

# A New Record of *Penicillium cainii* from Soil in Korea

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**Abstract** Twenty *Penicillium* isolates were recovered during the investigation of fungal community in the soil samples collected from Wando (Jeonnam Province, Korea). Among them, one species was identified and described as *P. cainii* based on phylogenetic analysis of internal transcribed spacer and  $\beta$ -tubulin (BT2) genes and morphological characteristics. This is a first report of *P. cainii* in Korea.

**Keywords** Morphology, *Penicillium cainii*, Sequence analysis, Soil fungi

Highly diverse groups of fungi are presented in soil and have important and complex physiological and ecological functions in ecosystem [1]. *Penicillium* species are detected frequently by using dilution plate techniques in soil analyses [2]. Many *Penicillium* species have been found from different soil resources and varied with soil depth and nutrient conditions [3-6]. Identification of *Penicillium* based on phenotype is useful for species with recognized distinctions; however, it is difficult for closely related species with similar morphology. Molecular approaches have been popular in recent years for the identification, especially phylogenetic analysis using different genes [7, 8]. Twenty *Penicillium* isolates were recovered during the investigation of fungal community in the soil samples from Wando, (Jeonnam Province, Korea). Among them, one species, *P. cainii* was identified based on molecular and morphological characteristics. The species is a new record of *Penicillium* in Korea.

Soil samples were collected from different places in Wando, Jeonnam Province, Korea during the period of

Aug. 2012. Each sample was taken from 10~15 cm depth, kept in sterile polyethylene tubes, and stored at 4°C until examination. Soil fungi were obtained by soil dilution plate method [9]. Soil dilution (100  $\mu$ g/L) using distilled water was spread on dichloran rose bengal chloramphenicol agar and incubated at 25°C for 3~7 days. Individual colonies of filamentous fungi were picked up and pure cultures were transferred in potato dextrose agar (PDA; Difco, Detroit, MI, USA) slant tubes and deposited in the Culture Collection of Chungnam National University (CNU) Fungi Herbarium.

Genomic DNA was isolated from mycelia collected from PDA plate using the method with a modification of Park *et al.* [10]. For the amplification of internal transcribed spacer (ITS) region and  $\beta$ -tubulin (BT2) gene of the *Penicillium* species (isolate CNU 114236), primers ITS5 and ITS4 [11], and primers Bt2a and Bt2b [12] were used respectively. The resulting sequences and relevant sequences available in the GenBank database were initially aligned with the CLUSTAL X program [13]. Sequences of the two genes were combined and completed with manual adjustment. The combined dataset was analyzed using RAxML software [14]. Maximum likelihood analysis was performed using the GTRGAT model of nucleotide substitution. The robustness of tree shown in Fig. 1 was evaluated by 1,000 bootstrap replications. ITS and BT2 gene sequences (accession Nos. KC424615 and KC424616, respectively) of the isolate CNU 114236 were identical to the type strain DAOM 239914 of *P. cainii* [15]. Meanwhile, in the phylogenetic tree, the isolate placed in a clade comprising reference isolates of *P. cainii* with 100% bootstrap values support (Fig. 1). The results indicated that the isolate CNU 114236 was *P. cainii*.

To confirm the molecular result, morphology of the isolate CNU 114236 was determined. Cultural features were

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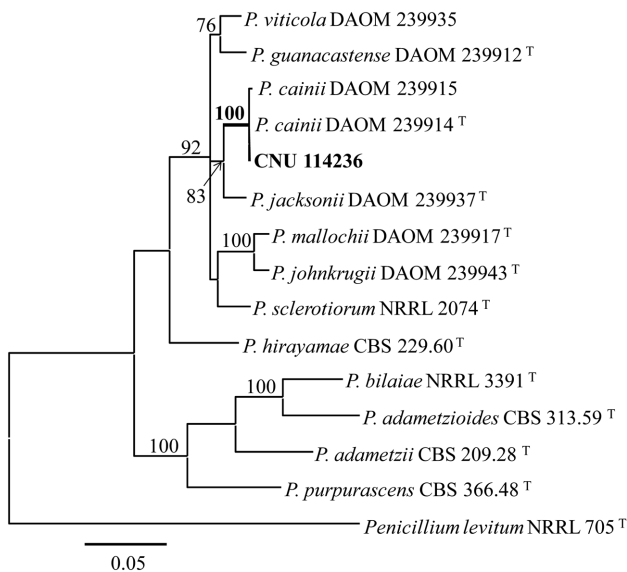
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**Fig. 1.** Maximum likelihood tree for *Penicillium cainii* using RAxML based on a combined internal transcribed spacer and  $\beta$ -tubulin sequence data. Bootstrap values (>70%) of the maximum likelihood analysis are presented at the nodes. The bar indicates the number of substitutions per site. The mark 'T' indicates type strain.

observed on Czapek yeast extract agar (CYA;  $K_2HPO_4$  1 g, Czapek concentrate 10 mg, yeast extract 5 g, sucrose 30 g, agar 15 g, distilled water 1 L), malt extract agar (MEA; Oxoid, Basingstoke, UK), and yeast extract sucrose agar (YES; yeast extract 20 g, sucrose 150 g,  $MgSO_4 \cdot 7H_2O$  0.5 g,

$CuSO_4 \cdot 5H_2O$  0.005 g,  $ZnSO_4 \cdot 7H_2O$  0.01 g, agar 20 g, distilled water 1 L). Three-point inoculated in 9 cm plastic Petri dishes plates using a dense conidial suspension were incubated in the dark at 25°C for 7 days. Conidial morphology on MEA media was measured and compared with the previous description [15]. Morphology of the present isolate agreed with the previous description of *P. cainii*. Morphological structures of the isolate CNU 114236 are shown in Table 1 and Fig. 2.

***Penicillium cainii* K. G. Rivera, Malloch & Seifert, 2011 (Fig. 2).**

**Colonies on CYA grown for 7 days at 25°C (Fig. 2A and B):** 37~40 mm in diameter, dense and velutinous, radially sulcae (8~16 sulcae) with several wrinkles, glaucous grey to pale olivaceous grey with 1~2 mm white mycelia at the margins, golden yellow in the center covered with clear or pale yellow droplets of exudates, soluble pigment not produced, margin entire, reverse yellowish white to golden yellowish.

**Colonies on MEA grown for 7 days at 25°C (Fig. 2C and D):** 36~38 mm in diameter, dense and velutinous, radially sulcate (7~11 sulcate), usually with some wrinkles, sclerotia not produced, grayish sky blue to pale greenish grey with white mycelia at the margins, olivaceous buffer in the center covered with yellowish exudates, soluble pigment not produced, margin entire, reverse pale luteous to orange.

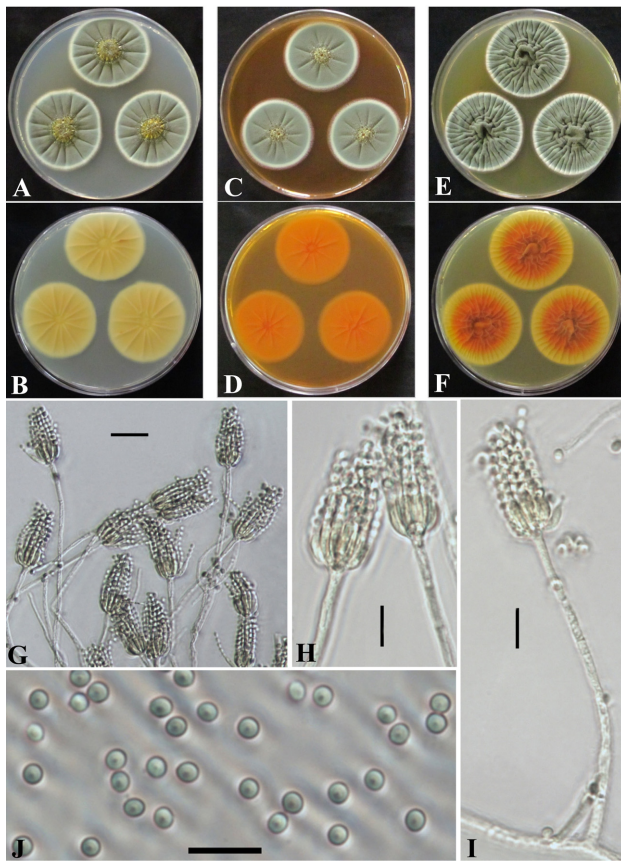
**Colonies on YES grown for 7 days at 25°C (Fig. 2E and F):** 39~42 mm in diameter, dense and velutinous, many irregularly radial sulcae, densely sulcae near the edges with

**Table 1.** Comparison of cultural and morphological characteristics between the present isolate CNU 114236 and *Penicillium cainii* described previously

	Present isolate	<i>P. cainii</i> <sup>a</sup>
Colony		
CYA	Glaucous grey to pale olivaceous grey with 8~16 sulcae, yellow exudates produced, reverse yellow-white to golden-yellow	Deep glue to deep green with 14~18 sulcae, yellow exudates produced by some strains, reverse golden yellow and brown-yellow
MEA	Grayish sky blue to pale greenish grey with 7~11 sulcae, yellowish exudates produced, reverse pale luteous to orange	Greenish grey and dull green, exudates not produced, orange to dark orange
YES	Glaucous grey to olivaceous grey with many irregularly radial sulcae, exudates absent, reverse pale luteous to orange	Greenish grey with 21~23 sulcae, negligibly dark orange exudates produced in some strains, reverse brown-orange to red-orange
Size (in diameter)	37~44 mm on CYA 36~38 mm on MEA 39~42 mm on YES	23~29 mm on CYA 28~35 mm on MEA 31~34 mm on YES
Stipe	Septate, rough-walled, normally 35~85 × 2~3 (~3.3) $\mu$ m, vesicle 3.5~5 (~5.5) $\mu$ m wide	Septate, rough-walled, 70~80 × 2.5~3 $\mu$ m, vesicle 3.5~5 $\mu$ m wide
Phialide	Ampulliform, 7~10 (~12) × 1.5~2.5 $\mu$ m	Ampulliform, 7.5~10 × 2~3 $\mu$ m
Conidia	Subglobose to globose, smooth-walled or finely roughened, 2~3 (~3.5) $\mu$ m in diam.	Globose, finely roughened, 2~2.5 $\mu$ m in diam.

CYA, Czapek yeast extract agar; MEA, malt extract agar; YES, yeast extract sucrose agar.

<sup>a</sup>Sources of description [15].



**Fig. 2.** *Penicillium cainii* CNU 114236. Colonies grown on Czapek yeast extract agar (A, B), malt extract agar (C, D), and yeast extract sucrose agar (E, F) for 7 days at 25°C, conidiophores (G~I), conidia (J) (scale bars: G = 20  $\mu$ m, H~J = 10  $\mu$ m).

many wrinkles, glaucous grey to olivaceous grey with 1~2 mm white mycelia at the margins, exudates absent, soluble pigment not produced, margin entire, reverse pale luteous to orange.

Conidiophores (Fig. 2G~I) were mostly monoverticillate on MEA, arising from felted hyphae or agar surface. Stipes were simple, septate, rough-walled, normally 35~85  $\times$  2~3 (~3.3)  $\mu$ m, sometimes over 100  $\mu$ m long, with vesicles 3.5~5.0 (~5.5)  $\mu$ m wide. Phialides were ampulliform, 7~10 (~12)  $\times$  1.5~2.5  $\mu$ m with conspicuous neck 1~2.5  $\times$  0.5~1  $\mu$ m in size. Conidia (Fig. 2J) were born in chains, subglobose to globose, smooth-walled or finely roughened, 2~3 (~3.5)  $\mu$ m in diameter.

**Culture examined.** CNU 114236, isolated from soil samples, Wando, Jeonnam Province, Korea.

**Note:** Rivera and Seifert [15] designated the species in the *Penicillium sclerotiorum* complex. They also demonstrated that the species was easily recognized by short and rough-stiped conidiophores combined with the association of host plants in the family Juglandaceae. However, the present isolate was collected from soil and produced yellowish

exudates in the center of colonies on MEA media. Phylogenetically, the species is closely related to *Penicillium jacksonii* but is distinguished by the conspicuously roughened conidiophores [15]. The fungus is only known from nuts of *Juglans nigra* and *Carya ovata* in Canada. It was firstly reported from soil in Korea.

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