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Article

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 of plants and provide direct and indirect benefits to the plant. Although Mongolia is rich in medicinal plants, due to climatic and anthropogenic reasons the resources are being depleted and many species are under the threat of gradual extinction, while the endophytic fungi of Mongolian plants are largely unknown. Understanding the diversity and metabolic potential of endophytic fungi is of great significance in terms of the utilization of beneficial fungal strains in plant propagation and the discovery of bioactive compounds. 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 to 6 genera: *Fusarium* (8), *Clonostachys* (7), *Penicillium* (6), *Alternaria* (1), *Aspergillus* (1), and *Madurella* (1). The antimicrobial activity was assessed by 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*, 8 against *Aspergillus niger*, respectively. All fungal strains were screened for their ability to solubilize complex phosphorus and zinc minerals and for their production of indole-3-acetic acid (IAA). The strains were preserved in the national culture collection for further sustainable exploitation of their biotechnological potential.

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

1. Introduction

To implement the Convention on Biological Diversity [1] and the Law on Genetic resources of Mongolia, which entered into force on the 30th 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. As the nation's growing and largest culture collection, we are committed to exploring, preserving, and supplying microbial resources and associated data for research, education and industrial applications. Mongolia is one of the countries most affected by climate change, with the temperature increase of 2.14°C confirmed between 1940 and 2008 [3], which can lead to the loss of biodiversity. In addition to climatic reason, overexploitation makes medicinal plants more vulnerable. Therefore, we focus on endophytic microorganisms, isolate the key culturable representatives residing 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]. Endophytes can improve the uptake of both macronutrients such as phosphorus, nitrogen and potassium and micronutrients such as zinc, iron and copper from soil and organic matter [7]. Phosphorus is the second important nutrient for overall plant development and productivity. It required for biological growth and development of plants and also performs a significant part in metabolic processes including photosynthesis, transfer of energy, signal transduction and respiration [8]. However, its structural and chemical characteristics make it a limiting nutrient for plant growth since they reduce its free availability. Zinc is one of the essential micronutrients for plants, and a deficiency of this element causes a slowdown in a number of processes, such as photosynthesis and the synthesis of biomolecules (carbohydrates, RNA and proteins), which leads to a decrease in the quality of the crop [9]. 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 [7,9]. Endophytic fungi produce phytohormones such as auxins, gibberellins (GAs) and cytokinins. A kind of auxin known as indole-3-acetic acid (IAA) generally affects plant cell division, nodule formation, seed initiation and tuber germination, development of xylem and root, photosynthesis, and provides resistance to plants against various stressful conditions and is the main auxin produced by endophytes [5,7].

Over the past 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 [10]. 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, xanthenes, and many others that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties [11–14]. 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 [15].

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 [16]. This plant usually parasitizes on 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 in traditional Chinese medicine [17]. In the traditional Mongolian medicine, it has been used to treat kidney diseases, high blood pressure, liver and bile dysfunction, diabetes, weakness, dropsy, nervousness and constipation [18]. 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 have 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 of this plant 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 [17]. Despite extensive research of 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 *C. songaricum* and its host *N. tangutorum* [19]. Further investigations revealed significant correlations between differential secondary metabolites and endophytic fungi in *C. songaricum* distributed across different locations [20], and moreover, plant species and lifestyle, as well as local environment strongly influenced the abundance and diversity of the endophytic fungal species in *C. songaricum* and its host *N. tangutorum* [21].

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

The medicinal and vulnerable plant samples of *Cynomorium songaricum* Rupr. were collected in July 2021 in Govi-Altai province (45°34'43" N, 98°13'12" E, h1730m), Mongolia. The aboveground and underground parts, fresh and old rhizomes on the root of host plant *Nitraria sibirica* Pall. were collected (Figure 1).

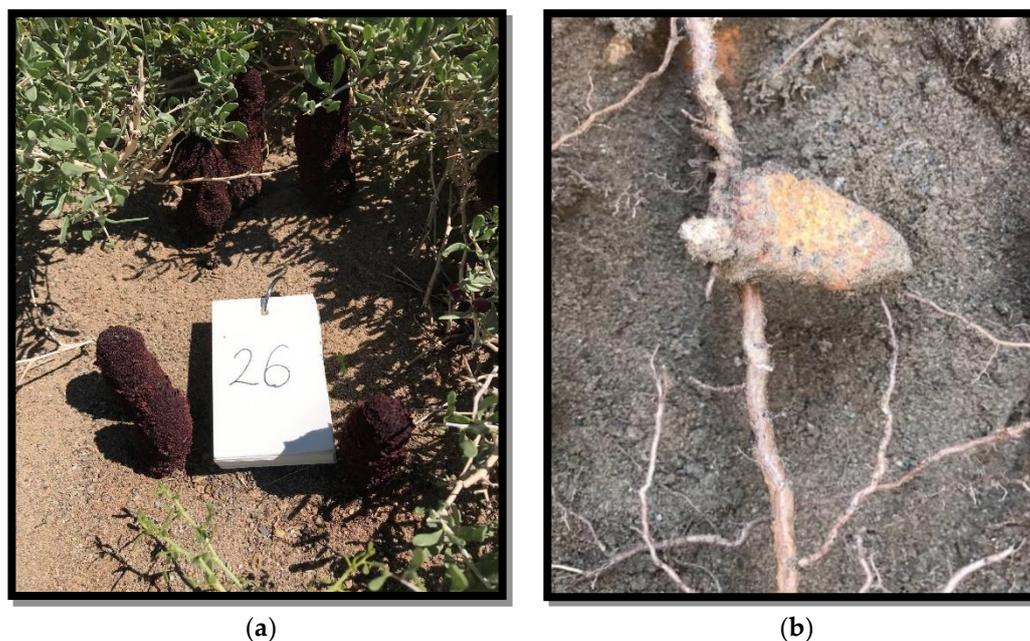


Figure 1. Habitat of the collected plant samples. *Cynomorium songaricum* (a) growing in sandy soil, and rhizome of *Cynomorium songaricum* on the root of host plant *Nitraria sibirica* Pall. (b).

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. [22] with minor modification. 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 2-4 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 potato dextrose agar (PDA, Biolab Diagnostics Laboratory Inc., 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 Low carbon agar (LCA) medium composed of glucose 1 g/L, potassium dihydrogen phosphate 1 g/L, magnesium sulphate 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 40-500x magnifications, and spore-forming fungi were preliminary identified by morphological features, such as conidia, conidiophores, and hyphae.

Molecular identification was carried out using fungal isolates grown on PDA at 28°C for 7 days. Total genomic DNA was extracted using the PrepMan™ Ultra Sample Preparation Reagent (Thermo Fisher Scientific Inc., 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 was amplified using the primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') as described previously [23]. 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 sec, and extension at 68°C for 90sec; 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, Korea), and sent to Macrogen, Korea for commercial sequencing. The sequences were analyzed by BLAST similarity search at the website of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST>) based on their identity values.

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

2.4. Antimicrobial Activity Test

The endophytic fungal isolates were screened using the 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)]. 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), yeast and fungal inoculums were seeded on 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, and 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 [24–27].

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 [28]. Agar plugs (6 mm), cut from 14 days 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) of endophytic fungi was determined using the following colorimetric assay. Fungal isolates were incubated in 5mL PDB supplemented with 5mM 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 FeCl₃, 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 5mM L-tryptophan and Salkowski reagent was used as a control. Development of pink color indicated IAA production and the pink to red color produced by the isolates was categorized into low, medium, and high [8]. 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 [29].

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 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 6 genera: *Fusarium*, *Clonostachys*, *Penicillium*, *Alternaria*, *Aspergillus*, and *Madurella* (Table 1). The genus *Fusarium* had the highest number of isolates (8), followed by *Clonostachys* with 7 isolates, *Penicillium* with 6 isolates, while the genera *Alternaria*, *Aspergillus*, and *Madurella* all had one isolate each.



Figure 2. 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	<i>Fusarium equiseti</i> CB33-4 (MT558601)	99.81
P26-H1-3	LC769421	Aboveground	<i>Clonostachys rosea</i> MR44 (KY320599)	99.65
P26-H2-1	LC769422	Aboveground	<i>Fusarium solani</i> GBC-Fungus 27	100

			(MN077430)	
P26-H2-2	LC769423	Aboveground	<i>Fusarium solani</i> N-49-1 (MT560378)	100
P26-R1-1	LC769424	Underground	<i>Fusarium equiseti</i> NL-374-D (OQ561206)	99.63
P26-R1-3	LC769425	Underground	<i>Alternaria</i> sp. INM5 (KY781740)	99.30
P26-R2-1	LC769426	Underground	<i>Fusarium solani</i> N-13-2 (MT560338)	100
P26-R2-2	LC769427	Underground	<i>Clonostachys rosea</i> MR44 (KY320599)	99.13
P26-ZN1-1	LC769428	Fresh rhizome	<i>Penicillium chrysogenum</i> MZC-0 (MN069559)	99.66
P26-ZN1-2	LC769429	Fresh rhizome	<i>Madurella fahalii</i> 332- pus (OQ421454)	98.96
P26-ZN1-3	LC769430	Fresh rhizome	<i>Aspergillus tabacinus</i> fung8 (MT635280)	100
P26-ZN2-2	LC769431	Fresh rhizome	<i>Penicillium chrysogenum</i> MZC-0 (MN069559)	99.83
P26-ZN2-3	LC769432	Fresh rhizome	<i>Penicillium roseopurpureum</i> IHEM:28005 (OU989457)	99.83
P26-ZN2-4	LC769433	Fresh rhizome	<i>Fusarium</i> sp. GFR18 (MT447523)	100
P26-ZN2-5	LC769434	Fresh rhizome	<i>Penicillium vinaceum</i> 533 (DQ681340)	100
P26-ZN2-6	LC769435	Fresh rhizome	<i>Penicillium roseopurpureum</i> G5-2 (MN206951)	100
P26-ZO1-1	LC769436	Old rhizome	<i>Penicillium</i> sp. FP-027-A7 (MH102087)	99.49
P26-ZO1-3	LC769437	Old rhizome	<i>Clonostachys rosea</i> daef27 (MH550497)	99.12
P26-ZO1-4	LC663164	Old rhizome	<i>Clonostachys rosea</i> Potato root (MT448899)	100
P26-ZO1-5	LC769438	Old rhizome	<i>Clonostachys</i> sp. 1R1D (OR365747)	99.82
P26-ZO1-6	LC769439	Old rhizome	<i>Fusarium</i> sp. GFR18 (MT447523)	99.82
P26-ZO2-1	LC769440	Old rhizome	<i>Fusarium proliferatum</i> CBB-6 (MT560216)	100
P26-ZO2-2	LC769441	Old rhizome	<i>Clonostachys rosea</i> MR44 (KY320599)	99.83

P26-ZO2-3	LC769442	Old rhizome	<i>Clonostachys rosea</i> N25 (MH259861)	100
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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 having 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 7 strains belonging to *Clonostachys* and 6 out of 8 strains belonging to *Fusarium* had antimicrobial activity. Strain P26-ZN1-2 belonging to *Madurella* inhibited growth of *E. coli* along with *B. subtilis* and *S. aureus* with inhibitory zone of 7.5 ± 0.7 , 9.5 ± 2.1 and 15.5 ± 0.7 , respectively. Moreover, 8 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 2 strains (P26-H2-2, P26-R2-1) displayed strong inhibition to pathogenic fungi. None of the strains inhibited the *Candida albicans* test (Table 2).

Table 2. Antimicrobial Activity of Endophytic Fungi.

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

Note: Diameter of the inhibitory zone: mm, -: 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. All 28 strains exhibited positive results for one or more traits, IAA production was seen in 9 strains in PDB medium supplemented with 5mM L-Tryptophan (Figure 3), whereas phosphate solubilization, and zinc oxide solubilization activity was noticed in 3, and 21 strains respectively. 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 the solubilization index (SI) (2.16 ± 0.02 cm). This strain also showed the most significant zinc solubilizing activity on mineral salt agar with the solubilization index (SI) (3.4 ± 0.1 cm).

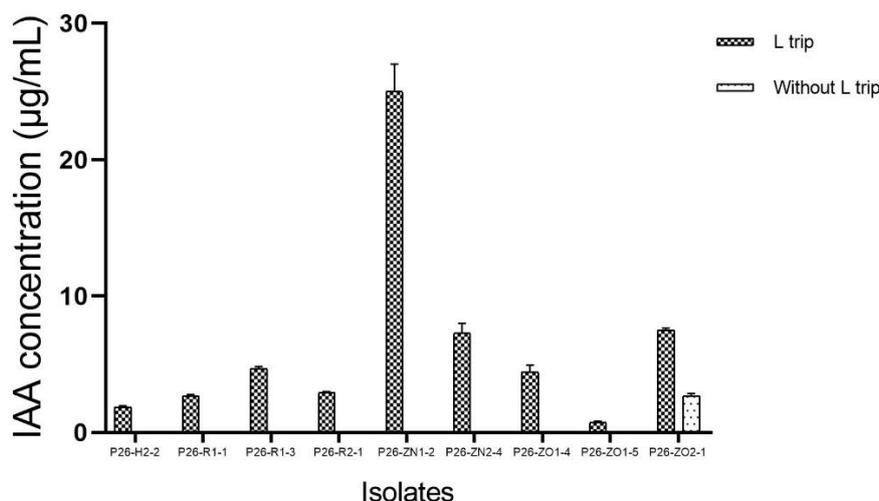


Figure 3. Production of IAA by endophytic fungal strains.

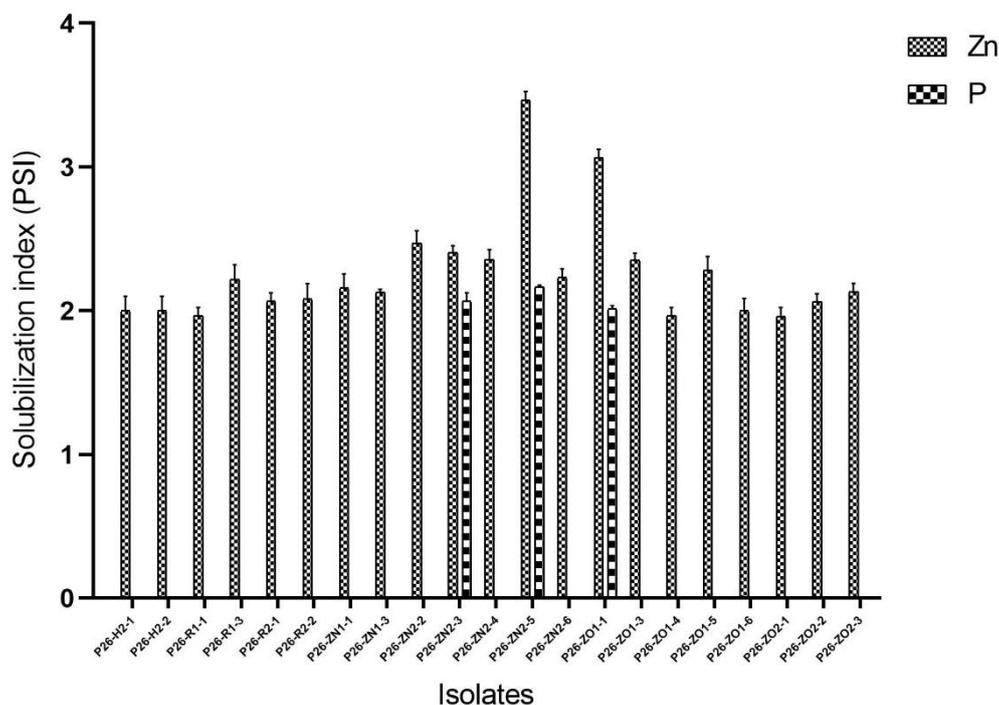


Figure 4. Phosphate and zinc solubilization by endophytic fungal strains.

4. Discussion

It has been shown that the diversity of endophytic fungi is influenced by the plant genotype [30,31] or both the host genotype and geography combined [32]. A recent study by Miao et al. on speciation and genetic diversity of endophytic fungi from their host plants, *C. songaricum*, parasitic *N. tangutorum* and non-parasitic *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 [21].

A possible exchange of endophytic fungi between *C. songaricum* and its host *N. tangutorum* was previously suggested [19], so the endophytic fungal composition of *C. songaricum* parasitizing another host, *N. sibirica* Pall, growing in the Gobi Desert was of great interest. Although 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 [19,33,34,] culturable endophytes are potential source for applications in biotechnology, medicine, agriculture and beyond.

In our study, representatives of the taxa *Fusarium*, *Clonostachys*, and *Penicillium* were predominated; 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 on the roots of *N. tangutorum* [19], and among the fungal genera from plants reported in general [11,35,36].

The second prevalent genus isolated from *C. songaricum* parasitizing the roots of *N. sibirica* was *Clonostachys*, with 7 isolates having 99.13–100% similarity to *Clonostachys rosea* based on ITS sequences. *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 [37,38]. 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 [39]. Our isolates, assigned to the genera *Fusarium* and *Clonostachys*, exhibited high antibacterial and antifungal activity.

Representatives of the genus *Penicillium* were isolated exclusively from fresh rhizomes. They did not show antimicrobial activities, but solubilized both phosphate and zinc. *Penicillium* spp. are known to be excellent solubilizers of phosphate [41–43], and zinc solubilizing efficiency in less extent [44]. The *Penicillium* spp. solubilizing minerals found in fresh rhizomes suggest that they may play a role in promoting 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 [45]. However, in recent years, several *Madurella* strains have been isolated from different plant species as endophytes [46–48]. *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.

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