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RESEARCH ARTICLE

First Report of *Gongronella guangdongensis* Isolated from Soil in Korea

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ABSTRACT

A fungal isolate designated KNU16-033 was isolated from a soil sample in Daegu, Korea. White, short, and felt-like aerial mycelia appeared on the surface of colonies of the isolate. Colonies with a smooth texture developed slowly and reached a diameter of 78 mm after 21 days of incubation on potato dextrose agar. This isolate displayed globose, colorless or light yellow sporangiospores, which differed morphologically from the sporangiospores of *Gongronella butleri*, which were hyaline, oval to flattened on one side or almost reniform. Based on these morphological characteristics and phylogenetic analysis using internal transcribed spacer regions, the isolated fungus was identified as *G. guangdongensis* belonging to Cunninghamellaceae. To our knowledge, this is the first record of *G. guangdongensis* in Korea.

Keywords: Cunninghamellaceae, *Gongronella guangdongensis*, Soil-inhabiting fungi

INTRODUCTION

Many of the fungi in the genus *Gongronella* are economically significant representatives of the class Zygomycetes. The cell walls of Zygomycetes are composed of chitosan [1]. Chitosan is also commercially produced from crustacean shells that are waste materials of food industries. The pin mold *Gongronella butleri* (Lendner) Peyronel & Dal Vesco is a well-known and important *Zygomycetes* fungus used in the production of chitosan. *G. butlei* reportedly produces the highest yield of chitosan [2]. *Gongronella* species have been used in biotechnological applications such as the production of antifungal proteins and enzymes [3-5]. Members of the genus *Gongronella* exclusively inhabit the soil [6-8] and grow relatively slowly between 25°C and 27°C [6]. Recent studies have focused on the genus *Gongronella*, and *G. butleri*, *Gongronella orasabula* and *Gongronella koreana* have been newly reported in Korea [9-11]. In this study, a fungal isolate designated KNU16-033, which was obtained from crop field soil in Korea, was identified as a member of the genus *Gongronella*. Based on the morphological and molecular characteristics, the isolate was identified as *Gongronella guangdongensis.* The isolation and identification of this strain, which is reported for the first time in Korea, is described in this report.

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MATERIALS AND METHODS

Sampling and fungal isolation

Fungal isolate KNU16-033 was isolated from a soil sample collected in Daegu, Korea (N 35°53′41.6″, E 128°35′10.1″). The soil sample was collected from the ground, air-dried, and stored in a plastic bag at 4°C until analysis. A conventional dilution plating technique was applied to isolate the fungus [12]. One gram of the soil sample was suspended in 10 mL of sterile distilled water. The suspension was vortexed, diluted, and a defined volume was spread on potato dextrose agar (PDA; Difco, Detroit, MI, USA). To examine the growth rate of fungal colonies, petri dishes containing the inoculated PDA were incubated at 25°C for 3 days. Individual colonies that developed on the agar were purified by subculture on fresh PDA and incubation at 25°C until mycelium development. The pure cultures were preserved on PDA slants at 4°C.

Morphological characterization

For morphological analysis, strain KNU16-033 was grown on PDA at 25°C for 21 days. After incubation colony characteristics such as color, size, and shape were recorded. For the micro observation, samples were observed using a model BX-50 light microscope (Olympus, Tokyo, Japan).

DNA extraction, PCR and sequencing analysis

Genomic DNA of strain KNU16-033 was extracted using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) following the manufacturer's instructions. To amplify the internal transcribed spacer (ITS) regions, ITS 1F/ITS 4 [13] primer pairs, and the product was purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced (SolGent, Daejeon, Korea). The similarities of obtained sequences were analyzed using the BLAST in NCBI and GENETYX-WIN (ver. 3.2) program. The obtained sequences from KNU16-033 was deposited in NCBI GenBank as accession no. LC372499.

Phylogenetic analysis

To perform the phylogenetic analysis, allied *Gongronella* species were retrieved from GenBank. MEGA 6 software [14] was used for the alignment and the phylogenetic tree constructed by the maximum parsimony method with bootstrap analysis of 1,000 replications.

RESULTS AND DISCUSSION

Gongronella guangdongensis F. Liu, T.T. Liu & L. Cai, Cryptogamie Mycologie 36: 137 (2015)

Morphology of isolate KNU16-033

The KNU16-033 had a slow growth rate, formed white colonies and reached a diameter of 78 mm after 21 days of incubation on PDA at 25°C. Other features included erect and branched sporangiophores, globose sporangium, columellae with a globose apophysis, and one-celled sporangiospores. The morphology of the isolate was compared with previous descriptions of *G. guangdongensis* and *G. butleri* [9, 15]. These species differ morphologically based on sporangiospore characteristics. The sporangiospores of *G. guangdongensis* are globose and colorless or light yellow, while those *G. butleri* sporangiospores are hyaline, and oval to flattened on one side or almost reniform [16]. Taxonomic descriptions and microphotographs of the morphological structures of isolate KNU16-033 are presented in Table 1 and Fig. 1. Colonies having a smooth texture with a white, short, and felt-like aerial mycelium developed slowly and attained a diameter of 78 mm after 21 days of incubation on PDA (Fig. 1A, 1B). On malt extract agar (MEA), growth was slow and the colonies that formed were pale yellow to brown with an irregular margin. The colonies typically reached a diameter of 59 mm after 6 days of incubation (Fig. 2C, 2D). Columellae were hemispherical, spherical or ovoid, smooth, often constricted at the attachment to apophyses, and 2.8~11.7 × 2.5~11.9 μm in size (Fig. 1E, 1G). The sporangia were pale to pale mouse grey and always globose with many spores. An apophysis was always present and abortive sporangia were sometimes present. The sporangial wall was thin, smooth, and approximately 15~22.9 μm in diameter (Fig. 1H). Sporangiospores were

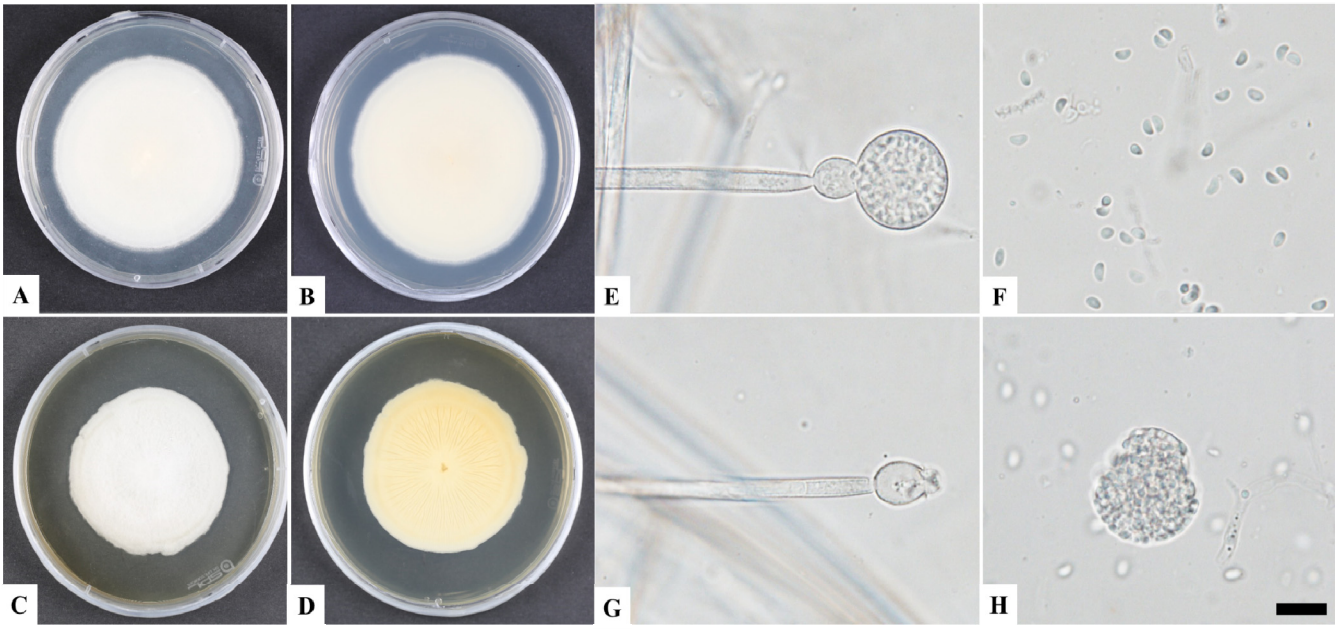

Fig. 1. Cultural and morphological characteristics of *Gongronella guangdongensis* KNU16-033. A, B, colonies on potato dextrose agar; C, D, colonies on malt extract agar; E, columellae and sporangium; F, sporangiospores; G, columellae; H, sporangium (scale bar = 10 μm).

globose, hyaline or light yellow, smooth, and 2.6~3 μm in size (Fig. 1F). Zygospores were not observed. These morphological characteristics of KNU16-033 were similar to those of *G. guangdongensis* and were distinct from those of *G. butleri* (Table 1).

Molecular phylogeny of isolate KNU16-033

After the sequencing analysis, 605 bp was obtained from isolate KNU16-033. As shown in Fig. 2, the KNU16-033 isolate clustered together with the *G. guangdongenesis* group with a 99% bootstrap value and was distinct from other *Gongronella* spp. in the phylogenetic tree constructed based on ITS region sequences (Fig. 2). Furthermore, it was revealed that KNU16-033 displayed 99% similarity with *G. guangdongensis* KC462739 that was isolated from soil. In this reason, phylogenetic analysis result support that isolate KNU16-033 was *G. guangdongensis*.

Gongronella species are valuable in the production of chitosan. Chitosan is an important component in the cell walls of Zygomycetes. Some *Gongronella* species produce a large

Table 1. Morphological characteristics of isolate KNU16-033 with reference to *Gongronella guangdongensis* and *Gongronella butleri*

Characteristics		KNU16-033[a]	*Gongronella guangdongensisis* [15]	*Gongronella butleri* [9]
Colony	Color	White or pale, colony reverse puff to honey	White or pale, colony reverse puff to honey	White to greyish or smokey brown, pale with brownish zone
	Size	61 mm in diameter after 13 days, and 78 mm in diameter after 21 days of incubation on PDA	50 mm in diameter after 13 days, and 70 mm after 21 days of incubation on PDA	N/A
	Shape	Margin irregular	Margin irregular	N/A
	Texture	Smooth	N/A	Woolly to cottony
Sporangia	Size (diam.)	15~22.9 μm	14~21.5 μm	7.0~12 × 5.5~7.7 μm
	Shape and position	Pale to pale mouse grey, globose, many spored always with an apophysis, then olivaveous to brown vinaceous with age, always with an apophysis, abortive sporangia sometimes present; sporangial wall thin and smooth	At first pale to pale mouse grey, then olivaceous to brown vinaceous with age, always globose, many spored, always with an apophysis, abortive sporangia sometimes present; sporangial wall thin and smooth	Globose or spherical, about 22.8 μm in diameter, wall smooth and soluble
Columellae	Size (diam.)	2.8~11.7 × 2.5~11.9 μm	2.5~12 × 2~12 μm	N/A
	Shape and position	Hemispherical, spherical or ovoid, smooth, often constricted at attachment to apophyses	Hemispherical, spherical or ovoid, smooth, often constricted at attachment to apophyses	N/A
Sporangio-spores	Size (diam.)	2.6~3 μm	2~3 μm	11.5~15.5 × 20.0~24.4 μm
	Shape and position	Globose, hyaline or light yellow, smooth	Globose, hyaline or light yellow, smooth	Smooth oval to flattened on one side to reinform

[a]Fungal strain studied in this paper.
N/A, not available in previous references.
Sources of descriptions [9, 15].

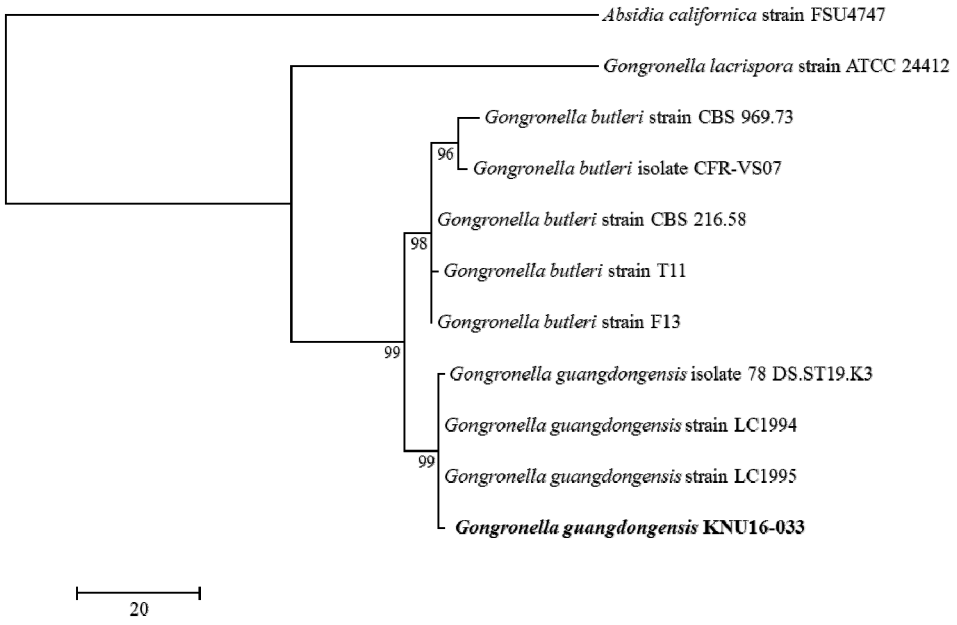

Fig. 2. One of the ten most parsimonious trees generated from maximum parsimony analysis of obtained sequences of the internal transcribed spacer rDNA region. *Absidia californica* strain FSU4747 was an outgroup. The KNU16-033 strain is in bold. The scale bar indicates the number of nucleotide substitutions.

amount of chitosan, which is economically significant in agriculture, medical, biological, and industrial applications [2, 17-19]. The chitosan-producing capability of *G. guangdongensis* will be the subject of future studies. *G. guangdongensis* is uniquely characterized by the presence of globose, hyaline or light yellow sporangiospores. The size and shape of sporangiospores are clearly distinguishing characteristics for other species of *Gongronella*, such as *G. orasabula and G. koreana* [10, 11]. To our knowledge, this is the first report of *G. guangdongenesis* in Korea.

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