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

Kallol Das, Seung-Yeol Lee, and Hee-Young Jung

**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 (*TEF1a*) 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.

**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 *Pestalotiopsisphaeria* 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 factor 1-alpha (*TEF1a*) 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. 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...
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: sp. nov

Type strain: KNU-PT-1804

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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 are isolated from Podocarpus macrophyllus 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 pycnidal 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 or 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), 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 doliform, 12.8–16.7 μm long (x ± SD = 14.4 ± 1.0), 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;
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

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

KNU-PT-1804 produces smaller conidia compared with *P. kenyana*, *P. neglecta*, and *P. teleopeae*, 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. teleopeae*. 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

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

| Species                  | Strain Numbers | ITS Accession | TUB2 Accession | TEF1α Accession |
|-------------------------|----------------|---------------|----------------|-----------------|
| *NeoPestalotiopsis saprophytica* | MFLUCC12-0282 | JX398982      | JX399017       | JX399048        |
| *Pestalotiopsis arecuthobii* | CBS 434.65T    | KM199341      | KM199427       | KM199516        |
| *P. arenaria*            | CBS 331.92T    | KM199340      | KM199426       | KM199515        |
| *P. australiasi*         | CBS 114126T    | KM199297      | KM199409       | KM199499        |
| *P. australis*           | CBS 1141931T   | KM199332      | KM199383       | KM199475        |
| *P. bicilata*            | CBS 790.68     | KM199305      | KM199400       | KM199507        |
| *P. camelliae*           | CBS 443.62     | KM199336      | KM199424       | KM199512        |
| *P. chamaerops*          | CBS 186.71     | KM199326      | KM199391       | KM199473        |
| *P. colombiensis*        | CBS 118553T    | KM199307      | KM199421       | KM199488        |
| *P. disseminata*         | CBS 118552     | MHS53986      | MHS54652       | MHS54410        |
| *P. grevilleae*          | CBS 114127T    | KM199300      | KM199407       | KM199504        |
| *P. hawaiiensis*         | CBS 114491T    | KM199339      | KM199428       | KM199514        |
| *P. hollandica*          | CBS 114126    | KM199297      | KM199409       | KM199499        |
| *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 114193    | KM199306      | KM199411       | KM199482        |
| *P. neglecta*            | TAP99M112      | AB482211      | AB453882       | AB453853        |
| *P. oryzae*              | CBS 353.69T    | KM199299      | KM199398       | KM199496        |
| *P. papuana*             | CBS 331.96T    | KM199321      | KM199413       | KM199491        |
| *P. parva*               | CBS 265.37     | KM199312      | KM199404       | KM199508        |
| *P. portugalica*         | CBS 393.48T    | KM199335      | KM199422       | KM199510        |
| *P. scoparia*            | CBS 176.25T    | KM199330      | KM199393       | KM199478        |
| *P. spathulata*          | CBS 356.86T    | KM199338      | KM199423       | KM199513        |
| *P. telopeae*            | CBS 114161T    | KM199296      | KM199403       | KM199500        |
| *Pestalotiopsis kaki*    | KNU-PT-1804T   | 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: Tamagawa University, Tokyo, Japan; KNU: Kyungpook National University, Daegu, Korea.

**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*     | 15.8–18.3 × 4.0–4.2      | 19.4–26.3 × 4.4–6.3 | In this study |
| 2       | *P. kenyana*  | 10.0–25.0 × 2.0–5.0       | 23.0–28.0 × 7.0–9.0 | [7] |
| 3       | *P. neglecta* | N/A                      | 27.0 × 9.0    | [26] |
| 4       | *P. teleopeae*| 5.0–15.0 × 2.0–9.0        | 24.5–31.0 × 6.0–8.0 | [7] |
| 5       | *P. australiasi* | 15.0–30.0 × 3.0–9.0    | 24.5–29.0 × 6.5–8.0 | [7] |
| 6       | *P. oryzae*   | 10.0–25.0 × 3.0–7.0       | 24.5–29.0 × 6.0–8.0 | [7] |
| 7       | *P. bicilata* | 10.0–45.0 × 2.0–5.0       | 22–28.5 × 6.0–7.5 | [7] |
| 8       | *P. disseminata* | 7.0–24.5 × 2.0–5.0   | 15.0–26.5 × 4.5–8.0 | [27] |
| 9       | *P. grevilleae* | 5.0–25.0 × 2.0–8.0     | 22.5–28.0 × 7.5–9.0 | [7] |
| 10      | *P. knightiae*| 10.0–30.0 × 2.0–10.0      | 22.0–27.0 × 8.5–10.5 | [7] |
| 11      | *P. parva*    | 5.0–18.0 × 2.0–4.0       | 16.5–20.0 × 5.0–7.0 | [7] |

N/A: not available.
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 KoRL1046122, and P. telepaea CBS 114137. The TUB2 gene displayed 98.62 to 99.77% similarity with P. telepaea CBS 113,606 P. leucadendri CBS 121,417 P. disseminata PSH20001-066, 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 mycobiont 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

![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.](image-url)
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 macrophyllus 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 Camelli sp. 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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