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RESEARCH NOTE

Neocosmospora rubicola, an Unrecorded Endophytic Fungus Isolated from Roots of *Glycyrrhiza uralensis* in Korea

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Abstract

Through a survey of symbiotic endophytic fungi, we isolated an endophyte fungal strain from the roots of *Glycyrrhiza uralensis* in Korea. The isolated fungal strain was identified using its morphological characteristics and through phylogenetic analysis of the internal transcribed spacer, the large subunit rDNA region, and the translation elongation factor region. The strain was identified as *Neocosmospora rubicola*. This species has not been previously reported in Korea. In this study, we report its isolation from the roots of *Glycyrrhiza uralensis* in Korea, followed by the characterization and identification of the strain.

Keywords: Endophyte, *Glycyrrhiza uralensis*, Licorice, *Neocosmospora rubicola*

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Licorice (*Glycyrrhiza uralensis* Fisch.) is a perennial legume and it is known to be an important medicinal plant that contains a variety of physiologically active substances[1]. Licorice root has been widely used as a pharmaceutical agent because of its anti- inflammatory and hepatoprotective effects. Some key components of licorice root are glycyrrhizin, liquiritin, licoricidin, glycyrrhetinic acid, and saponin[1,2]. Importantly, licochalcone A, a phenolic component of licorice root, is known for its anti-proliferative and anti-inflammatory properties[3]. In Korea, consumption of licorice is largely dependent on imports, as the domestic production of licorice could not satisfy its demand for consumption; moreover, few studies have been conducted in this regard.

Endophytic fungi refer to the fungi living inside of the tissues of plants, such as roots, leaves, and stems, without obvious disease symptoms[4]. They are often closely related to pathogens but have limited pathogenic effects; moreover, endophytes may help protect the host plant against pathogens[5]. They are transmissible from the host to other plants[6]. Endophytic fungi stimulate seed germination[7] but also provide resistance to pathogens that may be harmful to the host plants[8]. The study of endophytic fungi is essential since they form symbiotic associations with various plants, ranging from terrestrial herbaceous

and woody plants to aquatic plants, providing beneficial help to the hosts[9]. Because licorice is an important medicinal plant, it is essential to study the symbiotic presence of endophytic fungi within licorice roots. In this study, we report an unrecorded species of fungi identified while isolating endophytic fungi from licorice roots collected in Korea.

Licorice roots cultivated in Jecheon, Korea (N 37° 18′, E 128° 22′) were collected and transported to the laboratory within 48 hours. Only roots without apparent disease symptoms were selected. The roots were rinsed thoroughly in running tap water and their surfaces were sterilized with 70% ethanol for 1 min, and 3% NaClO for 1 min and 30 sec. Then, they were washed three times with sterile water and treated with streptomycin and chloramphenicol dissolved in sterilized water at a concentration of 100 μm/mL for 10 min. After removing the remaining moisture from the roots with sterilized filter paper, they were cut into pieces of 0.5 cm in length. Four root pieces were placed on water agar (WA) medium and cultured in a dark place at 25°C. The hyphae growing out from the root fragments were transferred to potato dextrose agar (PDA) medium. The isolate was stored in 20% glycerol at −80°C at the Mycology Laboratory of Korea National University of Education (Strain 15C026), Cheongju, Korea and deposited as a glycerol stock at the Culture Collection of National Institute of Biological Resources (NIBR), Incheon, Korea (accession number NIBRFG0000499910).

The isolates were cultured on two different media, potato dextrose agar (PDA) and malt extract agar (MEA). Their characteristics were determined after growth at 25°C for 7 days. For the study of conidia, the strain mycelium was cultured using a slide culture method and examined under an optical microscope (AXIO Imager A1; Carl Zeiss, Oberkochen, Germany).

Genomic DNA was isolated from the fungal mycelium using the DNeasy Plant mini kit (Qiagen, Germantown, MD, USA). A PCR was performed using the following fungal specific primers: 1) ITS1F and ITS4 to amplify the internal transcribed spacer (ITS) region [10]; 2) LR0R and LR16 to amplify the large subunit (LSU) region of rDNA[11]; and 3) EF-526F and EF-1567R to amplify translation elongation factor region (tef1)[12]. The PCR products were electrophoresed on a 1.5% agarose gel for 20 minutes and then sequenced by the company SolGent (Daejeon, Korea). The obtained sequences were deposited in GenBank (accession numbers KY554751) and compared with the pool sequences available in GenBank using BLAST. Phylogenetic analysis was conducted using the neighbor-joining method in MEGA 6[13].

Neocosmospora rubicola L. Lombard & Crous, Studies in Mycology 80: 227 (2015) (Fig. 1, Table 1)

Colony grown on PDA at 25°C for 7 days was 35~40 mm in diameter. Its color was white on both sides. The colony shape was uniformly round and raised at the margin. The surface of the colony was flat and smooth and there was no exudate. Dense mycelial growth was observed. Colony grown on MEA at 25°C for 7 days was 20~25 in diameter. It was white color on both sides, and its shape was uniformly round at the margin. Dense mycelial growth was observed, with aerial growth at the margins. Ascomatal state and chlamydiospores were not observed. The conidiophore was fibrous, simple, and with no branches.

Fibrous conidia were 6~10 μm long and they had a cylindrical shape with a pointed end either with or without septum. Macroconidium was not found and microconidia were 0~or 1-septum, fusiform, or elliptical in shape, and 8~22 × 3~6 μm in size.

Specimen examined. Korea, Jecheon, N 37° 18′, E 128° 22′, August 14, 2015, isolated from roots of *Glycyrrhiza uralensis*, J. Kim, 15C026 (NIBRFG0000499910, GenBank accession number: KY554751).

Comparison of the ITS seguence of the Korean strain with the sequences of GenBank using BLAST showed that it shares a 99% similarity to that of *Neocosmospora rubicola* KM231800. The LSU region showed a 98% similarity to that of KM231667, and the EF1 region showed a 98% similarity to that of KM231928. A phylogenetic tree showed that *N. rubicola* 15C026 isolated from *G. uralensis* in this study was closely related to CBS320.73 and CBS101018 (Fig. 2).

Neocosmospora rubicola was recently described as a new species by Lombard et al.[14] after the taxonomic re-evaluation of the large family Nectriaceae (Hypocreales, Pezizomycotina,

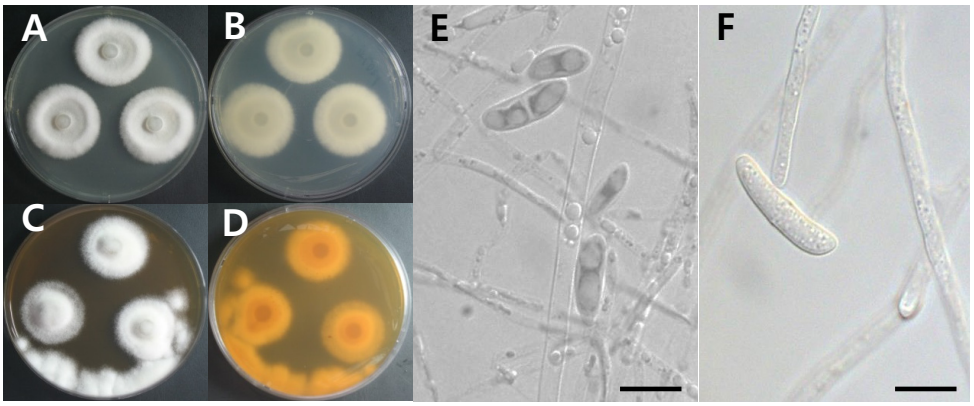

Fig. 1. Colony of *Neocosmospora rubicola* 15C026 grown on potato dextrose agar (PDA) (A, front; B, reverse) and malt extract agar (MEA) (C, front; D, reverse) for 7 days at 25°C, macrocondium grown on MEA (C) and PDA (D) at the same condition. Microconidia with 1-septum (E) and 0-septum (F) (scale bars: E, F = 10 μm).

Table 1. Morphological characteristics of *Neocosmospora rubicola*

Characteristics	*Neocosmospora rubicola* 15C026	*Neocosmospora rubicola*[a]
Colony	PDA, 25°C, 7 days; white, reverse concolorous 35~40 mm in diam, arranged in concentric rings, richly sporulating on the aerial mycelium	PDA, 24°C, 7 days; abundant white to pale luteous aerial mycelium, reverse concolorous, 35~40 mm in diam, arranged in concentric rings, richly sporulating on the aerial mycelium
Conidiophores	Simple conidiophores, 20~30 μm long, 3~7 μm at the base, hyaline, aseptate or septate	13~129 μm long, 3~7 μm at the base, hyaline, aseptate or septate
Microconidia	0~1-septum, 8~22 × 3~6 μm, ellipsoidal, fusiform or obovoid, straight to slightly curved, apex acutely rounded	0~1(~2)-septate, 11~22 × 4~5 μm, ellipsoidal, fusiform or obovoid, straight to slightly curved, apex acutely rounded, base sometime flattened

PDA, potato dextrose agar.
[a]Original description by Lombard et al.[14]

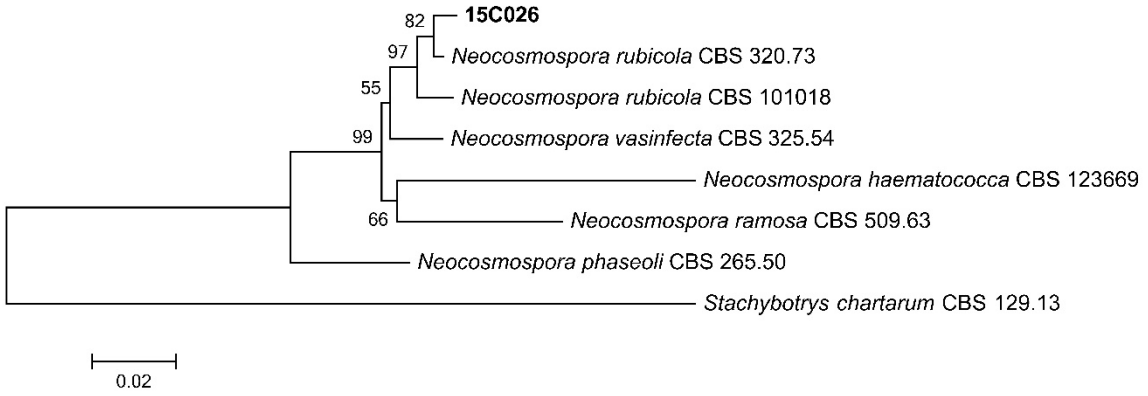

Fig. 2. Phylogenetic tree using neighbor-joining method based on a combined alignment of internal transcribed spacer (ITS), large subunit (LSU) and translation elongation factor 1-α (EF1) sequences. *Stachybotrys chartarum* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). Fungal strain isolated in this study is in bold.

Ascomycota). Multi-gene phylogenetic analysis based on DNA sequences of ten loci as well as morphological characteristics were used to identify the new species. *N. rubicola* has been isolated from raspberry (*Rubus idaeus*) and soil, and it has not been reported to cause any disease in animals or plants[14].

Licorice has an important use as a medicinal plant. However, endophytic fungi within licorice have not been studied in Korea. These studies are crucial to secure biodiversity and fungal resources. In addition, these endophytic fungi may be used as a natural source of pharmacologically active substances. In fact, some studies report that endophytic fungi produce secondary metabolites with anti-inflammatory activities. Therefore, further efforts to identify endophytic fungi in medicinal plants will allow a better understanding of the symbiotic relationship between plants and fungi.

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