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Phialocephala lagerbergii: A New Record from Crop Field Soil in Korea

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ABSTRACT : A unrecorded hyphomycete species of *Phialocephala* was isolated for the first time during the investigation of fungal community in the soil samples collected from different regions of Korea. The fungal isolate was identified as *Phialocephala lagerbergii*, based on the morphological characteristics and phylogenetic analysis of the ribosomal DNA sequence. In addition, cultural and micro-morphological features were described in detail.

KEYWORDS : Korea, Micromorphology, *Phialocephala lagerbergii*, rDNA

Introduction

The hyphomycete genus *Phialocephala* belongs to the family Vibrisseaceae. This fungus is characterized by a pigmented stipe, a terminal sporogenous head comprising fine hairs of metulae and phialides [1]. *Phialocephala* species contribute to a small proportion of the fungal biota, and are closely related to the genus *Phialophora*. *Phialocephala* species produce conidia in phialides during the peaks of mononematous conidiophores [2]. Conidiophores of *Phialocephala* are complex with irregular branching and are obconic to globose in shape [3]. *Phialocephala* species are known to produce a wide variety of extracellular enzymes such as polyphenyl oxidases, laccases, and proteases [4].

Phialocephala lagerbergii covers a wide range of ecological niches with diverse morphological characteristics [5]. Color of the colony, pigmentation in the culture, and outgrowths on the stipes are the common morphological features of *P. lagerbergii* [5]. *P. lagerbergii* is mainly isolated from wood and wood pulp [6]. Moreover, *Phialocephala* species are widely known as endophytes of roots and grow well on agar media [7]. In this study, *P. lagerbergii* was first isolated from the crop field soil during our fungal diversity study in Samcheok city, Korea. The purpose of this study was to (i) elucidate the morphological of *P. lagerbergii* by comparing with the previous descriptions of *Phialocephala* species and (ii) determine its phylogenetic status, based on ribosomal DNA (rDNA) sequence analysis.

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Materials and Methods

Sampling and isolation

Soil samples were collected from various locations in Samcheok city (37° 26' 87.04 N, 128° 90' 04.62 E), Gangwon-do, Korea. Fungal strains were isolated from the soil samples by the conventional dilution technique [8]. After dilution, the isolates were cultured on a potato dextrose agar (PDA; Difco, Detroit, MI, USA), supplemented with 100 μg chloramphenicol (bacteriostat/L PDA) for 5~7 days at 25°C, until the growth of fungal colony was observed. Then, a representative isolate (KNU14-11) was preserved at 20°C on PDA slants until further use.

Morphological characterization

Four different types of media were used for the morphological characterization of the study isolate (KNU14-11): potato dextrose agar (PDA), oatmeal agar (OA), yeast extract sucrose agar (YES), and malt extract agar (MEA). All the growth media were prepared according to Samson [9]. The isolate KNU14-11 was cultured on 9 cm petri dishes with three point inoculation and incubated in dark at 25°C for 7 days. Obverse and reverse colony colors, as well as the degree of speculation, were determined. An HK 3.1 CMOS digital camera (KOPTIC, Seoul, Korea), attached to an Olympus BX50F-3 microscope (Olympus, Tokyo, Japan), was used for capturing microscopic pictures of the fungal isolate. Scanning electron microscope (LEO Model 1450VP Variable Pressure Scanning Electron Microscope; Carl Zeiss, Oberkochen, Germany) was used for scanning and capturing the micro-morphological features of the fungal isolate.

DNA extraction, PCR amplification, sequencing, and data analysis

Genomic DNA was extracted from 1 week old colonies grown on PDA media, using a DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. The internal transcribed spacer region (ITS) was amplified using ITS1 (5'- TCCGTAGG TGAACCTGCG-3') and ITS4 (5'- TCCTCCGCTTATTG ATATGC-3') primers [10]. The amplified PCR products were sequenced using the Applied Biosystems 3730 DNA analyzer (Foster city, CA, USA). The sequences were compared with the reference ITS sequences, retrieved from GenBank, National Center for Biotechnology Information (NCBI), using the basic local alignment search tool (BLAST) software [11]. The annotated nucleotide sequence of KNU14-11 isolate was deposited in GenBank, with the accession number KP055600. All sequences were aligned using the Molecular Evolutionary Genetics Analysis (MEGA 6.0) software [12]. Phylogenetic tree was generated by the neighbor-joining method, utilizing the Kimura 2-parameter model with a bootstrap analysis of 1,000 replications for each clade.

Results and Discussion

Morphology of the KNU14-11 isolate

Colony morphology: Detailed morphological features of the fungal isolate KNU14-11 are shown in Fig. 1. The KNU14-11 isolate grown on PDA media attained a diameter of 9~12 mm within 7 days at 25°C. The dorsal side of the mycelium was white in color, whereas the ventral side was hairy green in the middle (Fig. 1A and 1E). Sporulation was moderately dense, and the conidia were seen in mass, having irregular form and smooth surface. The KNU14-11 isolate grown on OA media attained a diameter of 11~16 mm within 7 days at 25°C. The front and the rear sides of the mycelium were dark black with a circle (Fig. 1B and 1F). Sporulation was moderate, and the conidia were in mass, irregular form and rough surface. The KNU14-11 isolate grown on YES media attained a diameter of 8~10 mm within 7 days at 25°C. The front side of the mycelium was white on the margin and black in the center, and the rear was black in color (Fig. 1C and 1G). Sporulation was moderate to dense and the conidia were in mass, having irregular form and smooth surface. On MEA media, the KNU14-11 isolate attained a diameter of 14~17 mm within 7 days at 25°C. The front and rear sides of the mycelium were black in color (Fig. 1D and 1H).

Micromorphology: The conidiophores were macronematous, unbranched, erect, septate, smooth, and dark brown in color. Conidiophores were observed to be paler towards the apex. Conidiophores were 40~109 μm high and 3~4.8 μm wide (Fig 1I, 1M, and 1N). Hyphae were hyaline and smooth of about 1.5~2.5 μm wide. Hyphae were observed to be brown near the conidiophores. Conidia were catenate, non-septate, smooth, hyaline with rounded apices and truncated bases, tapering modestly to narrower bases, and 2~2.8 × 1~1.5 μm in size (Fig 1L, 1O, and 1P). Phialides were 2~4 in number and 4.8~24 × 1~7.2 μm in size (Fig 1J and 1K). Stipe comprised single to multiseptate and were 4.9~700 μm long. Comparisons of the morphological characteristics of KNU14-11 isolate with those of previously reported species of *Phialocephala* are described in Table 1.

Molecular phylogeny

The KNU14-11 isolate was sampled from the crop field soil of Samcheok city, Gangwon-do, Korea. The isolates used for constructing the phylogenetic tree are shown in Table 2, along with their respective GenBank accession numbers. The KNU14-11 isolate was most closely related to *P. lagerbergii* (CBS 266.336) and formed a monophyletic group, supported by a bootstrap value of 99% (Fig. 2). Phylogenetic analysis revealed that the isolate was *P. lagerbergii*.

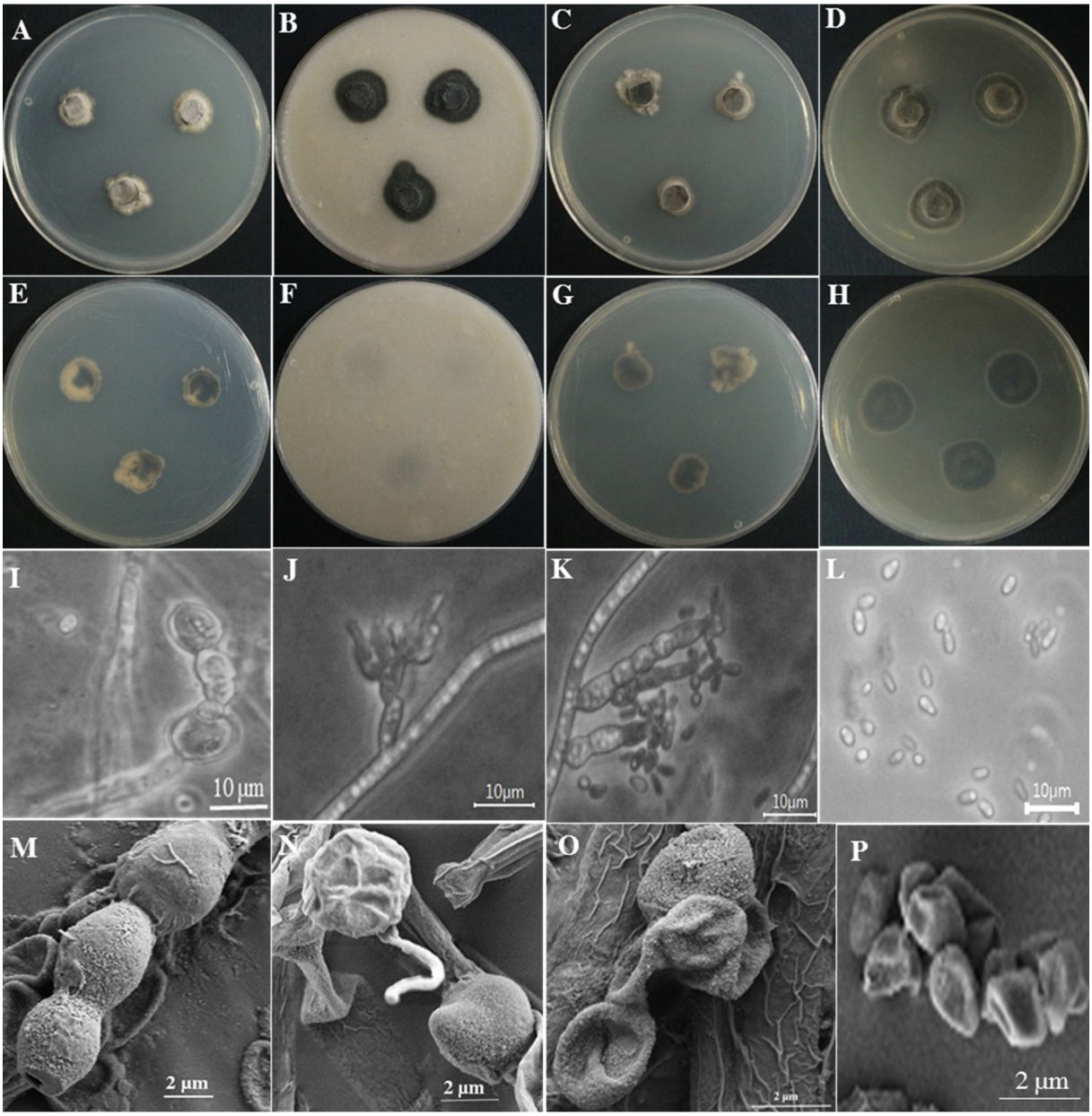

Fig. 1. Morphological characteristics of *Phialocephala lagerbergii* KNU 14-11 isolate grown for 7 days on potato dextrose agar, oatmeal agar, yeast extract sucrose agar, and malt extract agar media at 25°C. A~D, obverse colony from left to right; E~H, reverse colony from left to right; I~K, light microscopic picture of conidiophores; L, light microscopic pictures of conidia; M, N, scanning electronic pictures of conidiophores; O, P, scanning electronic pictures of conidia.

The KNU14-11 isolate belonged to the *Phialocephala* genus, based on its characteristics (colony, conidia, and conidiophores) that were similar to those of the *Phialocephala* genus. In the KNU14-11 isolate, the micromorphological structures were similar to those described by Jacobs et al. [13]. *Phialocephala* species closely resembles the *Sporendocladia fumosa* in terms of conidial arrangement [14]. Moreover, the arrangement of phialides and branching patterns of conidiophores, as well as the structure of phialides of *P. lagerbergii*, as described by Day et al. [6], was found to be identical to that of the KNU14-11 isolate (Fig. 1I-1K). This confirmed that our fungal isolate KNU14-11 was *P. lagerbergii*. In addition, the ITS sequence of CBS 266.33 was in close proximity (99% similarity) with that of *P. lagerbergii*. Based on the ITS sequence comparison, the present isolate was found to be closely related with *P. lagerbergii*, which was also strongly supported by their morphological comparison. Produc-

Table 1. Morphological characteristics of *Phialocephala lagerbergii* KNU 14-11 isolated in this study

Characteristics		Study isolate *Phialocephala lagerbergii*	*P. lagerbergii*[a]
Colony	Texture	Colonies on PDA, OA, YES and MEA are slow in growth.	Colonies on MEA are effuse hairy in the middle.
	Color	White in front and black in front with hairy green in the middle	Honey to primrose
Conidia	Size	2~2.8 × 1~1.5 μm	2~3 × 1~1.5 μm
	Shape	Catenate, non-septate, smooth hyaline, round apices and truncate bases	Catenate, non-septate, hyaline, round apices and truncate bases
	Surface	Tapering modestly	Tapering modestly
Conidiophores	Size	40~109 μm high and 3~4.8 μm wide	40~110 μm high and 3~5 μm wide
	Shape	Macronematous, unbranched, erect, septate, obconic, globose	Macronematous, unbranched, erect and septate, obconic to globose
Phialides	Structure	Solitary phialides formed directly on the mycelium	Solitary phialides formed directly on the mycelium
	Size	6.9~9.8 μm long and 3~3.7 μm wide	7~10 μm long and 3~4 μm wide
	Shape	Thick walled, smooth, lageniform	Thick walled, smooth, lageniform

[a]Source of description [13].
PDA, potato dextrose agar; OA, oatmeal agar; YES, yeast extract sucrose agar; MEA, malt extract agar.

Table 2. Sequences of *Phialocephala lagerbergii* and allied species used in this study, along with their GenBank accession numbers

S.N.	Species	Isolate No.	GenBank accession No.
1	*Phialocephala lagerbergii*	CBS266.33	NR119426
2	*Phialocephala aylmerensis*	DAOM250106	NR136124
3	*Phialocephala dimorphospora*	CBS 300.62	NR135931
4	*Phialocephala repens*	MUCL1849	EU434847
5	*Phialocephala mallochii*	DAOM250113	NR136123
6	*Phialocephala catenospora*	DAOM250108	NR136122
7	*Phialocephala nodosa*	NB475	NR136121
8	*Phialocephala oblonga*	NB565	KP768317
9	*Phialocephala glacialis*	UAMH 10852	NR136120
10	*Acephala macrosclerotiorum*	CBS 123555	NR121349
11	*Phialocephala piceae*	UAMH 10851	NR111319
12	*Phialocephala fortinii*	CBS 443.86	NR103577
13	*Acephala applanata*	CBS 109321	NR119482
14	*Variocladium giganteum*	CBS 508.71	NR111206
15	*Mollisia dextrinospora*	NR119489	NR119489
16	*Leohumicola verrucosa*	NR121306	NR121306
17	*Leohumicola lenta*	DAOM231149	NR111180
18	*Myxotrichum carminoparum*	UAMH 1597	NR111038
19	*Byssoascus striatosporus*	UAMH 3572	NR111040
20	*Phialocephala scopiformis*	CBS 468.94	NR119460
21	*Phlyctema vincetoxici*	CBS 123727	KJ663857
22	*Pezicula neosporulosa*	CBS 101.96	NR138003
23	*Pezicula sporulosa*	CBS 225.96	KR859262
24	*Phialocephala sphaeroides*	UAMH 10279	NR121302
25	*Phialocephala hiberna*	BPI 842235	NR119465
26	*Phialocephala urceolata*	UAMH 10827	NR111285
27	*Collembolispora barbata*	CBS 115944	NR111443
28	*Phialophora finlandia*	CBS 444.86	AF486119
29	*Catenulifera luxurians*	CBS 647.75	NR121470
30	*Vestigium trifidum*	DAOM240321	NR121556
31	*Phialocephala virens*	CBS 452.92	AF586132

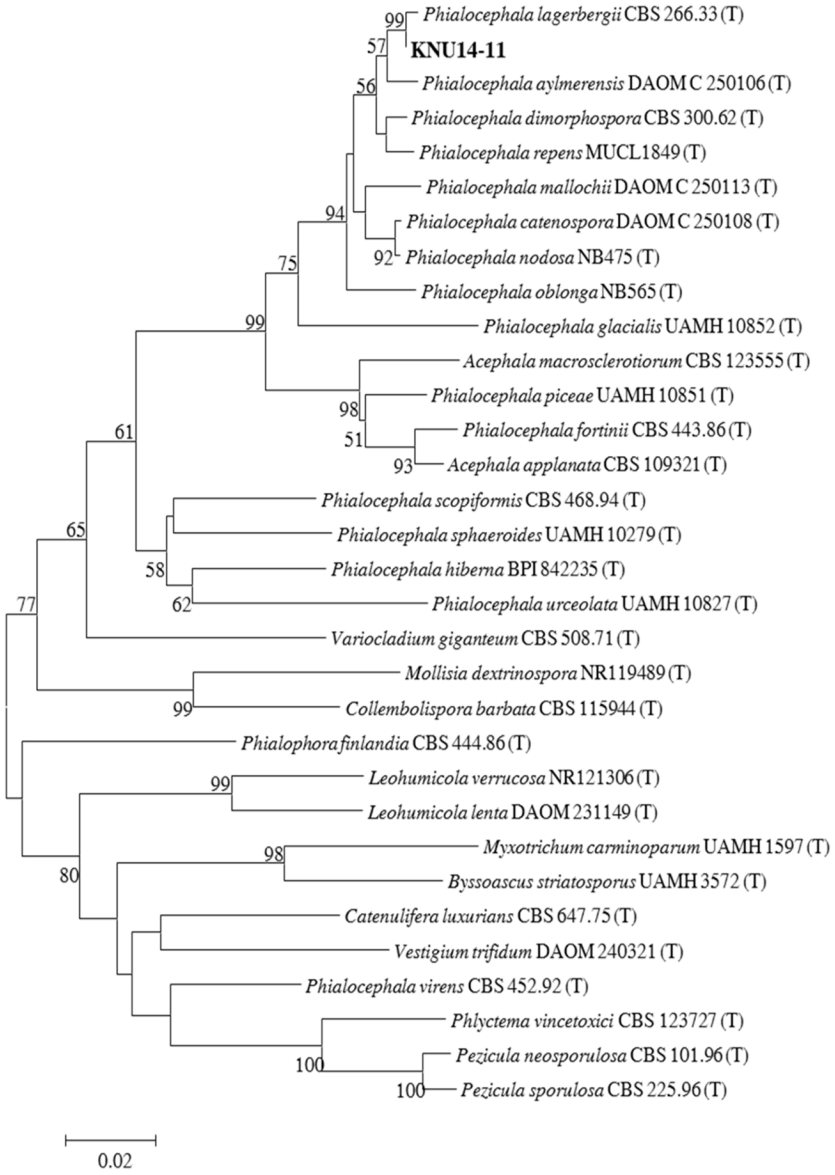

Fig. 2. Phylogenetic analysis based on the neighbor-joining method using the partial 18S-ITS1-5.8S-ITS2-28S rDNA region of *Phialocephala lagerbergii* KNU 14-11 isolate obtained from the crop field soil in Korea. Numerical values (> 50) on branches are the bootstrap values with 1,000 replicates.

tion of a wide variety of extracellular enzymes such as polyphenyl oxidases, laccases, and proteases by the *Phialocephala* species reflects its importance in the field of biotechnology. Further studies on its biotechnological importance are worthwhile in the future.

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