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RESEARCH ARTICLE

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Pestalotiopsis kaki sp. nov., a Novel Species Isolated from Persimmon Tree (*Diospyros kaki*) Bark in Korea

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ABSTRACT

During the screening of Korean microflora, a fungal strain (KNU-PT-1804) belonging to the genus *Pestalotiopsis* was isolated from persimmon tree (*Diospyros kaki*) bark collected from North Gyeongsang Province, Korea. The strain, KNU-PT-1804, produced smaller conidia compared with related species *P. kenyana*, *P. neglecta*, and *P. telopeae*. The novelty of the strain was confirmed based on phylogenetic analysis using molecular datasets of internal transcribed spacer (ITS) regions, β-tubulin (*TUB2*), and translation elongation factor 1-alpha (*TEF1α*) genes. Molecular phylogeny strongly supports that the strain is distinct from previously known *Pestalotiopsis* species, and we proposed the novel species, *Pestalotiopsis kaki* sp. nov., and provide a detailed description and illustration.

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1. Introduction

The genus, *Pestalotiopsis* Steyaert, was introduced by Steyaert (1949) and is placed in the Pestalotiopsidaceae [1]. *Pestalotiopsis* is characterized by moderately fusiform conidia, each with a basal hyaline cell, three pigmented median cells, and an apical hyaline cell with two or more apical appendages [2]. Species in the genus are important pathogens of plants [3,4]. The genus includes about 300 names and various reports show that *Pestalotiopsis* species produce a diverse array of chemical compounds [4,5]. Strains are historically identified by their host associations [6].

Fungi in the genus, *Pestalotiopsis,* are among the most frequently encountered in tropical and temperate regions. Teleomorphs of the fungi are taxonomically classified as *Pestalotiosphaeria* spp., based on some inference. Some strains are productive plant endophytes, and others cause disease on rainforest plants, such as banana and tea trees [4]. Nevertheless, taxonomic affinities of *Pestalotiopsis* species are unclear since morphological characteristics overlap substantially [4]. A combination of internal transcribed spacer (ITS), partial *β*-tubulin (*TUB2*), and partial translation elongation factor1-α (*TEF1α*) gene sequences provided better resolution of taxonomic relationships when compared to single-gene analysis [7].

The objective of this investigation is the identification and classification of novel fungal species in Korea. Molecular phylogenetic analyses are used to identify novel species, along with the characteristics in laboratory culture and morphology. In this present study, isolated fungi are described, and illustrated as novel fungal species. One such species is described in detail.

2. Material and methods

2.1. Soil sample collection and fungal isolation

The sample of persimmon tree (*Diospyros kaki*) bark was collected from North Gyeongsang Province (35°40'13.0"N, 128°35'52.9"E), Korea. The bark was transferred to the laboratory and stored at 4 °C until use. Symptomatic bark was directly scrapped onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates and incubated 2–3 days at 25 °C. Single colonies were transferred to new PDA plates and incubated for 4–5 days at 25 °C. Strain was selected for further molecular analyses based on different characteristics in culture. Fungal strain was maintained in 20% glycerol at −80 °C for further study.

2.2. Culture and morphology

Culture characteristics and morphological observations were recorded using different media – potato dextrose agar (PDA), malt extract agar (MEA; Difco), and oatmeal agar (OA; Difco) with

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incubation for 7–21 days at 25 °C [7]. Fungal growth was measured, and colony characteristics, such as color, shape, and size were recorded. Morphological characteristics were examined using a light microscope (BX-50; Olympus, Tokyo, Japan).

2.3. Genomic DNA extraction, PCR amplification, and sequencing

Fungal mycelia were grown on PDA plates for 4–5 days at 25 °C. Mycelia were scraped off from the PDA surface with the sterile blade. Genomic DNA was extracted using a HiGene Genomic DNA prep kit (BIOFACT, Daejeon, Korea) following the manufacturer's instructions; DNA extracts were stored at −20 °C before use. The PCR amplification process used a fragment of ITS region (ITS1F/ITS4) [8,9]; *TEF1α* (translation elongation factor 1-alpha gene, EF1-526F/EF1-1567R) [10]; *TUB2*, a partial *β*-tubulin gene region (BT2a/BT2b) [11,12]. The PCR yields were verified on 1% agarose gels using ethidium bromide. Amplified PCR products were purified with EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced by Macrogen Co. Ltd. (Daejeon, Korea). Sequence data were adjusted using SeqMan Lasergene software (DNAStar Inc., Madison, Wisconsin, USA).

2.4. Molecular phylogenetic analysis

The phylogenetic analyses were constructed with sequences retrieved from the National Center for Biotechnology Information (NCBI). Ambiguous regions were deleted from alignments and evolutionary distance matrices for the neighbor-joining (NJ) algorithm were calculated using Kimura's two-parameter model [13]. Exact taxonomic position was determined using maximum likelihood and maximum parsimony methods. This analysis also identified nodes with filled circles in the NJ [14] phylogenetic tree. Open circles showed corresponding nodes from maximum likelihood [15] or maximum parsimony [16] algorithms. The NJ method was inferred by tree topology using MEGA7 software with bootstrap values based on 1,000 replications [17].

3. Results

3.1. Taxonomical analysis of Pestalotiopsis kaki sp. nov

Strain KNU-PT-1804 showed distinct morphological characteristics compared with allied species of *Pestalotiopsis* and is therefore described as a new species.

Pestalotiopsis kaki **K. Das, S.Y. Lee and H.Y. Jung, sp. nov.** (Figure 1)

MycoBank: MB 835966

Etymology: *kaki* = Japanese name and specific epithet of the host plant (*Diospyros kaki*).

Typus: North Gyeongsang Province (35°40'13.0"N, 128°35'52.9"E), isolated from Persimmon (*Diospyros kaki*) bark. A stock metabolically inactive culture was deposited in the National Institute of Biological Resources (NIBRFGC000502249).

Ecology and Distribution: The members of this genus are considered pathogens, endophytes and saprophytes, and are widely dispersed in tropical and temperate ecosystems. Some species were isolated from *Podocarpus macropyllus* in south China, seeds of *Podocarpus falcatus*, leaf blight on Japanese spicebush (*Lindera obtusiloba*) in Japan, and leaf spot disease of Proteaceae from Zimbabwe. The proposed novel species, *Pestalotiopsis kaki*, was collected from Persimmon tree (*Diospyros kaki*) bark in Korea.

Cultural characteristics: Colonies on PDA were fast-growing, white, with abundant aerial mycelia, attaining a diam. of 81.1–86.2 mm after seven days at 25 °C; reverse white to light yellowish (Figure 1(A)). On MEA, colonies were fast-growing, whitish, reaching a diam. of 76.2–81.3 mm after seven days at 25 °C; reverse white to light yellowish (Figure 1(B)). On OA, colonies were also fast-growing, whitish, growing with margins and obtained a diam. of 72.2–80.1 mm. after seven days at 25 °C; reverse yellowish (Figure 1(C)). Conidiomata pycnidial in culture on PDA, globose, superficial to immersed, scattered or gregarious, up to 500 µm diameter, black conidial masses (Figure 1(D,E)).

Morphological characteristics: Conidiophores were hyaline to light brown, indistinct, often reduced to conidiogenous cells. Conidiogenous cells were discrete, ampulliform or lageniform, hyaline to brown, solitary to aggregated, 15.8–18.5 × 4.0–4.2 µm, collarette present (Figure 1(F–H)). Conidia fusoid, ellipsoid, straight to slightly curved, 4-septate, 19.4–26.3 × 4.4–6.3 µm (x ± SD = 22.3 ± 1.5 × 5.3 ± 0.46 µm), with the average diameter of 22.3 × 5.3 µm ($n = 100$); basal cell obconic, with a truncate base, hyaline or pale gray, thin-walled, 4.1–5.7 µm long; three median cells doliiform, 12.8–16.7 µm long (x ± SD = 14.4 ± 1.0 µm), wall verruculose, concolourous, or median cell darker than other median cells, mid-brown to brown, septa darker than the rest of cells (second cells from the base 4.1–5.4 µm long; third cells 4.3–5.8 µm long; fourth cells 4.0–5.7 µm long); apical cell 3.8–5.9 µm long, hyaline, subcylindrical; with 2–4 tubular apical appendages (mostly 3), arising from an apical crest, unbranched, filiform, 11.5–21.0 µm long (x ± SD = 15.6 ± 2.6 µm;

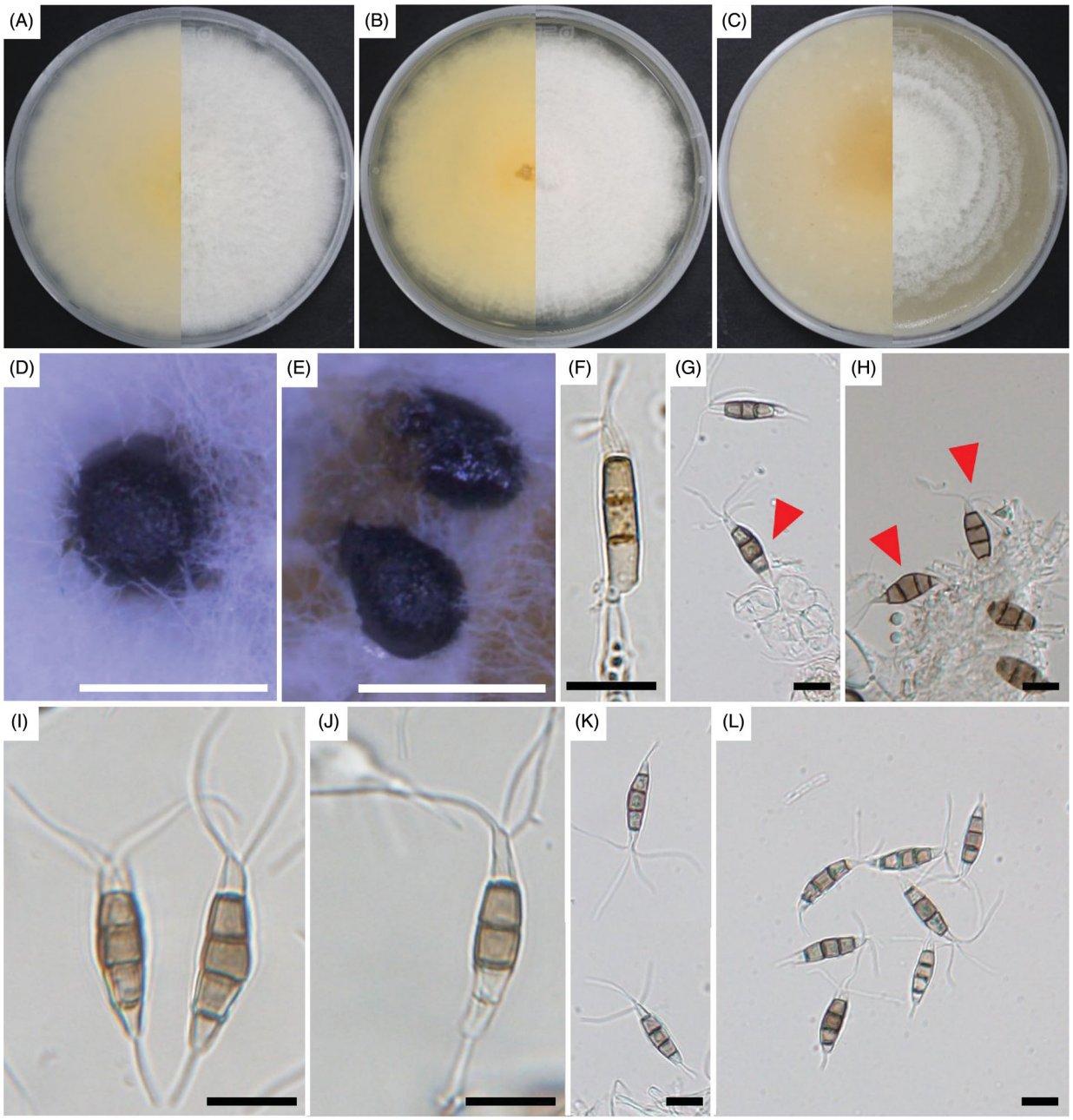

Figure 1. Culture characteristics and morphology of strain KNU-PT-1804. Colonies on potato dextrose agar (A); malt extract agar (B); oatmeal agar (C) after incubation for 7 days at 25 °C. Conidiomata on PDA (D,E); Conidiogenous cells (F–H); Conidia (I–L). Arrows indicate conidiogenous cells. Scale bars: D,E = 500 µm; F–L = 10 µm.

basal appendage single, tubular, unbranched, centric, 5.1–8.7 µm long (Figure 1(I–L)).

Note: The size of conidiogenous cells of strain KNU-PT-1804 (15.8–18.5 × 4.0–4.2 µm) were smaller than cells from the most closely related species, *P. kenyana,* (10.0–25.0 × 2.0–5.0 µm), but larger than *P. telopeae* (5.0–15.0 × 2.0–9.0 µm. In contrast, the description of *P. neglecta* does not mention diameter of conidiogenous cells (Table 2). KNU-PT-1804 produced smaller conidia (19.4–26.3 × 4.4–6.3 µm), than *P. kenyana* (23.0–28.0 × 7.0–9.0), *P. neglecta* (27.0 × 9.0), and *P. telopeae* (24.5–31.0 × 6.0–8.0) (Table 2). KNU-PT-1804 also displayed three smaller median cells with diameters of 12.8–16.7 µm long (x ± SD = 14.4 ± 1.0 µm); diameters of the closest strain, *P. kenyana* (15.5–18.5 µm long, x ± SD = 17 ± 0.7 µm), and *P. telopeae* (16–18.5 µm long, x ± SD = 17.1 ± 1 µm). Thus, three median cells of strain KNU-PT-1804 were smaller compared with related strains, *P. kenyana* and *P. telopeae*. Moreover, KNU-PT-1804 produced cells with differences in diameters of second cells from the base, 4.1–5.4 µm long; third cells, 4.3–5.8 µm long; fourth cells, 4.0–5.7 µm long. In contrast, similar measurements in *P. kenyana* are: second cells from the base, 4.5–6.0 µm long; third cells, 5.5–7.5 µm long; fourth cells, 3.5–4.5 µm long. Similar measurements for *P. telopeae* were 4.5–7.0 µm long, 5.0–7.5 µm long, and 5.0–7.0 µm long for second, third, and fourth cells, respectively. Thus, diameters of KNU-PT-1804 cells

Table 1. List of species used in phylogenetic analyses along with their GenBank accession numbers.

Species	Strain Numbers	GenBank Accession Numbers		
		ITS	*TUB2*	*TEF1α*
NeoPestalotiopsis saprophytica	MFLUCC12-0282	JX398982	JX399017	JX399048
Pestalotiopsis arceuthobii	CBS 434.65^T	KM199341	KM199427	KM199516
P.arengae	CBS 331.92^T	KM199340	KM199426	KM199515
P. australasiae	CBS 114126^T	KM199297	KM199409	KM199499
P. australis	CBS 114193^T	KM199332	KM199383	KM199475
P. biciliata	CBS 790.68	KM199305	KM199400	KM199507
P. camelliae	CBS 443.62	KM199336	KM199424	KM199512
P. chamaeropis	CBS 186.71	KM199326	KM199391	KM199473
P. colombiensis	CBS 118553^T	KM199307	KM199421	KM199488
P. disseminata	CBS 118552	MH553986	MH554652	MH554410
P. grevilleae	CBS 114127^T	KM199300	KM199407	KM199504
P. hawaiiensis	CBS 114491^T	KM199339	KM199428	KM199514
P. hollandica	CBS 265.33^T	KM199328	KM199388	KM199481
P. humus	CBS 336.97	KM199317	KM199420	KM199484
P. kenyana	CBS 911.96	KM199303	KM199396	KM199503
P. knightiae	CBS 114138	KM199310	KM199408	KM199497
P. malayana	CBS 102220^T	KM199306	KM199411	KM199482
P. neglecta	TAP99M112	AB482211	AB453882	AB453853
P. oryzae	CBS 353.69^T	KM199299	KM199398	KM199496
P. papuana	CBS 331.96^T	KM199321	KM199413	KM199491
P. parva	CBS 265.37	KM199312	KM199404	KM199508
P. portugalica	CBS 393.48^T	KM199335	KM199422	KM199510
P. scoparia	CBS 176.25^T	KM199330	KM199393	KM199478
P. spathulata	CBS 356.86^T	KM199338	KM199423	KM199513
P. telopeae	CBS 114161^T	KM199296	KM199403	KM199500
Pestalotiopsis kaki	**KNU-PT-1804^T**	**LC552953**	**LC552954**	**LC553555**

MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; CBS: Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; TAP: TAP: Tamagawa University, Tokyo, Japan; KNU: Kyungpook National University, Daegu, Korea.
ITS: Internal transcribed spacer regions of the rDNA; *TUB2*: partial beta-tubulin gene; *TEF1α*: partial translation elongation factor gene.
Strains identified in this study are indicated in bold.

Table 2. Morphological comparison of *Pestalotiopsis kaki* sp. nov. with closely related species.

Sl. No.	Strain Name	Conidiogenous cells (μm)	Conidia (μm)	References
1	*P. kaki* KNU-PT-1804^T	15.8–18.5 × 4.0–4.2	19.4–26.3 × 4.4–6.3	In this study
2	*P. kenyana* CBS 442.67^T	10.0–25.0 × 2.0–5.0	23.0–28.0 × 7.0–9.0	[7]
3	*P. neglecta* CCTU 12	N/A	27.0 × 9.0	[26]
4	*P. telopeae* CBS 114161^T	5.0–15.0 × 2.0–9.0	24.5–31.0 × 6.0–8.0	[7]
5	*P. australasiae* CBS 114126^T	15.0–50.0 × 3.0–9.0	24.5–29.0 × 6.5–8.0	[7]
6	*P. oryzae* CBS 353.69^T	10.0–25.0 × 3.0–7.0	24.5–29.0 × 6.0–8.0	[7]
7	*P. biciliata* CBS 124463^T	10.0–45.0 × 2.0–5.0	22–28.5 × 6.0–7.5	[7]
8	*P. disseminata* CBS 143904	7.0–24.5 × 2.0–5.0	15.0–26.5 × 4.5–8.0	[27]
9	*P. grevilleae* CBS 114127^T	5.0–25.0 × 2.0–8.0	22.5–28.0 × 7.5–9.0	[7]
10	*P. knightiae* CBS 114138^T	10.0–30.0 × 2.0–10.0	22.0–27.0 × 8.5–10.5	[7]
11	*P. parva* CBS 278.35^T	5.0–18.0 × 2.0–4.0	16.5–20.0 × 5.0–7.0	[7]

N/A: not available.

were less than comparable cells from *P. kenyana* and *P. telopeae*.

KNU-PT-1804 produces smaller conidia compared with *P. kenyana*, *P. neglecta*, and *P. telopeae*, and comparisons with these closest certain species in the genus show smaller and larger conidiogenous cells. KNU-PT-1804 also produces three smaller median cells and second cells from the base, third cells, and fourth cells compared with *P. kenyana* and *P. telopeae*. Morphology of KNU-PT-1804 strain is thus distinct from previously identified species of *Pestalotiopsis*.

3.2. Molecular phylogeny of strain KNU-PT-1804

The phylogenetic relationship strain KNU-PT-1804 from ITS regions, *TUB2*, and *TEF1α* sequences were analyzed and compared with sequences retrieved

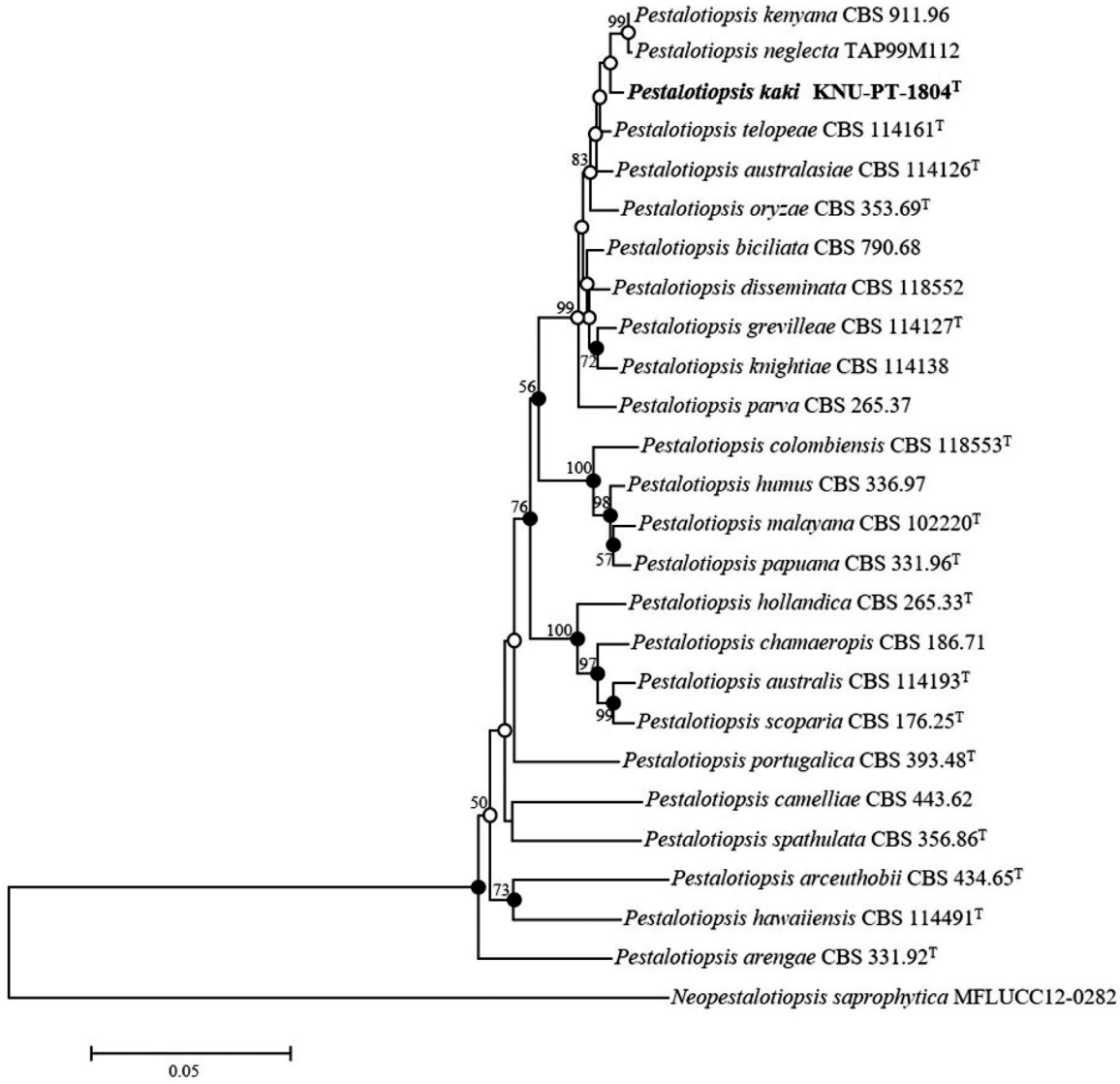

Figure 2. Neighbor-joining phylogenetic tree of strain KNU-PT-1804 based on combined sequences (ITS+*TUB2*+*TEF1α*), showing its phylogenetic position among related *Pestalotiopsis* species. The tree was rooted using *NeoPestalotiopsis saprophytica* MFLUCC12-0282 as an outgroup. Bootstrap values greater than 50% (percentage of 1,000 replications) are shown at branching points. Bar, 0.05 substitutions per nucleotide position.

from NCBI (Table 1). Sequences of 613, 441, and 1043 bp were obtained from ITS regions, *TUB2*, and *TEF1α* genes, respectively. BLAST search results for ITS regions showed 100% similarity with *P. oryzae* CL107, *P. neglecta* UMAS 7_2, *P. kenyana* KoRLI046122, and *P. telopeae* CBS 114137. The *TUB2* gene displayed 98.62 to 99.77% similarity with *P. telopeae* CBS 113,606 *P. leucadendri* CBS 121,417 *P. disseminata* PSH2000I-066, and *P. biciliata* CBS 124463. Finally, *TEF1α* showed 98.84 to 99.89% similarity with *P. kenyana* LC3633, *P. rhodomyrtus* LC3413, and *P. photinicola* YB28-2. The taxonomic position of KNU-PT-1804 was determined using combined sequences of ITS regions, *TUB2* and *TEF1α* genes and also by nodes in the NJ phylogenetic tree along with filled nodes in maximum likelihood and maximum parsimony trees (Figure 2). Corresponding nodes were also recovered using maximum likelihood or maximum parsimony algorithms, as indicated by open circles. A combination of sequences was used for phylogenetic analyses based on maximum parsimony (tree length = 755, consistency index = 0.53, retention index = 0.65, and composite index = 0.47) to determine the taxonomic position of strain KNU-PT-1804. This position is distinct from the other identified species of *Pestalotiopsis* (Figure 2). Consequently, the strain KNU-PT-1804 is proposed as a new species of mycobiota in the genus of *Pestalotiopsis*.

4. Discussion

In this present study, the morphologically distinct strain, KNU-PT-1804, was isolated from persimmon tree (*Diospyros kaki*) bark collected in Korea. Though several strains were isolated from the persimmon bark, but there were no promising candidate for the novels species or unreported species in

Korea. The isolated several fungal strains were selected based on different cultural and molecular characteristics. Among them, there was no similar strain that could be used for comparing the molecular variation. As a results, only one strain was proposed as a novel species. The strain exhibits morphological differences from previously identified, closely related species, based descriptions of the latter in the literature (Table 2).

Pestalotiopsis is a species-rich genus containing pathogens, endophytes and saprophytes [18]. Members of the genus, *Pestalotiopsis,* are common in tropical and temperate ecosystems [7]. *Pestalotiopsis* spp. cause a variety of plant diseases and are often isolated as plant endophytes or saprobes [19]. Many species are named to reflect their host association [4]. Fifteen endophytic *Pestalotiopsis* species were isolated from *Podocarpus macropyllus* in south China [20], five from seeds of *Podocarpus falcatus* (Thunb.) Mirb. in Ethiopia [21]. Most species in the genus *Pestalotiopsis* are pathogens that cause leaf blight in many plant species [4,5,22]. For example, species produce leaf blight on Japanese spicebush (*Lindera obtusiloba*) (*P. microspore*) [23], and leaf spot disease on Proteaceae (*Pestalotiopsis* sp. in Zimbabwe) [24]. Eight novel species in *Pestalotiopsis* and three novel species in *Pseudopestalotiopsis* were described from the symptomatic and asymptomatic tissues of *Camellia sinensis* and other *Camellia* spp. in China [25].

In conclusion, morphological characteristics and phylogenetic analyses indicate a strain distinct from previously identified species of the genus, *Pestalotiopsis. Pestalotiopsis kaki* sp. nov. is thus proposed as a novel species. Considering all aspects of this new member of the genus, further investigation is essential to determine its distribution and pathogenicity and to characterize its ecological importance based on Korean soils and environmental conditions.

Disclosure statement

The authors declare that they have no potential conflicts of interest.

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