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Two New Records of *Penicillium* Associated with Blue Moldy Bulbs of Lily in Korea

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Two new records of *Penicillium* from blue moldy bulbs of lily are reported in Korea. The Korean isolates of *P. albocoremium* (Frisvad) Frisvad and *P. tulipae* Overy and Frisvad were phylogenetically identical to the reference species based on DNA sequence of the β-tubulin gene. *P. albocoremium* and *P. tulipae* are described and illustrated.

KEYWORDS: Colony characteristics, Micromorphology, β-Tubulin gene, *Penicillium albocoremium, Penicillium tulipae*

The large number of species which constitute the genus *Penicillium* occupy a wide spectrum of habitats in our environment. As a consequence, many have become economically important in either harmful or useful roles. Some species cause deterioration of wide range of stored products and decay of bulbs and root vegetables (Frisvad and Samson, 2004).

The genus *Penicillium* contains approximately 225 species, among which 45 species are associated with the teleomorph genus *Eupenicillium*, and 24 with *Talaromyces* (Pitt *et al.*, 2000). Recently, 17 additional species have been reported (Peterson *et al.*, 1999; Kong, 2000; Peterson and Sigler, 2002; Kong and Liang, 2003; Peterson *et al.*, 2003; Peterson, 2004; Wang *et al.*, 2004; Frisvad and Samson, 2004). Of the four subgenera of *Penicillium*, subgenus *Penicillium* now consists of 58 species with a high diversity of morphology and production of secondary metabolites (Frisvad and Samson, 2004). Most of these species occur on food, feeds, bulbs or root vegetables.

Identification of *Penicillium* species is not easy. Many common species look alike to the uninitiated. At the same time, there is a great deal of variability within the species. In recent years, molecular approaches have been used increasingly in identification and phylogenetic classification of filamentous fungi and their application has led to the reconsideration of several genera (Bruns *et al.*, 1991). In the genus *Penicillium*, the region spanning of the nuclear ribosomal internal transcribed spacer (ITS 1, ITS 2 and 5.8S rDNA) has been investigated to clarify the subdivision within the genus and to evaluate phylogenetic relationship of some species. However, the ribosomal DNA gene has too few informative differences to reveal the phylogeny of *Penicillium*. Among the protein-coding genes, β-tubulin gene has proven useful for identification of closely related *Penicillium* species (Seifert and Louis-Seize, 2000; Samson *et al.*, 2004).

During our studies on the genus *Penicillium* from Korea, we encountered many species of *Penicillium* previously unreported in Korea. In this paper, we report on two new records of *Penicillium* from blue moldy bulbs of lily that produce terverticillate penicilli.

Materaials and Methods

Isolation. *Penicillium* isolates were isolated from blue moldy bulbs of lily (*Lilium longiflorum* Thumb.) collected from Taean area, Chungnam Province, Korea. The conidia assumed to be *Penicillium* were picked up from blue molds of bulbs and transferred to malt extract agar (MEA; malt extract 20 g, peptone 1.0 g, glucose 20 g, agar 20 g, distilled water 1 liter) and grown for 7 days at 25°C.

Culture. Isolates were then three point inoculated onto Czapek yeast extract agar (CYA; K_2HPO_4 1.0 g, Czapek concentrate 10 mg, yeast extract 5 g, sucrose 30 g, agar 15 g, distilled water 1 liter), MEA and 25% glycerol nitrate agar (G25N; K_2HPO_4 0.75 g, Czapek concentrate 7.5 *ml*, yeast extract 3.7 g, glycerol 250 g, agar 12 g, distilled water 750 *ml*). Colony appearance, exudate production, pigmentation and reverse coloration were assessed and colony diameters were measured and recorded after one week of growth at 25°C.

DNA extraction. The isolates were grown in liquid shake culture in potato dextrose broth medium for 3~4 days at 25°C. Mycelia were collected from the cultures by filtration and transferred to 1.5 *ml* tubes. These samples

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were frozen at −70°C. DNA was extracted by method of Cubero *et al.* (1999).

PCR amplification and sequencing. For amplification of the β-tubulin gene, primers Bt2a (5'-GGTAAC-CAAATCGGTGCTGCTTTC-3') and Bt2b (5'-ACCCT-CAGTGTAGTGACCCTTGGC-3') (Glass and Donaldson, 1955) were used. PCR mixture contained 0.5 pmol of each primer, 0.2 mM of dNTP's, 10 mM Tris-HCl, 50 mM KCl, 1.5 $MgCl_2$, 2.5 U *Taq* polymerase and 15 ng of template DNA. PCR cycling conditions were as follow: an initial denaturation step of 94°C for 4 min followed by 25 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min. A final elongation step of 72°C for 10 min was performed.

The PCR product was purified by using a Wizard PCR prep kit (Promega, Madison, WI, U.S.A.). Purified double stranded PCR fragments were directly sequenced with BigDye terminator cycle sequencing kits (Applied Biosystems, Forster City, CA, U.S.A.) following to the manufacturer's instructions. Same primer sets with PCR amplification were used to sequence both DNA strands. The gel electrophoresis and data collection were performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Forster City, CA, U.S.A.). The sequences were proofread, edited and merged into comparable sequences using the PHYDIT program version 3.0 (Chun, 1995; available at http://plaza.sun.ac.kr/~jchun/phydit). Sequences generated from materials in this study and retrieved from GenBank were initially aligned using the program CLUSTAL X (Thompson *et al.*, 1997), and then alignment was refined manually using the PHYDIT program version 3.0 (Chun, 1995). Ambiguously aligned regions were excluded from the following analyses.

Results and Discussion

Sequence analysis of β-tubulin gene. The partial β-tubulin gene from 7 isolates of *Penicillium* from blue moldy bulbs of lily in Korea were amplified. Amplification of the β-tubulin gene with primers Bt2a and Bt2b yields fragment of approximately 500 bp. BLAST database searches were performed with partial β-tublin gene as queries to reveal relationship to published sequences. In a distance analysis with neighbor-joining method, sequences of CNU-05002, CNU-05042, CNU-05044 and CNU-05050 isolates were 100% identical to those of *P. albocoremium* CBS 472.84, with a bootstrap value of 63% (Fig. 1, Table 1). Isolates CNU-05024, CNU-05025, CNU-05026 and *P. tulipae* CBS 109555 were belonged to the same group. Sequence similarity among them was 100%, which was supported by a bootstrap values of 81% (Fig. 1, Table 1). Sequence similarity among *P. albocoremium*, *P. tulipae* and related species ranged from 96.2~98.9%.

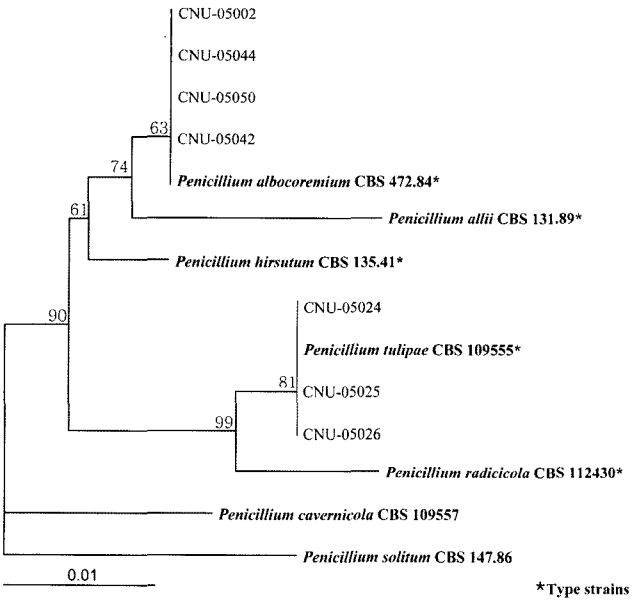

Fig. 1. Neighbor-joining tree based on phylogenetic analysis of β-tubulin gene sequences. The number below each branch indicate bootstrap values of distance. The bootstrap values were obtained after a bootstrap test of 1000 replications. References, which were taken from GenBank database, are in bold type.

The β-tubulin gene has been widely used for phylogenetic analysis in fungi. The amount of variation is suitable for studying phylogenetic relationships among closely related species of *Penicillium* (Samson *et al.*, 2004) and filamentous fungi (Glass and Donaldson, 1995). In this study, the phylogenetic tree inferred from the sequences of β-tubulin gene correlated well with the species that were defined by cultural and morphological characteristics.

Taxonomic description

***Penicillium albocoremium* (Frisvad) Frisvad** **Fig. 2**

Int. Mod. Tax. Meth. Pen. Asp. Clas.: 275, 2000

Syn.: *P. hirsutum* var. *albocoremium* Frisvad, Mycologia 81: 856, 1989.

Colonies on CYA 25~35 mm diam, slightly radially sulcate, surface texture floccose to fasciculate; exudate clear to pale yellow droplets on surface; conidiogenesis moderate to heavy, grey green to dark dull green; reverse dark brownish orange to brownish yellow. Colonies on MEA 30~40 mm diam, plane, texture floccose, exudate clear or pale yellow; conidiogenesis light to moderate, pale green to grey green; reverse brownish orange to reddish orange. Colonies on G25N 25~40 mm diam, plane, texture floccose, exudate pale brown droplets; conidiogenesis moderate to heavy, blue green to grey green; reverse yellow orange to brownish orange.

Table 1. DNA similarity matrix for β-tubulin gene sequences of *P. albocoremium*, *P. tulipae* and their related species

	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	100												
3	100	100											
4	100	100	100										
5	100	100	100	100									
6	97.9	97.9	97.9	97.9	97.9								
7	97.9	97.9	97.9	97.9	97.9	100							
8	97.9	97.86	97.9	97.9	97.9	100	100						
9	97.9	97.9	97.9	97.9	97.9	100	100	100					
10	98.1	98.1	98.1	98.1	98.1	96.5	96.5	96.5	96.5				
11	97.6	97.6	97.6	97.6	97.6	96.8	96.8	96.8	96.8	96.3			
12	98.9	98.9	98.9	98.9	98.9	97.9	97.9	97.9	97.9	97.6	97.6		
13	97.3	97.3	97.3	97.3	97.3	98.66	98.66	98.66	98.7	96	96.3	97.3	
14	97.1	97.1	97.1	97.1	97.1	96.3	96.3	96.3	96.2	95.7	96.8	97.1	95.7

1, *P. albocoremium* CNU-05002; 2, *P. albocoremium* CNU-05042; 3, *P. albocoremium* CNU-05044; 4, *P. albocoremium* CNU-05050; 5, *P. albocoremium* CBS 472.84 (from GenBank database); 6, *P. tulipae* CNU-05024; 7, *P. tulipae* CNU-05025; 8, *P. tulipae* CNU-05026; 9, *P. tulipae* CBS 109555 (from GenBank database); 10, *P. allii* CBS 131.89 (from GenBank database); 11, *P. cavernicola* CBS 109557 (from GenBank database); 12, *P. hirsutum* CBS 135.41 (from GenBank database); 13, *P. radicicola* CBS 112430 (from GenBank database); 14, *P. solitum* CBS 147.86 (from GenBank database).

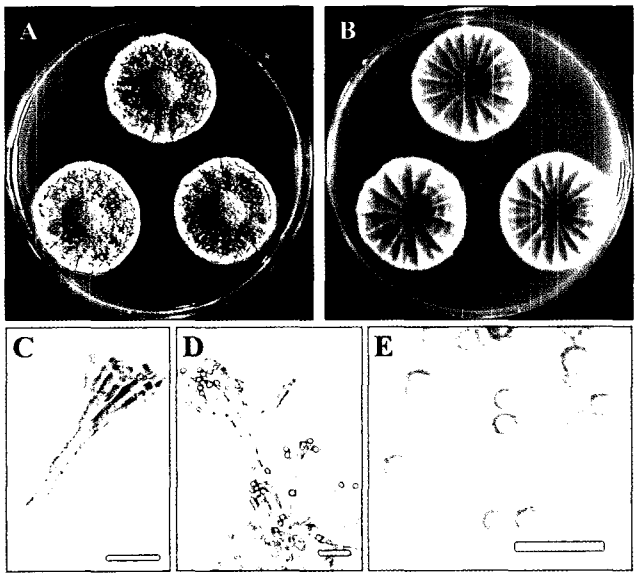

Fig. 2. *Penicillium albocoremium.* (A~B) 7-day-old colonies on CYA, (A) obverse, (B) reverse; (C~D) conidiophores; (E) conidia. Scale bar = 12 μm.

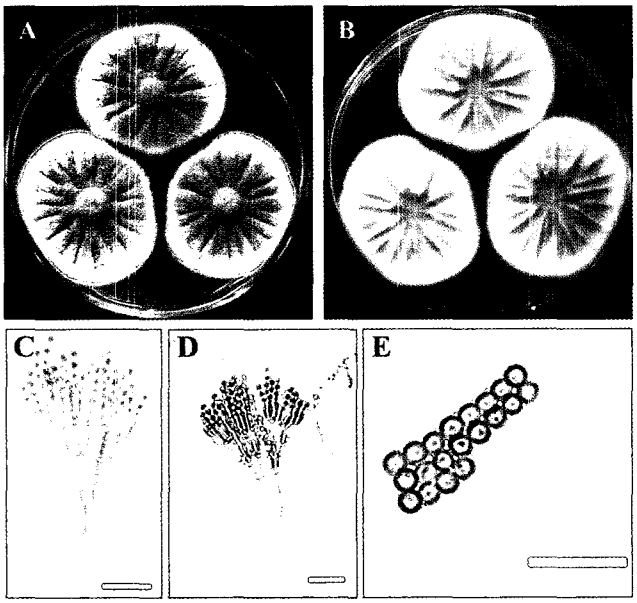

Fig. 3. *Penicillium tulipae.* (A~B) 7-day-old colonies on CYA, (A) obverse, (B) reverse; (C~D) conidiophores; (E) conidia. Scale bar = 12 μm.

Conidiophores borne singly or in fascicles, arising from the surface hyphae, stipes 150~500 μm long or of indeterminate length, very rough and warty, terverticillate or quarterverticillate; rami 10~25 μm long, 3~5 μm wide; metulae cylindrical, 8~19 μm long; phialides 8~12 μm long, with short collula; conidia globose to subglobose, smooth-walled, 3~5 μm.

Habitats: Bulbs of lily.

Materials examined: On bulbs of lily, CNUMH 05002 (15 April 2005), CNUMH 05042 (20 May 2005), CNUMH 05044 (20 May 2005), CNUMH 05050 (05 Oct. 2005).

Note: Colony characteristics and micromorphology of the species agreed well with the description of *P. albocoremium* (Overy and Frisvad, 2003; Frisvad and Samson, 2004). This species is most closely related to *P. tulipae*, but differs by its dark brownish orange reverse on CYA (Frisvad and Samson, 2004), and markedly sulcate colonies on G25N. *P. hirsutum* differs from *P. albocoremium* by its production of deep violet brown exudate on CYA (Frisvad and Samson, 2004). The species has been reported from *Fragaria*, *Apium*, *Allium*, *Zingiber*, cake

and indoor air in Denmark, England, Israel and Slovenia (Frisvad and Samson, 2004). This is the first record of *P. albocoremium* in Korea.

***Penicillium tulipae* Overy and Frisvad** **Fig. 3**

System. Appl. Microbiol. 26: 631, 2003.

Colonies on CYA 35~48 mm diam, radially sulcate, surface texture velutinous to slightly floccose; conidiogenesis very heavy, greyish green to dull green; exudate small, clear to yellow droplets on the surface; reverse light orange to orange in center slowly fading to light yellow to pale yellow. Colonies on MEA 25~38 mm diam, plane or slightly radially sulcate, surface texture velutinous to slightly floccose; conidiogenesis moderate, greyish green; exudate absent; reverse pale yellow to yellowish white. Colonies on G25N 25~35 mm diam, deeply radially sulcate, surface texture velutinous to floccose; conidiogenesis light to moderate, pale green; exudate absent; reverse light orange, orange in center.

Conidiophores arised from subsurface hyphae, stipes 150~1500 μm long, smooth- to finely rough-walled, terverticillate, rarely quarterverticillate; rami cylindrical, 10~25 μm long; metulae cylindrical, 8~15 μm long; phialides ampulliform tapering to a distinct collulum, 7~13 × 2.5~4.0 μm; conidia globose to subglobose, smooth-walled, 3~4.5 μm.

Habitats: Bulbs of lily.

Materials examined: On lily bulbs, CNUMH 05024 (18 Feb. 2005), CNUMH 05025 (18 Feb. 2005), CNUMH 05026 (18 Feb. 2005).

Note: Colony characteristics and micromorphology of the fungus agreed well with the description of *P. tulipae* (Frisvad and Samson, 2004). The fungus has been reported from *Lilium*, *Tulipa*, *Helianthus*, *Beta*, *Brassica*, *Chrysanthemum*, *Apium* and agricultural soil in Denmark, Germany, Korea and Netherlands (Frisvad and Samson, 2004). This is the first record of *P. tulipae* from lily in Korea, although the fungus has been reported from *Tulipa* sp. (Frisvad and Samson, 2004).

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