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RESEARCH NOTE

New Record of three *Penicillium* species isolated from the Freshwater Environment in Korea

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ABSTRACT

In this study, we aimed to observe the diversity of aquatic fungi by collecting deposits of soil, plants, and plant litter from streams and rivers. Three *Penicillium* strains were isolated from soil in streams. Based on the morphological characteristics and phylogenetic analysis using DNA sequences of the internal transcribed spacer, ß-tubulin and calmodulin genes, the isolates were identified as *Penicillium guanacastense, P. saturniforme*, and *P. scabrosum*. These three fungi have not yet been reported in Korea.

Keywords: Freshwater environment, *Penicillium guanacastense, P. saturniforme*, *P. scabrosum*

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The freshwater environment consists of various diverse microorganisms and provides plenty of habitat for fungi, such as plant litter, soil, and freshwater plants. In the environment, fungi play a role in controlling nutrient and carbon cycles as a decomposer [1].

The genus *Penicillium* belongs phylogenetically to Trichocomaceae, and the name is originated from penicillus, little brush by Link. *Penicillium* is a ubiquitous and widespread fungi, which can live in any environment such as soil, air, indoor and various foods, and produce beneficial secondary metabolite [2]. To our knowledge, over 100 species of *Penicillium* have been reported in Korea [3]. Recently, *P. piscarium* from Seungcheon reservoir and *P. pasqualens* and *P. sanguifluum* from Dokdo have been reported in Korea [3,4].

In this study, we isolated three *Penicillium* species from the freshwater environment in Korea. Through molecular phylogenetic analysis and morphological characterization, the three fungal strains were identified as *Penicillium guanacastense, P. saturniforme*, and *P. scabrosum.* Here, we present their mycological descriptions and phylogenetic relationships.

Fungal strains were collected from soil underwater at Namsaengi-mot in Jeju, Soyang-river in Chuncheon-si and Gamcheon in Gimcheon-si between 2016-2018 (Table 1). Soil samples were treated using the dilution plate method and then incubated at 20℃. Three-point inoculation was performed on potato dextrose agar (PDA; Difco, BD, Franklin Lakes, NJ, USA), Czapek yeast autolysate agar (CYA; Czapek concentrate 10 mL, sucrose 30 g, yeast extract 5 g, K_2HPO_4 1 g, trace elements stock solution 1 mL, agar 20 g, dH_2O 1 L), yeast extract sucrose agar (YES;yeast extract 20 g, sucrose 150 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, trace element stock solution 1 mL, agar 20 g, dH_2O 885 mL) and creatine sucrose agar (CREA; sucrose 30 g, creatine·$1H_2O$ 3 g, $K_3PO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, bromocresol purple 0.05 g, trace element stock solution 1 mL, agar 20 g, dH_2O 1 L) to observe the morphological characters of the isolated strains [2]. Colony diameter was measured after 7 days of inoculation, and morphological characteristics were observed using a model Eclipse Ni-U microscope (Nikon, Tokyo, Japan).

Table 1. Environmental factors of sampling site.

Site	Collection date	Location	GPS
Namsaengi-mot	2016.08.09	Jeju island	N33°31'57.7″E126°36'51.7″
Gamcheon	2016.03.23	Gimcheon-si, Gyeongsongbuk-do	N35°53'33.3″E127°59'2.6″
Soyang-river	2018.03.09	Chuncheon-si, Gangwon-do	N37°59'01.0"N127°49'05.0"E

Fungal genomic DNA was isolated using the NucleoSpin Plant II DNA extraction Kit (Macherey-Nagel, Duren, Germany). For the identification of the fungi, amplification of an internal transcribed spacer (ITS) region using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), ß-tubulin gene using Bt2a (5'-GGTAACCAAATCGGTGCT GCTTTCG-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGCG-3') and calmodulin gene using cmd5 (5'-CCGAGTACAAGGARGCCTTC-3') and cmd6 (5'-CCGATRGAGGTCATRACGTGG-3') were performed [5]. Similarity searches of the DNA sequences were carried out using BLASTn algorithms available at the National Center for Biotechnology Information (NCBI). For the phylogenetic analysis, MEGA7 software was used [6]. A phylogenetic tree was constructed by the neighbor-joining (NJ) method with 1,000 bootstrap replications. Reference sequences of other fungi were obtained from GenBank at NCBI (Table 2).

Taxonomy

Penicillium guanacastense K.G. Rivera, Urb & Seifert, Mycotaxon 119, 315-28 (2012) [7] (Fig. 1 and 2).

Mycobank No.: MB563044

Description:

Colonies on PDA texture floccose to granular, slight radial sulcate, front olive green to dark green, reverse dark brown to orange, 4.5 mm on 7 days at 25℃; **YES** texture velutinous to fasciculate, sulcate, some yellow mycelium, front dark green to pale grayish green, reverse light green to light yellow, 5 mm on 7 days at 25℃; **CYA** texture velutinous, exudate present orange droplet, radial sulcate, front center white, middle whitish green, margin light lemon, reverse dark to light orange, 4 mm on 7 days at 25℃; **CREA** high acid production.

The conidiophore monoverticillate, smooth-walled, short stipes, hyaline; **phialide** 9.3-12 µm length × 3.8-4.46 µm width, cylindrical; **conidia** 2.77-4.47 µm diameter, globose to subglobose.

Phylogenetic section: Sclerotiora

Table 2. GenBank accession numbers of isolates includes in this study.

Species	Strain	Genebank accession number		
		ITS	BT	CMD
Penicillium ardesiacum	DTO 093-C1	KM189565	KM088805	KM089190
Penicillium arianeae	DTO20B8	KC773833	KC773784	KC773811
Penicillium bilaiae	NRRL 3391	JN714937	JN625966	KC773811
Penicillium cainii	DAOM 2399914	JN686435	KC773787	KC773814
Penicillium citreonigrum	CBS 454.93	AY157489	KC773790	KC773816
Penicillium crocicola	DTO 104-E2	KM189581	KJ834445	KM089210
Penicillium daejeonium	KACC 46609	JX436489	JX436493	JX436491
Penicillium fuscum	NRRL 3008	AF033411	GQ367513	GQ367539
Penicillium glabrum	CBS:125543	GU981567	GU981619	GQ367545
Penicillium guanacastense	DAOM 239912	JN626098	JN625967	JN626010
Penicillium guanacastense	NNIBRFG1410	MT995061		
Penicillium herquei	CBS 336.48	JN626101	JN625970	JN626013
Penicillium hirayamae	CBS 229.60	JN626095	JN625955	JN626003
Penicillium jacksonii	DAOM 239937	JN686437	JN686368	JN686391
Penicillium jamesonlandense	CBS 102888	NR_119570	KY989126	KJ866985
Penicillium johnkrugii	DAOM 239943	JN686447	JN686378	JN686401
Penicillium jugoslavicum	CBS 192.87	KC773836	KC773789	KC773815
Penicillium kojigenum	CBS 345.61	NR_121253	KJ834463	KJ867011
Penicillium lividum	DTO 105-H6	KM189582	KM088825	KM089211
Penicillium madriti	CBS 347.61	MH858079	KJ834470	EU644076
Penicillium malachiteum	CBS 647.95	KC773838	KC773794	KC773820
Penicillium mallochii	DAOM 239917	JN626104	JN625973	JN626016
Penicillium montanense	DTO 090-I6	KM189551	KM088789	KM089174
Penicillium odoratum	CBS 294.62	KC411730	KJ834478	KM089363
Penicillium osmophilum	CBS 462.72	MH860531	AY674376	KU896846
Penicillium palmense	CBS 336.79	KJ834515	GQ367508	GQ367534
Penicillium quercetorum	DTO 091-A5	KM189556	KM088795	KM089180
Penicillium restingae	CMR H-12	KF803355	KF803349	KF803352
Penicillium samsonianum	AS3.15403	KJ668590	KJ668582	KJ668586
Penicillium saturniforme	AS3.6886	EU644081	EU644080	EU644062
Penicillium saturniforme	NNIBRFG5702	MT995063		
Penicillium scabrosum	CBS 683.89	KM023349	KM023336	FJ530987
Penicillium scabrosum	NNIBRFG1470	MT995062		
Penicillium sclerotiorum	CBS 287.36	JN626132	JN626001	JN626044
Penicillium spinulosum	NRRL 1750	AF033410	KJ834493	GQ367524
Penicillium swiecickii	CBS 119391	NR_121254	KJ834494	KJ866993
Penicillium thiersii	CBS 117503	MH863022	KJ834497	AY741726
Penicillium thomii	DTO 091-A9	KM189560	KM088799	KM089184
Penicillium vanoranjei	CBS 134406	KC695696	KC695686	KC695691
Penicillium viticola	FKI 4401	AB606414	AB540174	AB540173

ITS: Internal transcribed spacer; BT: ß-tubulin gene; CMD: Calmodulin gene

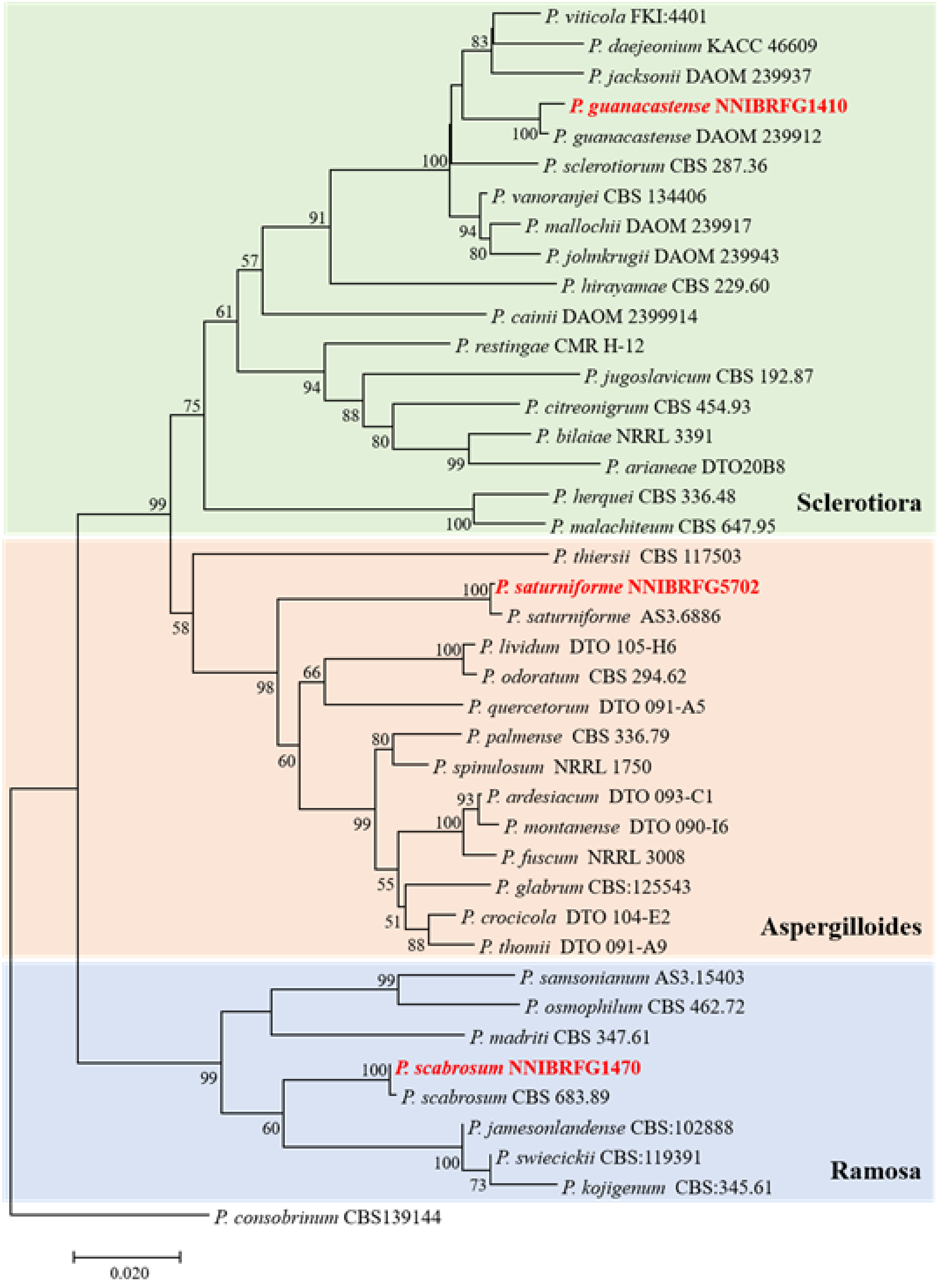

Fig. 1. Phylogenetic analysis using the neighbor-joining method based on internal transcribed spacer (ITS) region, ß-tubulin and calmodulin gene sequences of three *Penicillium* species. *P. consobrinum* was used to an outgroup. Bootstrap values more than 50% (1,000 replications) are shown at branches. The new isolates from the present study are shown in bold and red.

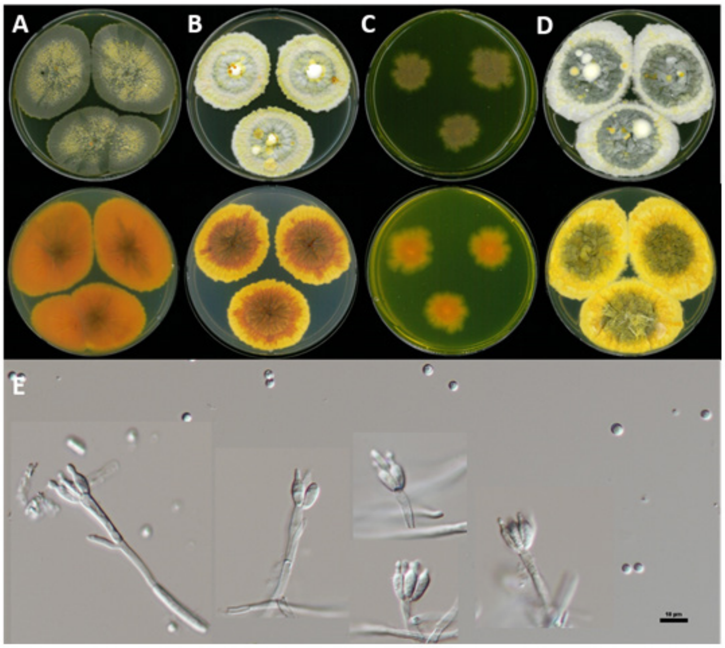

Fig. 2. *Penicillim guanacastense* NNIBRFG1410. Colony shape on A, potato dextrose agar (PDA); B, Czapek yeast autolysate agar (CYA); C, creatine sucrose agar (CREA); D, yeast extract sucrose (YES); E, conidiophores and conidia. Scale bar is 10 μm.

Habitat: Soil in stream

Specimen examined: Namsaengi-mot, Chocheon-uep, Jeju-do, Republic of Korea, 22 Mar 2016, NNIBRFG1410, Nakdonggang National Institute of Biological Resources

Note: NNIBRFG1410 is phylogenetically closely related to *P. guanacastense* within Sclerotiora clade. The molecular data of NNIBRFG1410 is identical to the strain of *P. guanacastense*,with high similarity in the ITS, ß-tubulin and calmodulin sequence data, respectively. We confirm NNIBRFG1410 as *P. guanacastense* based on the morphology and phylogeny, and report the freshwater environment. The morphology of NNIBRFG1410 and Ref. species are similar conidiophore, philade and conidia (Table 3).

Table 3. The morphology of *Penicillium* species.

Taxa	*Penicillium guanacastense*		*Penicillium saturniforme*		*Penicillium scabrosum*	
	NNIBRFG1410	Ref [7]	NNIBRFG5702	Ref [9]	NNIBRFG1470	Ref [10]
Conidiophore	monoverticillate, smooth-walled, short stipes, hyaline	monoverticillate, stipes septate, smooth to finely roughened,	mono or biverticillate, smooth-walled	mono or biverticillate, finely roughwalled, commonly with swollen apices	terverticillate, smooth-walled	bi- and terverticillate, a comparatively short
Rami					6.5–11.3 × 2.85–3.8 μm	10-20 × 2.5-4.0 μm
Metulae			10.15–13.18 × 3.3–3.79 μm	10–20 × 3.5–4.5 (–5.4) μm	6.19–8.6 × 2.65–3.3 μm	15-25 × 2.5-4.0 μm,
Phialide	9.3–12 x 3.8–4.46 μm , cylindrical	7–10(–16) × 2–3(– 4) μm, ampulliform with a collula	6.52–7.23 × 3.03–3.49 μm, cylindrical-shaped	7–11 × (2.0–) 2.5–3.5(–4.0) μm, ampulliform with short collula	7.6–9.4 × 2.3–2.6 μm, flash-shaped	7-11 × 2.0-2.5 μm, well-defined, abruptly narrowed collulum
Conidia	2.77–4.47 μm diameter, globose to subglobose	2.5–3.5 × 2–2.5 μm, globose to subglobose, finely roughened	3.19–3.94 μm diameter, globose to subglobose	(3.0–) 3.5–4.0 μm, sphaeroidal, ovoid to ellipsoidal	6.19–8.6 μm diameter, globose to subglobose	2.4-3.2 μm, globose to subglobose, rough-walled

Penicillium saturniforme (L. Wang & W.Y. Zhuang) Houbraken & Samson, Studies in Mycology 70: 48 (2011)[8] (Fig. 1and 3).

≡***Eupenicillium saturniforme*** L. Wang & W.Y. Zhuang, Mycopathologia 167 (6): 300 (2009).

Mycobank No.: MB541663

Description:

Colonies on PDA texture velutinous, radial slightly sulcate, front olive green to white, reverse pale yellow, 3 mm on 7 days at 25℃; **YES** texture floccose to fasciculate, slightly radial sulcate, front dark green to white, reverse light yellow, 3.5 mm on 7 days at 25℃; **CYA** texture floccose to granular, front dark green to white, reverse pale yellow, 2.5 mm on 7 days at 25℃

The conidiophore mono or biverticillate, smooth-walled; metulae 10.15-13.18 μm length×3.3-3.79 μm width; **phialide** 6.52-7.23 μm length×3.03-3.49 μm width, cylindrical-shaped; **conidia** 3.19-3.94 μm diameter, globose to subglobose.

Phylogenetic section: Aspergilloides

Habitat: Soil in the river

Specimen examined: Soyang-river, Chuncheon-si, Gangwon-do, Republic of Korea, 03 May 2018, NNIBRFG5702, Nakdonggang National Institute of Biological Resources

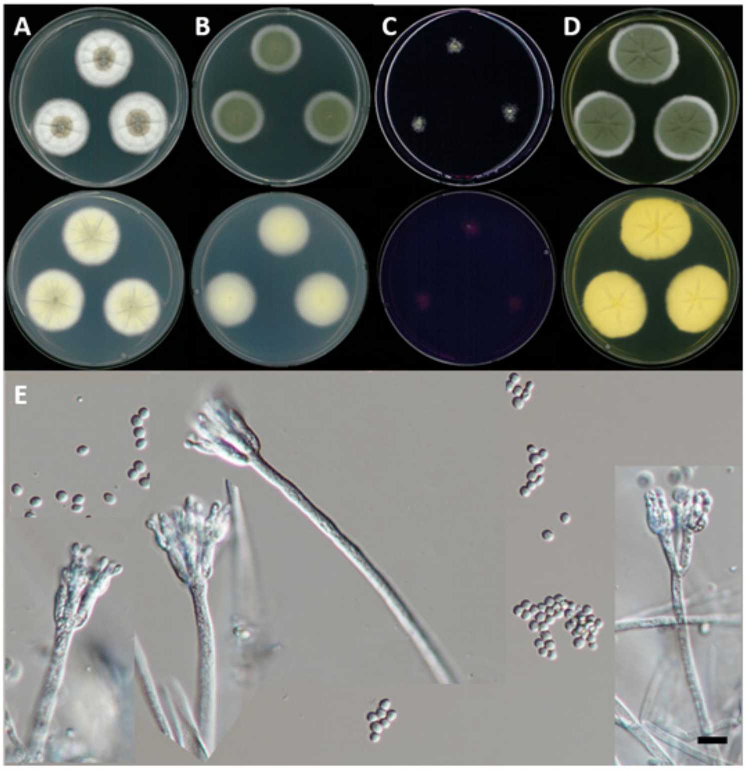

Fig. 3. ***Penicillim saturniforme*** **NNIBRFG5702.** Colony shape on A, potato dextrose agar (PDA); B, Czapek yeast autolysate agar (CYA); C, creatine sucrose agar (CREA); D, yeast extract sucrose (YES); E, conidiophores and conidia. Scale bar is 10 μm.

Note: NNIBRFG5702 is phylogenetically closely related to *P. saturniforme* within Aspergilloides clade. The molecular data of NNIBRFG5702 is identical to the strain of *P. saturniforme*,with high similarity in the ITS, ß-tubulin and calmodulin sequence data, respectively. We confirm NNIBRFG5702 as *P. saturniforme* based on the morphology and phylogeny, and report the freshwater environment. The morphology of NNIBRFG5702 and Ref. species are similar conidiophore, matulae, philade and conidia (Table 3).

Penicillium scabrosum Frisvad, Samson & Stolk, Persoonia 14 (2): 177 (1990)[9] (Fig. 1 and 4).

Mycobank No.: MB136735

Description:

Colonies on PDA texture floccose to fasciculate, radial sulcate, front pale green to dark green, reverse dark orange to light orange, 2.5 mm on 7 days at 25℃; **YES** texture floccose to granular, deeply radial sulcate, front grayish green to light moss green, reverse yellow, 2.5 mm on 7 days at 25℃; **CYA** texture floccose to granular, deeply radial sulcate, exudate pale yellow droplet, front light green, reverse dark grayish orange, 3.5 mm on 7 days at 25℃; **CREA** good acid production

The conidiophore terverticillate, smooth-walled; **Rami** 6.5-11.3 µm length×2.85-3.8 µm width; **metulae** 6.19-8.6 µm length×2.65-3.3 µm width; phialide, 7.6-9.4 µm length×2.3-2.6 µm width, flash-shaped; **conidia** 6.19-8.6 µm diameter, globose to subglobose.

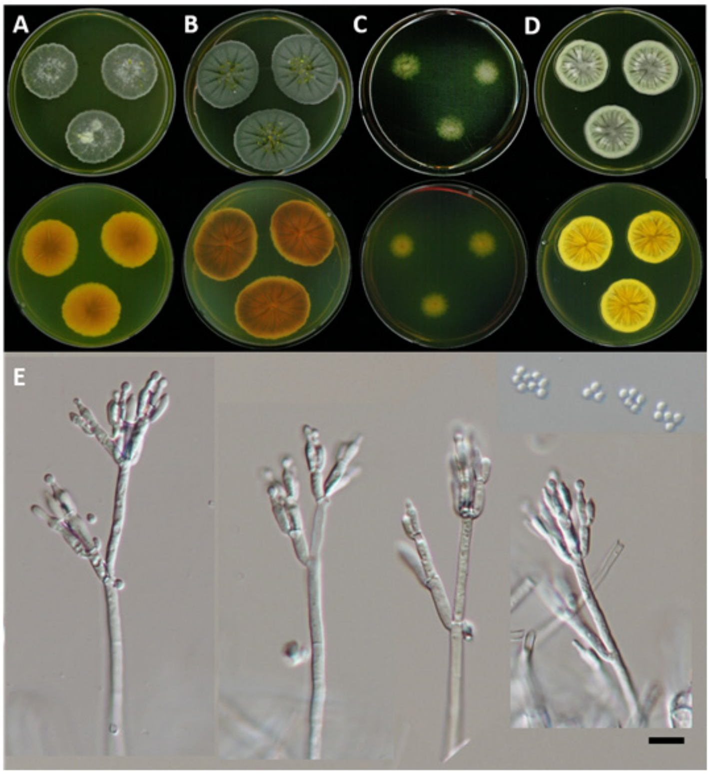

Fig. 4. *Penicillim scabrosum* NNIBRFG1470. Colony shape on A, potato dextrose agar (PDA); B, Czapek yeast autolysate agar (CYA); C, creatine sucrose agar (CREA); D, yeast extract sucrose (YES); E, conidiophores and conidia. Scale bar is 10 µm.

Phylogenetic section: Ramosa

Habitat: Soil in the stream

Specimen examined: Gamcheon, Gimcheon-si, Gyeongsangbuk-do, Republic of Korea, 23 Mar 2016, NNIBRFG1470, Nakdonggang National Institute of Biological Resources

Note: NNIBRFG1470 is phylogenetically closely related to *P. scabrosum* within Ramosa clade. The molecular data of NNIBRFG1470 is identical to the strain of *P. scabrosum*,with high similarity in the ITS, ß-tubulin and calmodulin sequence data, respectively. We confirm NNIBRFG1470 as *P. scabrosum* based on the morphology and phylogeny, and report the freshwater environment. The morphology of NNIBRFG1470 and Ref. species are similar conidiophore, rami, matulae and philade. Conidia size of NNIBRFG1470 are bigger than Ref. species (Table 3).

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