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First Report of *Neopestalotiopsis australis* Isolated from Soil in Korea

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ABSTRACT : Fungal strain KNU16-005 was isolated from field soil in *Jeollabuk-do,* Korea. Based on its morphological characteristics and phylogenic analysis using internal transcribed spacer regions of rDNA, β-tubulin and translation elongation factor 1-alpha gene sequences of KNU16-005 were identified as *Neopestalotiopsis australis*. This species has not been previously reported in Korea.

KEYWORDS : ***Neopestalotiopsis australis*, Phylogenetic analysis, Soil fungi**

The genus *Neopestalotiopsis* Maharachch was recently segregated from *Pestalotiopsis* Steyaert, which was separated into three genera, *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis*, based on morphological data and phylogenetic analysis of internal transcribed spacer (ITS), 28S nrRNA (LSU), β-tubulin (BT), and translation elongation factor 1-alpha (TEF) gene sequences [1]. The genus *Pestalotiopsis* was a heterogeneous group of coelomycetous fungi differentiated primarily based on conidial characteristics such as size, septation, presence or absence of appendages, and color of the median cells [2, 3]. *Neopestalotiopsis* species can be easily distinguished from the other two genera by its versicolorous median cells and indistinct conidiophores, which are often reduced to conidiogenous cells [1]. Numerous studies have shown that the relationship between plants and *Pestalotiopsis*-like fungi can be parasitic, symbiotic, or saprophytic [3, 4]. Pathogenic *Pestalotiopsis* species cause a variety of plant diseases worldwide, including fruit rot disease on grape, gray blight of tea plant, and canker on the medicinal plant *Acanthopanax divaricatus* in Korea [4-6]. *Neopestalotiopsis* species also exhibit pathogenicity and cause economic loss to various crops such as palm, coconut, and mango [1]. The type species of the *Neopestalotiopsis* genus, *N. protearum*, was isolated from leaf spot on *Leucospermum cuneiforme* and *N. vitis* was recently reported as the causal agent of grape leaf spot in China [7, 8].

During our studies of microbial communities in the field soil of Wanju, *Jeollabuk-do,* the Korea fungal strain KNU16-005 was isolated. Based on its morphological characteristics and phylogenetic analysis, this isolate was identified as *Neopestalotiopsis australis* Maharachch and designated as KNU16-005. This fungus has not been previously reported in Korea.

Collected soil samples (1 g) were suspended in 10 mL of sterile distilled water and the prepared suspension was vortexed, serially diluted, and spread onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates. The plates were incubated at 25°C for 3 days. Single colonies on these plates were purified by transferring colonies onto new plates followed by incubation on PDA at 25°C. One isolate, KNU16-005, was selected for further morphological and molecular phylogenetic analyses.

The isolate KNU16-005 was cultured at 25°C and colony characteristics such as color, shape, and size were recorded. After 12 days of incubation on PDA agar, the

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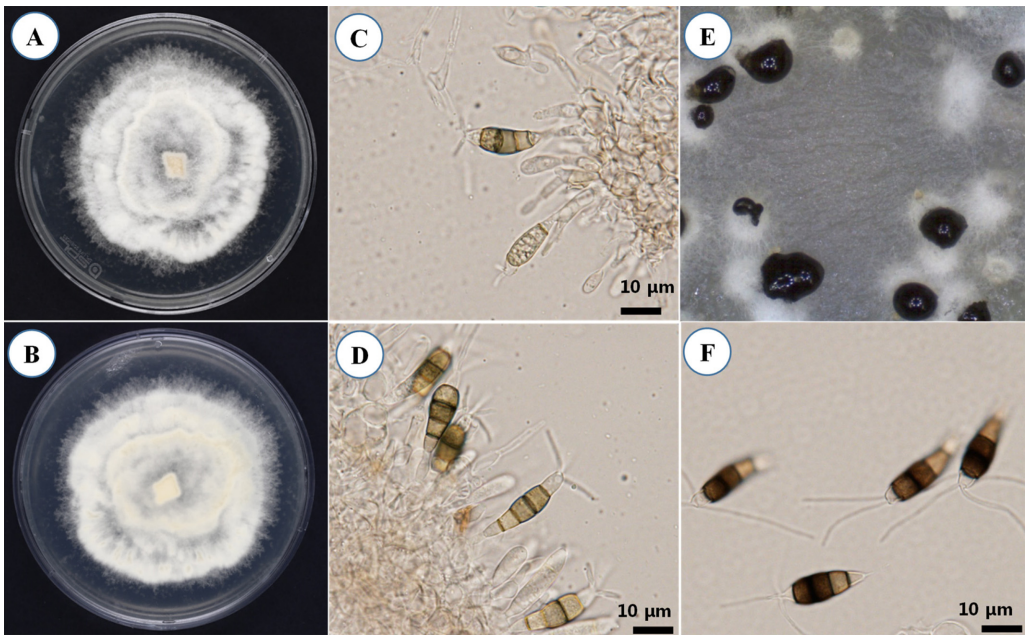

Fig. 1. Morphological characterization of *Neopestalotiopsis australis* KNU16-005 using a light microscope. A, colony in front; B, colony in reverse; C, D, microscopic pictures of conidiogenous cells; E, microscopic pictures of conidiomata; F, microscopic pictures of conidia.

colony was 6.5~6.8 cm in diameter, whitish to yellowish-white in color and had irregular edges, dense aerial mycelium on the surface, and black conidiomata (Fig. 1A, 1B, 1E). The morphology of the isolate was examined under an Olympus CX31 light microscope (Tokyo, Japan). Conidia were 21~25 × 6~8 μm, 4-septate, and straight to slightly curved (Fig. 1F). The basal cell was conical in shape with an obtuse end, hyaline, and thin-walled. Three median cells were versicolorus. The second cell was brownish, the third cell was dark brown, and the fourth cell was brown. Three apical appendages arising from the apex of the apical cell were 20~30 μm in length, while the basal appendage was small hyaline pedicel and 3~6 μm in length. As shown in Table 1, these morphological characteristics of isolate KNU16-005 agreed well with those for representatives of the genus *Neopestalotiopsis* and were most

Table 1. Morphological characteristics of *Neopestalotiopsis australis* isolated in this study

Characteristics		*Neopestalotiopsis australis* isolated in this study	*Neopestalotiopsis australis*[a]
Colony	Color	white to yellowish-white	pale honey
	Size	6.5~6.8 cm after 12 days on PDA	3.0~4.0 cm after 7 days on PDA
	Shape	irregular edge	lobate edge
Conidiomata	Shape	globose to clavate	globose to clavate
	Color	black	brown to black
Conidiophores		indistinct	indistinct
Conidia	Length (μm)	21~25	21~27
	Width (μm)	6~8	7.5~9
	Color of median cells	versicolorus	versicolorus
Apical appendages	Number	Mostly 3	3~4 (mostly 3)
	Length (μm)	20~30	21~32

PDA, potato dextrose agar.
[a]Source of description [1].

closely matched to the characteristics of *N. australis* [1].

To confirm the reliability of morphological identification, the isolate KNU16-005 was subjected to molecular identification based on amplification of three DNA regions that are widely used for phylogenetic analysis. For this purpose, genomic DNA was extracted from the mycelia using the HiGene Genomic DNA prep kit (BIOFACT, Daejeon, Korea). The ITS regions, including 5.8S, were amplified with the primers ITS1F and ITS4 [9]. To amplify the partial BT and TEF genes, the T1/Bt2b and EF-728F/EF2 primer pairs were used [10-13]. The amplified PCR products were sequenced using an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). ITS, BT, and TEF gene sequences of related *Neopestalotiopsis* and *Pestalotiopsis* strains were obtained from GenBank and used for phylogenetic analysis (Table 2). Evolutionary distance matrices for the neighbor-joining algorithm were calculated using Kimura's two-parameter model [14]. Tree topology was inferred by the neighbor-joining method in the program MEGA7 [15], with bootstrap values based on 1,000 replications. GenBank BLAST searching revealed that the ITS regions and BT and TEF gene sequences of KNU16-005 matched those of *N. australis* CBS 114159 (KM199348, KM199432, and KM199537) with 99.63%, 99.04%, and 99.59% similarities, respectively, clearly indicating that both strains belong to same *Neopestalotiopsis* species. The isolate KNU16-005 clustered together with *N. australis* CBS 114159 in the phylogenetic tree constructed based on the combined ITS, BT, and TEF sequences, confirming their close relationship at the species level (Fig. 2).

The results of morphological and phylogenetic characterizations clearly indicate that isolate KNU16-005 is *N. australis.* This fungal species has not been previously detected in Korea. Previous studies showed that *Pestalotiopsis*-like fungi produce many important bioactive secondary metabolites such as the anti-cancer drug taxol or antimycotic pestacin [3, 16]. Thus, further investigations of *N. australis* KNU16-005 as a producer of bioactive compounds should be conducted.

Table 2. *Neopestalotiosis* and *Pestalotiosis* strains used in this study and their GenBank accession numbers

Species	Strain	Accession no.		
		ITS	BT	TEF
Neopestalotiopsis australis	CBS 114159	KM199348	KM199432	KM199537
N. formicarum	CBS 115.83	KM199344	KM199444	KM199519
N. formicarum	CBS 362.72	KM199358	KM199455	KM199517
N. honoluluana	CBS 111535	KM199363	KM199461	KM199546
N. honoluluana	CBS 114495	KM199364	KM199457	KM199548
N. javaensis	CBS 257.31	KM199357	KM199437	KM199543
N. mesopotamica	CBS 299.74	KM199361	KM199435	KM199541
N. mesopotamica	CBS 336.86	KM199362	KM199441	KM199555
N. piceana	CBS 225.30	KM199371	KM199451	KM199535
N. piceana	CBS 254.32	KM199372	KM199452	KM199529
N. rosae	CBS 101057	KM199359	KM199429	KM199523
N. rosae	CBS 124745	KM199360	KM199430	KM199524
N. surinamensis	CBS 111494	JX556232	KM199462	KM199530
N. surinamensis	CBS 450.74	KM199351	KM199465	KM199518
Pestalotiopsis australis	CBS 114193	KM199332	KM199383	KM199475
P. australis	CBS 114474	KM199334	KM199385	KM199477
P. oryzae	CBS 111522	KM199294	KM199394	KM199493
P. oryzae	CBS 353.69	KM199299	KM199398	KM199496
P. parva	CBS 265.37	KM199312	KM199404	KM199508
P. parva	CBS 278.35	KM199313	KM199405	KM199509
Neopestalotiopsis australis[a]	KNU16-005	KY398730	KY398731	KY398732

ITS, internal transcribed spacer; BT, β-tubulin; TEF, translation elongation factor 1-alpha.
[a]Isolated in this study.

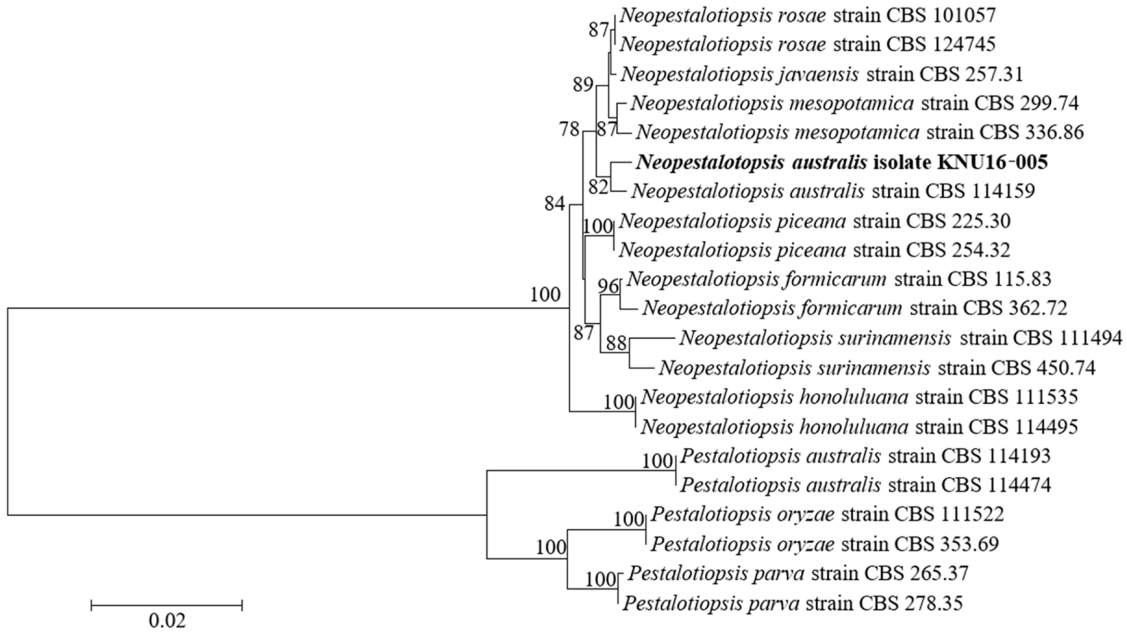

Fig. 2. Neighbor-joining phylogenetic tree based on the combined internal transcribed spacer (ITS), β-tubulin (BT), and translation elongation factor 1-alpha (TEF) sequences, showing the relationship between *Neopestalotiopsis australis* KNU16-005 and closest *Neopestalotiopsis* and *Pestalotiopsis* taxa. Bootstrap values (based on 1,000 replications) greater than 70% are shown at the branch points. Bar, 0.02 substitutions per nucleotide position.

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