MYCOBIOLOGY
2025, VOL. 53, NO. 3, 269–279
https://doi.org/10.1080/12298093.2025.2473141

RESEARCH ARTICLE

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Isolation and Characterization of Four Unreported *Penicillium* Species Isolated from the Freshwater Environments in Korea

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ABSTRACT

The fungal species of the genus *Penicillium* can be found across a diverse array of environments. The infrageneric classification of the genus *Penicillium* has been studied with comparison of morphological and phylogenetical features, derived into two subgenus, 32 sections, and 89 series. In this study, 11 fungal strains were isolated from freshwater environments, plant litter, and nearby substrates in Korea and were identified as previously unreported species. The internal transcribed spacer (ITS) regions, β-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II subunit (*RPB2*) genes were analyzed for phylogenetic analyses. A neighbor-joining tree was then constructed using the concatenated DNA sequences, and the strains were compared with closely related species of the genus *Penicillium*. The strain clustered into distinct phylogenetic lineages, confirming their classification as *P. contaminatum*, *P. jinfoshanicum*, *P. xuanhanense*, and *P. soppii*. NNIBRFG40229 exhibits monoverticillate conidiophores with flask-shaped phialides, characteristic of *P. contaminatum*; NNIBRFG1595 presents divaricate conidiophores, consistent with *P. jinfoshanicum*; NNIBRFG5602 shows a velutinous texture with orange pigmentation, resembling *P. xuanhanense*; and NNIBRFG4602 shows biverticillate conidiophores with cylindrical metulae, corresponding to *P. soppii*. This study provides the first report of these species in Korea, enhancing taxonomic understanding.

ARTICLE HISTORY
Received 13 January 2025
Revised 21 February 2025
Accepted 24 February 2025

KEYWORDS
Morphological characteristics; *Penicillium contaminatum*; *Penicillium jinfoshanicum*; *Penicillium soppii*; *Penicillium xuanhanense*

1. Introduction

The genus *Penicillium* is a widely recognized and prevalent fungal genus found in a vast array of environments, including soil, plants, air, indoor spaces, and various food items [1–3]. Due to its widespread presence across the globe, this species has been isolated from various environments [4]. In 1809, the name of *Penicillium* is derived from "penicillus", which means "little brush" [5]. The classification of *Penicillium* was started in 1901 introducing a three subgeneric classification including *Aspergilloides*, *Biverticillium*, and *Eupenicillium* [6]. Since its first introduction, the genus *Penicillium* has undergone multiple changes in classification systems from a classification system based on cultural characteristics to a classification system based on phylogenetic analysis [1,7,8]. Following the recent classification system, the teleomorphs or anamorphs of *Penicillium* had been changed into a system based on DNA sequence data deriving the genus *Penicillium* into two subgenera, 32 sections, and 89 series [9]. In the genus *Penicillium*, infrageneric ranks such as subgenera and sections are crucial for organizing its numerous species, which aids in understanding their evolutionary relationships and practical applications. This classification stabilizes the structure of the genus, enabling more straightforward identification and study of its many species [9]. At the time of this writing, approximately 535 species were discovered in the genus *Penicillium* from all over the world [10]. In Korea, native species belonging to the genus *Penicillium* are being studied and reclassified according to the principle of "one fungus-one name" [11]. Korean *Penicillium* species records in GenBank were inaccurate due to the reliance of ITS sequence data, thus reevaluation was conducted by using the concatenated sequences of not only ITS regions, but also *BenA*, *CaM*, and *RPB2* sequences to analysis for a more precise identification [12]. The aim of this study was to investigate the diversity of *Penicillium* strains from diverse environments in Korea, and the

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isolated strains were identified for the potential research. The morphological and molecular characteristics of isolated strains were recorded.

2. Materials and methods

2.1. Sample collection and fungal isolation

Samples from soil, water, and plants were collected from various locations in Korea (Table 1). Fungi were then isolated from the collected samples using the serial dilution method as described in a previous study [13]. Various fungal strains were isolated from different provinces. From the isolates, 11 fungal strains were chosen and then used for morphological, cultural, and phylogenetical analyses. Each strain was deposited at the Nakdonggang National Institute of Biological Resources under the accession numbers NNIBRFG25747, NNIBRFG40229, NNIBRFG1595, NNIBRFG1963, NNIBRFG5170, NNIBRFG44548, NNIBRFG48023, NNIBRFG5602, NNIBRFG5768, NNIBRFG25960, and NNIBRFG4602, respectively (Table 1).

2.2. Cultural and morphological characterization

To observe cultural characteristics, the isolates were cultured on four different media. The strains were cultured at three points on potato dextrose agar (PDA; Difco, Detroit, MI), malt extract agar (MEA; Difco, Detroit, MI), Czapek yeast extract agar (CYA; MB Cell, Seoul, South Korea), and yeast extract sucrose agar (YES; yeast extract, 4 g; sucrose, 20 g; KH_2PO_4, 1 g; $MgSO_4$, 0.5 g; agar, 15 g; distilled H_2O, 1000 mL), then incubated at 25 °C for seven days [14]. The cultures were maintained in darkness, and various characteristics were observed, including the size, color, and shape of the mycelium, as well as morphological features such as conidiophore, stipe, metula, phialide, conidia, and the arrangement of conidia. A light microscope (BX-50; Olympus, Tokyo, Japan) was used to study the morphological properties.

2.3. Genomic DNA extraction, PCR amplification, and sequencing

Total genomic DNA from each strain was collected from growing colony on PDA and extracted using the HiGene™ Genomic DNA Prep Kit (Biofact, Daejeon, South Korea) according to the manufacturer's instructions. The internal transcribed spacer (ITS) regions, β-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II subunit (*RPB2*) genes were amplified using the primer pairs ITS1F/ITS4, Bt2a/Bt2b, CMD5/CMD6, and RPB2-5f/RPB2-7cR, respectively [15–18]. Amplification was confirmed by electrophoresis using HP Agarose (BIOPURE, Cambridge, MA) 1.0% gels. Amplified

Table 1. Information of *Penicillium* isolates used in this study.

Species	Strain	Source	Year of isolation	Location
Penicillium contaminatum	NNIBRFG25747	Water	2019	Sincheon-ri, Hanbando-myeon, Yeongwol-gun, Gangwon, South Korea (37° 13′ 35″ N 128° 20′ 18″ E)
	NNIBRFG40229	Plant litter	2022	Sinjeom-ri, Yongmun-myeon, Yangpyeong-gun, Gyeonggi, South Korea (37° 32′ 42″ N 127° 35′ 2.45″ E)
Penicillium jinfoshanicum	NNIBRFG1595	Sediment	2016	Deoksan-ri, Daedeok-myeon, Gimcheon-si, Gyeongbuk, South Korea (35° 55′ 52.1″ N 127° 54′ 23″ E)
	NNIBRFG1963	Sediment	2016	Bugok-ri, Cheongsong-eup, Cheongsong-gun, Gyeongbuk, South Korea (36° 26′ 22.8″ N 129° 5′ 25.7″ E)
	NNIBRFG5170	Sediment	2019	Singung-ri, Naebuk-myeon, Boeun-gun, Chungbuk, South Korea (36° 30′ 35″ N 127° 38′ 5″ E)
	NNIBRFG44548	Sediment	2022	Mangmi-ri, Jipyeong-myeon, Yangpyeong-gun, Gyeonggi, South Korea (37° 25′ 54″ N 127° 39′ 50″ E)
	NNIBRFG48023	Water	2022	Dae-ri, Yeonghae-myeon, Yeongdeog-gun, Gyeongbuk, South Korea (36° 32′ 6″ N 129° 15′ 39″ E)
Penicillium xuanhanense	NNIBRFG5602	Water	2018	Singung-ri, Naebuk-myeon, Boeun-gun, Chungbuk, South Korea (36° 30′ 35″ N 127° 38′ 5″ E)
	NNIBRFG5768	Sediment	2019	Cheongpyeong-ri, Buksan-myeon, Chuncheon-si, Gangwon, South Korea (37° 59′ 1″ N 127° 49′ 5″ E)
	NNIBRFG25960	Water	2020	Jikdong-ri, Sohol-eup, Pocheon-si, Gyeonggi, South Korea (37° 44′ 56.3″ N 127° 9′ 57.8″ E)
Penicillium soppii	NNIBRFG4602	*Cypripedium macranthum*	2018	Gohan-ri, Gohan-eup, Jeongseon-gun, Gangwon, South Korea (37° 8′ 57.4″ N 128° 54′ 10.8″ E)

products were purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA) and sequencing services were provided by Macrogen (Seoul, South Korea).

2.4. Phylogenetic analyses

Taxa for phylogenetic analysis and outgroups were chosen according to previous study [10], and additional related species were added according to the Basic Local Alignment Search Tool (BLAST) results. Sequences were downloaded from the National Center for Biotechnology Information (NCBI) database (Table 2). Phylogenetic trees were constructed from the concatenated sequences of the ITS regions, *BenA*, *CaM*, and *RPB2* using the neighbor-joining (NJ) method in MEGA version 11.0 [19,20]. The evolutionary distance matrices for the NJ analysis were generated according to Kimura's two-parameter model with bootstrap values based on 1000 replications [21].

3. Results

3.1. Phylogenetic analysis

The phylogenetic relationships of the isolated *Penicillium* species were assessed through BLASTn sequence similarity searches and phylogenetic tree reconstruction based on concatenated sequence datasets of four loci (ITS, *BenA*, *CaM*, and *RPB2*). BLASTn analysis revealed that strains NNIBRFG25747 and NNIBRFG40229 shared high sequence similarity with *P. contaminatum* CBS 345.52^{T} exhibiting high sequence similarity across multiple loci (ITS: 99.7–99.8%, *BenA*: 100%, *CaM*: 99.8%, and *RPB2*: 100%; bootstrap support = 100%), while NNIBRFG1595, NNIBRFG1963, NNIBRFG5170, NNIBRFG44548, and NNIBRFG48023 were closely related to *P. jinfoshanicum* CS12-10^{T}, showing high sequence similarity (ITS: 100%, *BenA*: 99.7–99.8%, *CaM*: 99.4–99.8%, and *RPB2*: 99.9–100%; bootstrap support = 100%). Similarly, NNIBRFG5602, NNIBRFG5768, and NNIBRFG25960 exhibited strong sequence similarity to *P. xuanhanense* CS31-04^{T} (sequence similarity for ITS = 100%, *BenA* = 100%, *CaM* = 100%, and *RPB2* = 100%; bootstrap support = 100%), and NNIBRFG4602 showed a close phylogenetic relationship with *P. soppii* CBS 226.28^{T}, sharing high sequence similarity (ITS: 100%, *BenA*: 100%, *CaM*: 100%, and *RPB2*: 99.7%; bootstrap support = 100%). The phylogenetic tree, reconstructed using

Table 2. GenBank accession numbers used for phylogenetic analyses in this study.

		GenBank accession number			
Species	Strain number	ITS	*BenA*	*RPB2*	*CaM*
Penicillium aurantioviolaceum	CBS 137777^{T}	KM189756	KM089005	KM089779	KM089392
Penicillium austroafricanum	CBS 137773^{T}	KM189610	KM088854	KM089628	KM089241
Penicillium austrosinicum	CGMCC 3.18410^{T}	KX885061	KX885041	KX885032	KX885051
Penicillium cainii	DAOM 239914^{T}	JN686435	JN686366	MT156346	JN686389
Penicillium cartierense	CBS 137956^{T}	KM189564	KM088804	KM089576	KM089189
Penicillium chroogomphum	CBS 136204^{T}	KC594043	KP684056	MN969167	KP684057
Penicillium contaminatum	CBS 346.59^{T}	KM189782	KM089032	KM089806	KM089419
Penicillium contaminatum	**NNIBRFG25747**	**PQ771855**	**PQ772837**	**PQ772848**	**PQ772859**
Penicillium contaminatum	**NNIBRFG40229**	**PQ771856**	**PQ772838**	**PQ772849**	**PQ772860**
Penicillium crocicola	CBS 745.70^{T}	KM189581	KJ834445	JN406535	KM089210
Penicillium exsudans	HMAS 248735^{T}	KX885062	KX885042	KX885033	KX885052
Penicillium fusisporum	CBS 137463^{T}	KF769424	KF769400	MN969117	KF769413
Penicillium grevilleicola	CBS 137775^{T}	KM189630	KM088874	KM089648	KM089261
Penicillium guanacastense	DAOM 239912^{T}	JN626098	JN625967	KX961295	JN626010
Penicillium jejuense	CBS 138646^{T}	KF818464	KF818461	KF818467	KF818470
Penicillium jinfoshanicum	CS12-10^{T}	OQ870813	OR051074	OR051425	OR051253
Penicillium jinfoshanicum	**NNIBRFG1595**	**PQ771857**	**PQ772839**	**PQ772850**	**PQ772861**
Penicillium jinfoshanicum	**NNIBRFG1963**	**PQ771858**	**PQ772840**	**PQ772851**	**PQ772862**
Penicillium jinfoshanicum	**NNIBRFG5170**	**PQ771859**	**PQ772841**	**PQ772852**	**PQ772863**
Penicillium jinfoshanicum	**NNIBRFG44548**	**PQ771861**	**PQ772843**	**PQ772854**	**PQ772865**
Penicillium jinfoshanicum	**NNIBRFG48023**	**PQ771860**	**PQ772842**	**PQ772853**	**PQ772864**
Penicillium lenticrescens	CBS 138215^{T}	KJ775675	KJ775168	MN969123	KJ775404
Penicillium mallochii	DAOM 239917^{T}	JN626104	JN625973	KX961296	JN626016
Penicillium maximae	CBS 134565^{T}	EU427298	KC773795	MN969126	KC773821
Penicillium meliponae	CBS 142495^{T}	MF278315	MN969418	LT854653	LT854648
Penicillium roseoviride	CBS 267.35^{T}	KM189549	KM088787	KM089559	KM089172
Penicillium sclerotiorum	IMI 40569^{T}	JN626132	JN626001	JN406585	JN626044
Penicillium soppii	CBS 226.28^{T}	AF033488	MN969399	JN406606	KJ867002
Penicillium soppii	**NNIBRFG4602**	**PQ771865**	**PQ772847**	**PQ772858**	**PQ772869**
Penicillium thomii	CBS 225.81^{T}	KM189560	KM088799	KM089571	KM089184
Penicillium valentinum	CBS 172.81^{T}	KM189550	KM088788	KM089560	KM089173
Penicillium xuanhanense	CS31-04^{T}	OQ870873	OR051222	OR062086	OR051396
Penicillium xuanhanense	**NNIBRFG5602**	**PQ771863**	**PQ772845**	**PQ772856**	**PQ772867**
Penicillium xuanhanense	**NNIBRFG5768**	**PQ771862**	**PQ772844**	**PQ772855**	**PQ772866**
Penicillium xuanhanense	**NNIBRFG25960**	**PQ771864**	**PQ772846**	**PQ772857**	**PQ772868**
Penicillium yezoense	CBS H-21863^{T}	KM189553	KM088792	KM089564	KM089177
Hamigera avellanea	CBS 295.48^{T}	AF454075	EU021664	EU021627	EU021682

ITS: internal transcribed spacer regions; *BenA*: β-tubulin gene; *RPB2*: RNA polymerase II subunit gene; *CaM*: calmodulin gene.
^{T}Type strain. The strains isolated in this study are indicated in bold.

the NJ method, demonstrated that these isolates formed well-supported monophyletic groups with their respective reference strains. The concatenated alignment consisted of 1883 nucleotides (ITS: 352 bp, *BenA*: 366 bp, *CaM*: 404 bp, and *RPB2*: 761 bp), with consistently high bootstrap support values, confirming the taxonomic placement of the isolates (Figure 1).

3.2. Taxonomy

Penicillium contaminatum Houbraken, Studies in Mycology 78: 419 (2014) [MB#809962]

Strains NNIBRFG25747 and NNIBRFG40229 were found to be morphologically identical, and they clustered together with *P. contaminatum* CBS 345.52^T in respect to the molecular phylogeny. Thus, in this study, only the cultural and morphological characteristics of strain NNIBRFG40229 were described in this study since they were identical.

Cultural characteristics: On PDA at 25 °C for 7 d: moderately deep and radially sulcate with circular colonies having white mycelium; dull green conidia; margins entire; texture is velvety; no exudate present; no soluble pigments detected; coloration of

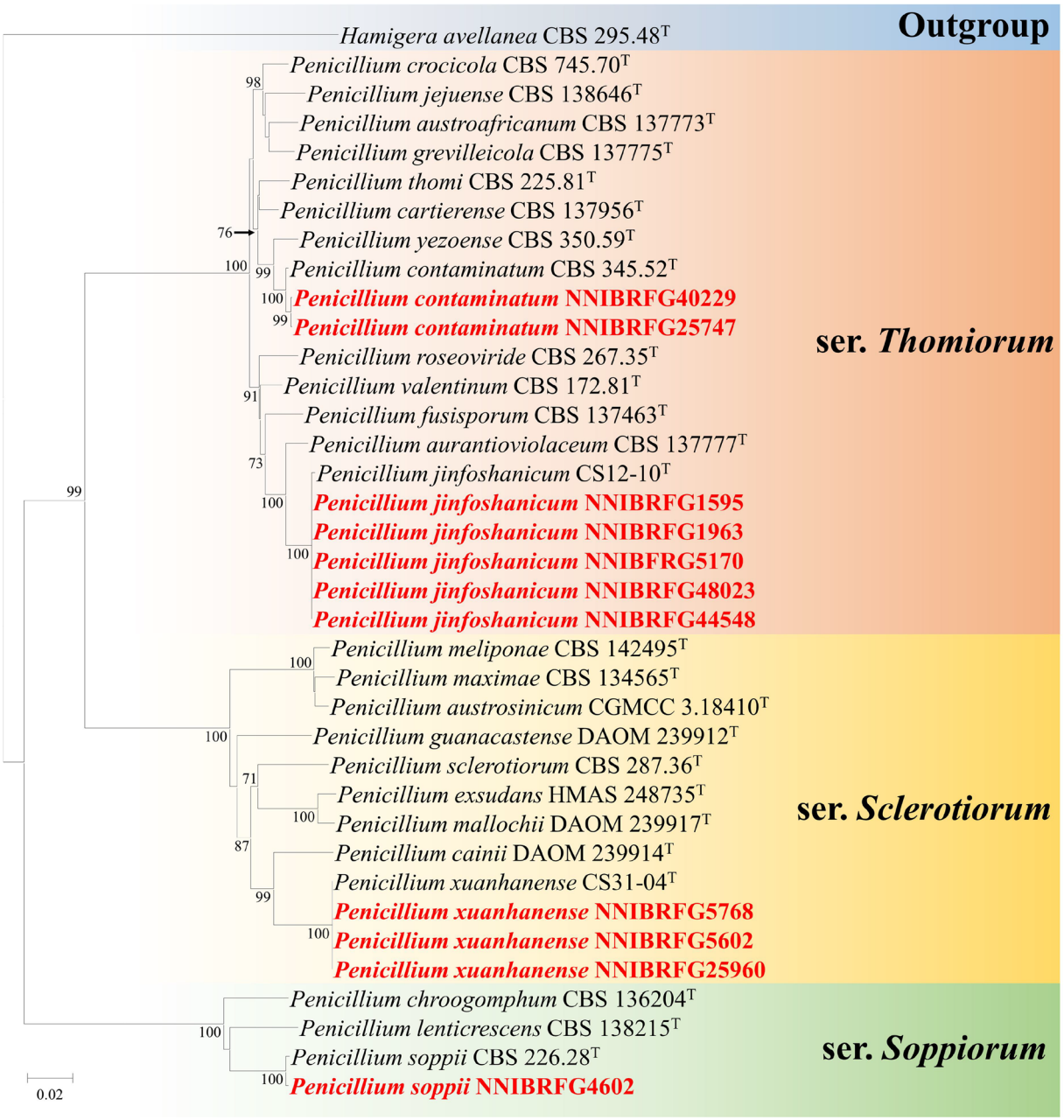

Figure 1. Neighbor-joining phylogenetic tree based on a combined dataset of partial sequences of ITS regions, *BenA*, *RPB2*, and *CaM* sequences, the different series of *Penicillium*, namely, *Thomiorum*, *Sclerotiorum*, and *Soppiorum*. *Hamigera avellanea* CBS 295.48^T was used as an outgroup. The numbers above/below the branches indicate bootstrap values (>70%) obtained from 1000 replicates. The unrecorded *Penicillium* species in Korea are highlighted in bold red. Bar = 0.02 substitutions per nucleotide position.

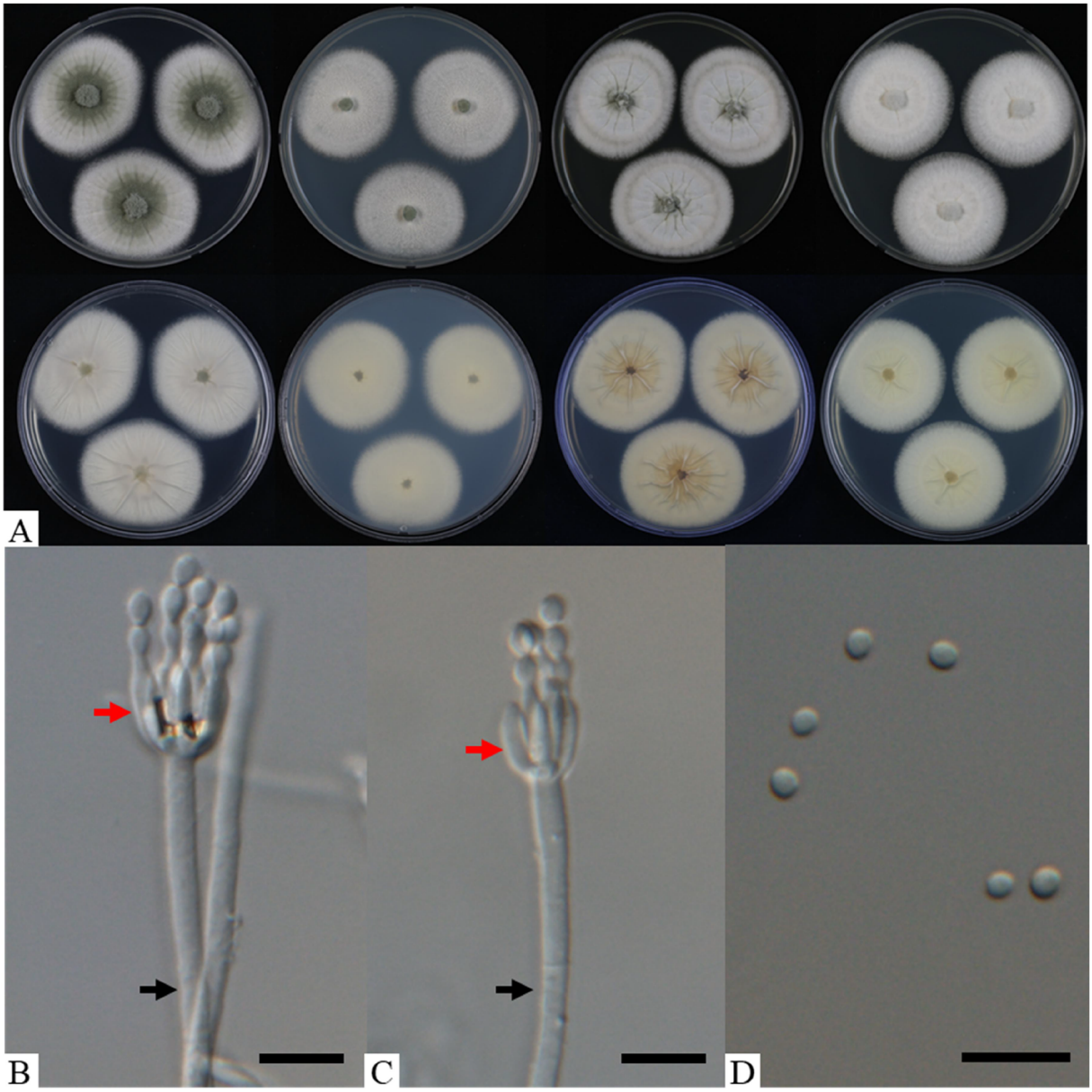

Figure 2. Morphological characteristics of *Penicillium contaminatum* NNIBRFG40229. (A) Colonies after seven days at 25 °C, from left to right (top row) PDA obverse, MEA obverse, CYA obverse, YES obverse; (bottom row) PDA reverse, MEA reverse, CYA reverse, YES reverse; (B, C) Conidiophore consists of the stipe (black arrows) and phialide (red arrows); (D) Conidia; scale bars = 10 µm.

reverse is white and pale (Figure 2(A)). On MEA at 25 °C for seven days: moderately deep and radially sulcate with circular colonies having white mycelium; dull green conidia; margins entire; texture is velvety to floccose; no exudate present; no soluble pigments detected; coloration of reverse is beige (Figure 2(A)). On CYA at 25 °C for seven days: moderately deep and radially sulcate with circular colonies having white mycelium; grey green conidia; margins entire to slightly irregular; texture is velvety; no exudate present; no soluble pigments detected; coloration of reverse is beige to yellow (Figure 2(A)). On YES at 25 °C for seven days: moderately deep and radially sulcate with circular colonies having white mycelium; dull grey conidia; margins entire; texture is velvety; no exudate present; no soluble pigments detected; coloration of reverse is beige (Figure 2(A)). Colony diameters after seven days at 25 °C are as follows: PDA 46–48; MEA 43–45; YES 44–48; CYA 45–46 (Figure 2).

Morphological characteristics: Conidiophores monoverticillate (Figure 2(B,C)); stipes rough walled, 155–230 × 2.6–3.6 µm (Figure 2(B,C)); phialides ampulliform with short narrow neck, 9.1–11.2 × 2.5–3.5 µm (Figure 2(B,C)); conidia long irregular columns, ellipsoidal, 3.3–4.2 × 2.8–3.3 µm (Figure 2(D)).

Habitat: Plant litter in freshwater.

Specimen examined: Sinjeom-ri, Yongmun-myeon, Yangpyeong-gun, Gyeonggi, South Korea; April 14 2022; NNIBRFG40229 (ITS = PQ771856; *BenA* = PQ772838; *CaM* = PQ772860; *RPB2* = PQ772849).

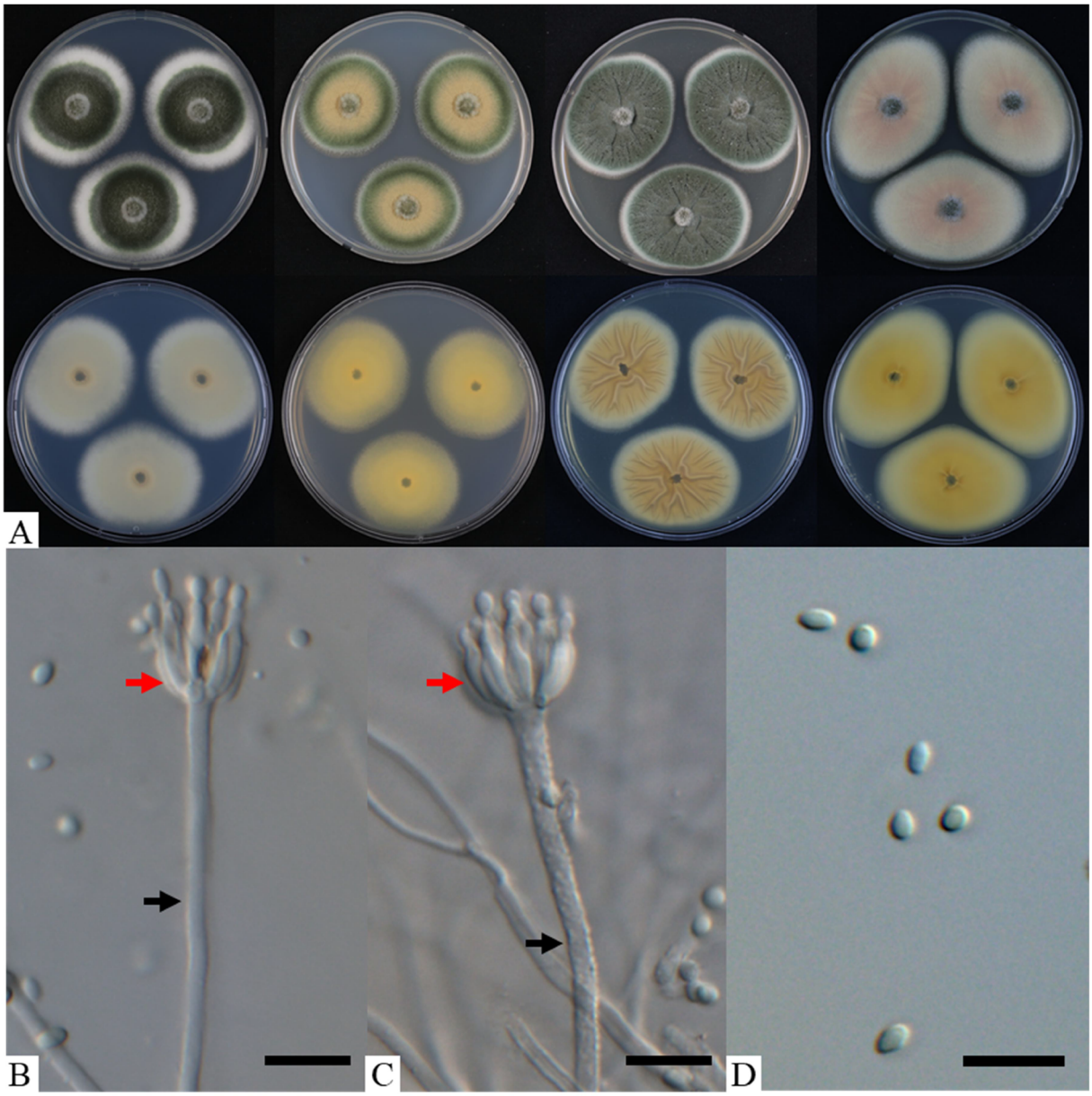

Figure 3. Morphological characteristics of *Penicillium jinfoshanicum* NNIBRFG1595. (A) Colonies after seven days at 25 °C, from left to right (top row) PDA obverse, MEA obverse, CYA obverse, YES obverse; (bottom row) PDA reverse, MEA reverse, CYA reverse, YES reverse; (B, C) Conidiophore consists of the stipe (black arrows) and phialide (red arrows); (D) Conidia; scale bars = 10 µm.

Note: *Penicillium contaminatum* was initially reported in 2014, isolated from the contaminant in the UK [22]. Comparing the Korean *P. contaminatum* NNIBRFG40229 and CBS 345.52^{T}, the cultural characteristics from MEA, CYA, and YES media are similar, but *P. contaminatum* isolated in Korea tends to grow slower than CBS 345.52^{T} grown on CYA (45–46 vs. 42–55 mm), MEA (43–45 vs. 46–50 mm), and YES (44–48 vs. 51–57 mm) (Figure 2(A)) [22]. For the morphological characteristics, both strains exhibit short, narrow, flask-shaped phialides at the ends of monoverticillate conidiophores (Figure 2(B,C)), producing long irregular columns or ellipsoidal conidia (Figure 2(D)) [22].

Penicillium jinfoshanicum X.C. Wang & W.Y. Zhuang, J. Fungi 9 (12, no. 1150): 79 (2023) [MB#571549]

Strains NNIBRFG1595, NNIBRFG1963, NNIBRFG5170, NNIBRFG44548, and NNIBRFG48023 were found to be morphologically identical, and they clustered together with *P. jinfoshanicum* CS12-10^{T} in respect to the molecular phylogeny. Thus, in this study, only the cultural and morphological characteristics of strain NNIBRFG1595 were described in this study since they were identical.

Cultural characteristics: On PDA at 25 °C for seven days: plain and protuberant at centers with circular colonies having white mycelium; dull green conidia; margins entire; texture is velutinous; no exudate present; no soluble pigments detected; coloration of reverse is cream to yellow (Figure 3(A)). On MEA at 25 °C for seven days: plain with nearly circular colonies having white mycelium; dull green conidia; margins slightly irregular; texture is velutinous to floccose; no exudate present; no soluble

pigments detected; coloration of reverse is cream (Figure 3(A)). On CYA at 25 °C for seven days: protuberant at centers and radially sulcate with circular colonies having white mycelium; dull green conidia; margins entire; texture is velutinous; no exudate present; no soluble pigments detected; coloration of reverse is cream to yellow (Figure 3(A)). On YES at 25 °C for seven days: radially sulcate with nearly circular colonies having white mycelium; margins fimbriate; texture is velutinous to floccose; no exudate present; no soluble pigments detected; coloration of reverse is yellow (Figure 3(A)). Colony diameters after seven days at 25 °C are as follows: PDA 45–46; MEA 40–42; CYA 46–48; YES 56–57 (Figure 3).

Morphological characteristics: Conidiophores monoverticillate, occasionally divaricate (Figure 3(B,C)); stipes rough walled, 60–170 × 3.2–3.7 µm (Figure 3(B,C)); phialides acerose to ampulliform, tapering into very thin neck, 9.0–13.2 × 2.5–3.3 µm (Figure 3(B,C)); conidia narrow ellipsoidal, smooth walled, 3.4–3.8 × 2.5–3.3 µm (Figure 3(D)).

Habitat: Sediment in freshwater.

Specimen examined: Gam-cheon, Deoksan-ri, Daedeok-myeon, Gimcheon-si, Gyeongbuk, South Korea; March 23 2016; NNIBRFG1595 (ITS = PQ771857; *BenA* = PQ772839; *CaM* = PQ772861; *RPB2* = PQ772850).

Note: *Penicillium jinfoshanicum* was first reported in 2023, isolated from the soil in China [23]. Comparing the Korean *P. jinfoshanicum* NNIBRFG1595 and CS12-10^{T}, the cultural characteristics from PDA, MEA, CYA, and YES media are similar; however, the strain NNIBRFG1595 exhibits slower growth on PDA (45–46 vs. 49–51 mm), but faster growth on YES (56–57 vs. 52–53 mm) (Figure 3(A)) [23]. For the morphological characteristics, both strains exhibit short, flask-shaped phialides at the ends of monoverticillate conidiophores (Figure 3(B,C)), producing narrow ellipsoidal of conidia (Figure 3(D)) [23].

Penicillium xuanhanense X.C. Wang & W.Y. Zhuang, J. Fungi 9 (12, no. 1150): 127 (2023) [MB#571574]

Strains NNIBRFG5602, NNIBRFG5768, and NNIBRFG25960 were found to be morphologically identical, and they clustered together with *P. xuanhanense* CS31-04^{T} in respect to the molecular phylogeny. Thus, in this study, only the cultural and morphological characteristics of strain NNIBRFG 40229 were described in this study since they were identical.

Cultural characteristics: On PDA at 25 °C for seven days: plain with circular colonies having white mycelium; grayish green conidia; margins entire; texture is velutinous; no exudate present; no soluble pigments detected; coloration of reverse is yellow to orange (Figure 4(A)). On MEA at 25 °C for seven days: protuberant at centers with circular colonies having white mycelium; dull green conidia; margins entire; texture is velutinous; no exudate present; no soluble pigments detected; coloration of reverse is yellow to red brown (Figure 4(A)). On CYA at 25 °C for seven days: plain and radially sulcate with circular colonies having white mycelium; dull green conidia; margins entire; texture is velutinous; no exudate present; no soluble pigments detected; coloration of reverse is orange to yellow (Figure 4(A)). On YES at 25 °C for seven days: radially sulcate with circular colonies having white mycelium; dull green conidia; margins entire; texture is velutinous; no exudate present; no soluble pigments detected; coloration of reverse is white to red (Figure 4(A)). Colony diameters after seven days at 25 °C are as follows: PDA 22–25; MEA 29–33; CYA 19–21; YES 29–33 (Figure 4).

Morphological characteristics: Conidiophores monoverticillate or divaricate (Figure 4(B,C)); stipes smooth to rough walled, 44.0–145.2 × 2.6–3.3 µm (Figure 4(B,C)); phialides ampulliform to acerose, tapering into very thin neck, 10.0–11.5 × 3.1–3.7 µm (Figure 4(B,C)); conidia narrow ellipsoidal, smooth walled, 2.9–3.7 × 2.0–2.7 µm (Figure 4(D)).

Habitat: Filtered freshwater.

Specimen examined: Singung-ri, Naebuk-myeon, Boeun-gun, Chungbuk, South Korea; March 9 2018; NNIBRFG5602 (ITS = PQ771863; *BenA* = PQ772845; *CaM* = PQ772867; *RPB2* = PQ772856).

Note: *Penicillium xuanhanense* was initially reported in 2023, isolated from the soil in China [23]. Comparing the Korean *P. xuanhanense* NNIBRFG5602 and CS31-04^{T}, the cultural characteristics from PDA, MEA, CYA, and YES media are similar, but the strain NNIBRFG5602 *P. xuanhanense* shows slower growth on PDA (22–25 vs. 27–28 mm), YES (29–33 vs. 35–36 mm), and CYA (19–21 vs. 30–31 mm) (Figure 4(A)) [23]. For the morphological characteristics, both strains exhibit long flask-shaped phialides at the ends of monoverticillate conidiophores (Figure 4(B,C)), producing narrow ellipsoidal of conidia (Figure 4(D)) [23].

Penicillium soppii K. Zaleski, Bull. Int. Acad. Polon. Sci., Cl. Sci. Math., Sér. B., Sci. Nat. 1927: 476 (1927) [MB#121424]

Cultural characteristics: On PDA at 25 °C for seven days: flat, and radially sulcate with irregular colonies having white mycelium; dull green conidia; margins undulate; texture is velutinous to floccose;

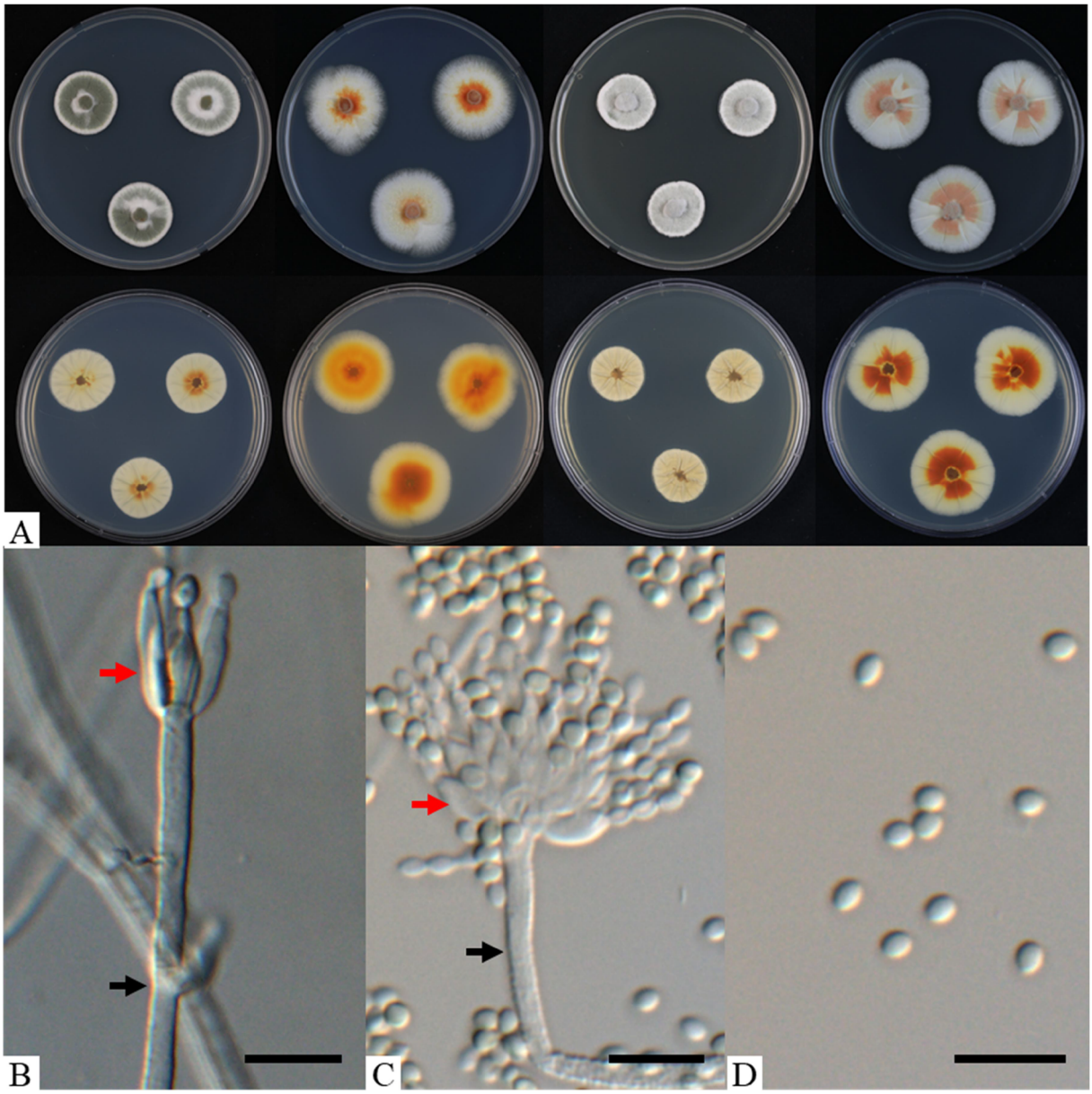

Figure 4. Morphological characteristics of *Penicillium xuanhanense* NNIBRFG5602. (A) Colonies after seven days at 25 °C, from left to right (top row) PDA obverse, MEA obverse, CYA obverse, YES obverse; (bottom row) PDA reverse, MEA reverse, CYA reverse, YES reverse; (B, C) Conidiophore consists of the stipe (black arrows) and phialide (red arrows); (D) Conidia; scale bars = 10 µm.

no exudate present; no soluble pigments detected; coloration of reverse is light brown to brown (Figure 5(A)). On MEA at 25 °C for seven days: flat, and radially sulcate with irregular colonies having white mycelium; dull green conidia; margins undulate; texture is velutinous; no exudate present; no soluble pigments detected; coloration of reverse is light brown (Figure 5(A)). On CYA at 25 °C for seven days: flat, and radially sulcate with irregular colonies having white mycelium; margins undulate; dull green conidia; texture is velutinous; no exudate present; no soluble pigments detected; coloration of reverse is light brown (Figure 5(A)). On YES at 25 °C for seven days: flat with irregular colonies having white mycelium; margins undulate; dull green conidia; texture is floccose; no exudate present; no soluble pigments detected; coloration of reverse is light brown to brown (Figure 5(A)). Colony diameters after seven days at 25 °C are as follows: PDA 20–25; MEA 17–21; CYA 27–31; YES 22–26 (Figure 5).

Morphological characteristics: Conidiophores biverticillate (Figure 5(B,C)); stipes smooth walled 320.0–450.0 × 2.5–4.0 µm (Figure 5(B,C)); metula 10.8–13.4 × 2.8–4.4 µm, cylindrical (Figure 5(B,C)); phialides parallel in the cluster, short tapered necks, 8–10 × 2.3–3 µm (Figure 5(B,C)); conidia globose to subglobose, smooth walled, 2.0–2.8 µm (Figure 5(D)).

Habitat: Endophyte from *Cypripedium macranthum*.

Specimen examined: Gohan-ri, Gohan-eup, Jeongseon-gun, Gangwon, South Korea; October 23

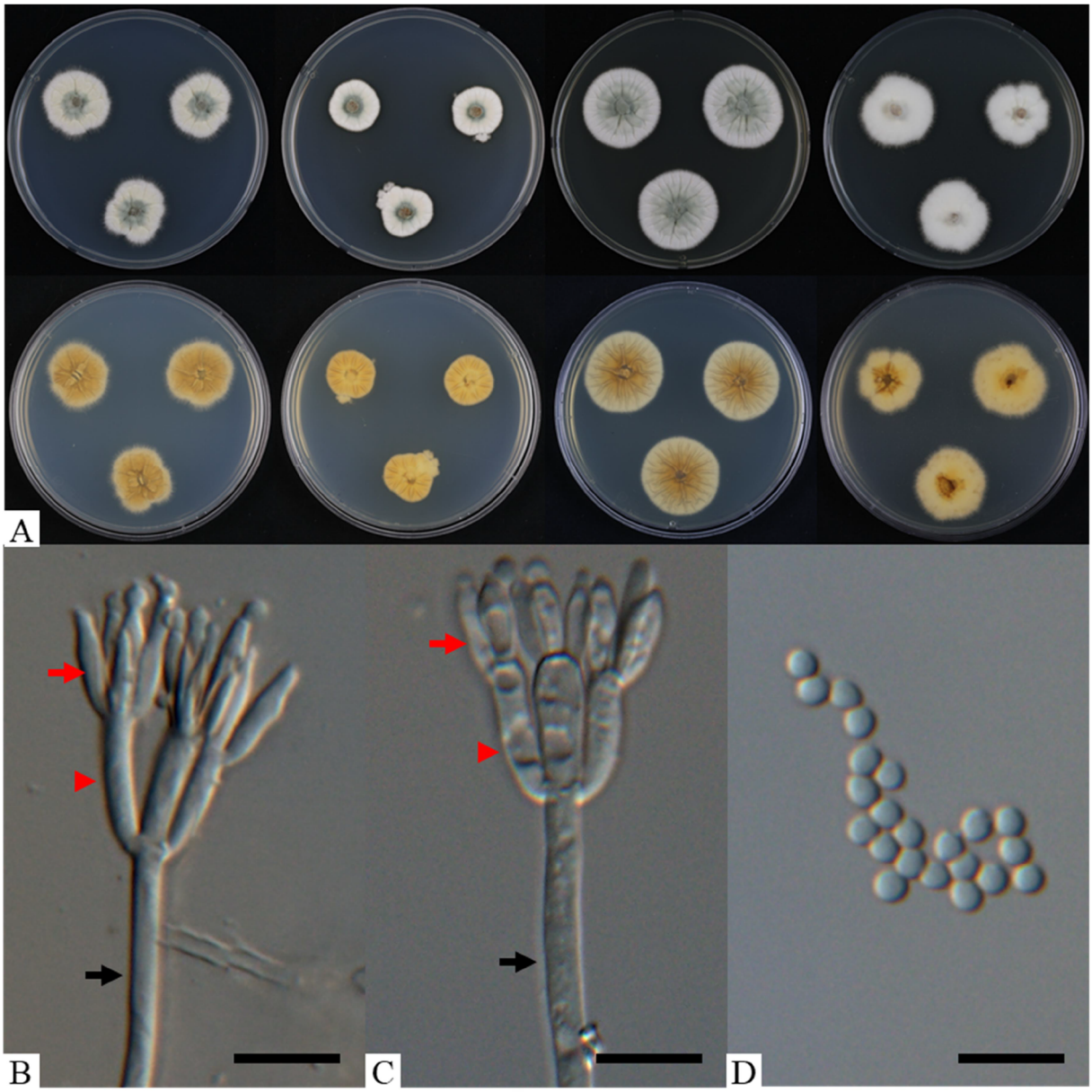

Figure 5. Morphological characteristics of *Penicillium soppii* NNIBRFG4602. (A) Colonies after seven days at 25 °C, from left to right (top row) PDA obverse, MEA obverse, CYA obverse, YES obverse; (bottom row) PDA reverse, MEA reverse, CYA reverse, YES reverse; (B, C) Conidiophore consists of the stipe (black arrows), metula (red arrows head), and phialide (red arrows); (D) Conidia; scale bars = 10 µm.

2015; NNIBRFG4602 (ITS = PQ771865; *BenA* = PQ772847; *CaM* = PQ772869; *RPB2* = PQ772858).

Note: The species *Penicillium soppii* was first reported in 1927 [24]. However, in Korea, *P. soppii* was first reported under the synonym *P. meleagrinum* var. *viridiflavum* [11]. Furthermore, according to the Mycobank database (http://www.mycobank.org/), it is revealed that *P. sumatraense* is the current name of *P. meleagrinum* var. *viridiflavum*. Hence, the discrepancy supports that *P. soppii* has not been reported in Korea yet. Comparing the Korean *P. soppii* NNIBRFG4602 and CBS 226.28^{T}, the cultural characteristics from MEA, CYA, and YES are similar (Figure 5(A)). For the morphological characteristics, both strains exhibit long flask-shaped phialides at the ends of cylindrical metula (Figure 5(B,C)). Metula are formed on the branching point of biverticillate conidiophores (Figure 5(B,C)), producing globes to subglobose of conidia (Figure 5(D)) [24].

4. Discussion

The morphological and phylogenetic analyses conducted on the four previously unrecorded *Penicillium* strains in this study revealed significant diversity within the genus, underscoring the potential for discovering unreported species in Korea. Phylogenetic analyses based on the ITS regions, *BenA*, *CaM*, and *RPB2* sequences successfully classified the strains, placing them into distinct subgenera and sections within *Penicillium*. In this study, all isolates were obtained from freshwater sources and their nearby substrates,

suggesting that these environments may serve as significant habitats for *Penicillium* species. The isolation of these fungi from aquatic habitats indicates a potential adaptation to the unique conditions. Ongoing taxonomic investigations have further expanded the known diversity of *Penicillium*, with novel and previously unreported species continuously being identified across various ecological niches, including freshwater ecosystems [25–34]. Recently, a lot of studies have been conducted on the species of *Penicillium*, and many new species that have not been recorded in aquatic and terrestrial environments in Korea have been continuously reported [25–34]. Among these, *P. annulatum*, *P. camponotum*, *P. echinulonalgiovense*, *P. globosum*, *P. limosum*, *P. onobense*, and *P. yunnanense* have been identified from freshwater and soil samples in 2021, contributing to the expanding diversity of *Penicillium* in Korea. Similarly, *P. aquadulcis*, *P. flavigenum*, and *P. lenticrescens* have been isolated from freshwater samples, further enhancing the understanding of their ecological roles in aquatic environments [26]. Throughout this study, these finding adds four new *Penicillium* species to the current list of native *Penicillium* species in Korea, and suggests that Korea's unique environment may harbor more unexplored fungal diversity, further offering insights into regional biodiversity. Consequently, the compiling of data on the genus *Penicillium* in Korea is essential for advancing our understanding of its adaptations and the potential applications of its bioactive properties [35]. More than 20 species of *Penicillium* such as *P. expansum*, *P. citrinum*, and *P. digitatum* were reported as the cause of post-harvest disease on crops in Korea [36]. Moreover, recent study indicated that *P. labradorum* were agents of disseminated fungal disease in a dog [37]. However, the species isolated from this study have not been studied for any pathogenicity tests. Furthermore, the identification of these unreported strains has implications for potential industrial applications, since *Penicillium* species can be sources of new bioactive compounds. *P. rubens* was first studied for its production of the antibacterial antibiotic penicillin [38], and the antibiotic production is being continuously studied with other various *Penicillium* species, such as *P. griseofulvin* and *P. brasiliensis*, which produced anti-inflammatory activities, antibiotic enzymes, and other pharmaceutical metabolites [39,40]. Furthermore, some of the *Penicillium* species such as *P. roqueforti* and *P. camemberti* are used in the production of many varieties of blue cheese [38]. Thus, future studies on these strains should focus on the pathogenicity, and biochemical properties to fully assess their potential applications. To the best of our knowledge, this is the first report of *P. contaminatum*, *P. jinfoshanicum*, *P. xuanhanense*, and *P. soppii* identified in Korea.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by a grant from the Nakdonggang National Institute of Biological Resources (NNIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NNIBR20251105).

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