacterial activity of endophytic fungi isolated from jambu bol (*Syzygium malaccense*). *Biodiversitas J. Biol. Divers.* **2021**, *22*, 5668–5677. [CrossRef]
55. Orole, O.O.; Adejumo, T.O.; Link, T.; Voegele, R.T. Molecular identification of endophytes from maize roots and their biocontrol potential against toxigenic fungi of Nigerian maize. *Sci. Prog.* **2023**, *106*, 368504231186514. [CrossRef]