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Two Species of *Penicillium* Associated with Blue Mold of Yam in Korea

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During 2007 survey of post-harvest diseases of yam performed in May and June, severe tuber loss caused by blue mold was observed in Iksan, Cheonbuk Province. Two species of *Penicillium* were isolated from the infected tubers. Based on β-tubulin gene sequence analysis, and cultural and morphological characteristics, the isolates were identified as *Penicillium sclerotigenum* and *P. polonicum*. *P. sclerotigenum*, which is a novel to Korea, is presently described and illustrated.

KEYWORDS : Blue mold, *P. polonicum*, *P. sclerotigenum*, β-tubulin gene, Yam tuber

Yam (*Dioscorea batatas* Decne) is a perennial vine whose tuber is used for food or as a medicinal supplement (Tae, 1998; Park *et al*., 2005). Yam tubers are harvested in Korea mostly between October and November, and most of which are stored in cold storage chamber. During a survey of yam post-harvest diseases in May and June of 2007, severe loss of tuber caused by *Penicillium* was observed in Iksan, Cheonbuk Province. The fungal pathogens penetrate through wounds in the tubers and infect the inner tissue. First symptom of the blue mold infection is soft, watery, discolored spots of varying size on the tuber surface. When an affected area is cut, severe brown to dark-brown lesions in the flesh tissue are seen (Fig. 1). At room temperature a white mold begins to grow on the surface of the lesions and the lesions rapidly become covered with bluish or blue-green spores and fungal mycelia (Fig. 1). Sporulation seldom develops while tubers are held in cool storage, but can develop after the tubers are keep in room temperature.

Penicillium spp. were isolated from tubers naturally infected with blue mold. They were identified based on analysis of β-tubulin gene sequence (Seifert and Louis-Seize, 2000; Samson *et al*., 2004), and morphological and cultural characteristics. In this paper, we report on two species of *Penicillium* associated with blue mold of the yam. One species has not previously been reported in Korea.

Materials and Methods

Isolation. Isolates of *Penicillium* spp. were obtained from yam tubers with blue mold collected from cold storage chamber in Iksan City, Cheonbuk Province, Korea (Fig. 1.). The conidia assumed to be *Penicillium* were picked up from blue molds of tuber 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 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 yeast extract sucrose agar (YES; sucrose 52.5 g, $MgSO_4 \cdot 7H_2O$ 0.175 g, $CuSO_4 \cdot 5H_2O$ 0.00175 g, $ZnSO_4 \cdot 7H_2O$ 0.0035 g, yeast extract 7 g, agar 7 g, distilled water 350 *ml*). Colony appearance, exudate production, pigmentation, and reverse coloration were assessed, and colony diameters were measured and recorded after a week of growth at 25°C.

DNA extraction. The isolates were cultured in potato dextrose broth for 3~4 days at 25°C with shaking. Mycelia were collected from the cultures by filtration and transferred to 1.5 *ml* tubes. These samples were frozen at −70°C. DNA was extracted as described previously (Cubero *et al*., 1999).

Polymerase chain reaction (PCR) amplification and sequencing. For amplification of the β-tubulin gene, primers Bt2a (5'-GGT AAC CAA ATC GGT GCT GCT TTC-3') and Bt2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3') (Glass and Donaldson, 1995) were used. The PCR mixture contained 0.5 pmol of each primer, 0.2 mM of dNTP's, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U *Taq* polymerase, and 15 ng of template DNA. PCR cycling conditions were as follows: an initial denaturation step of 94°C for 5 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 was performed for 10 min. The PCR product was purified using a Wizard PCR prep

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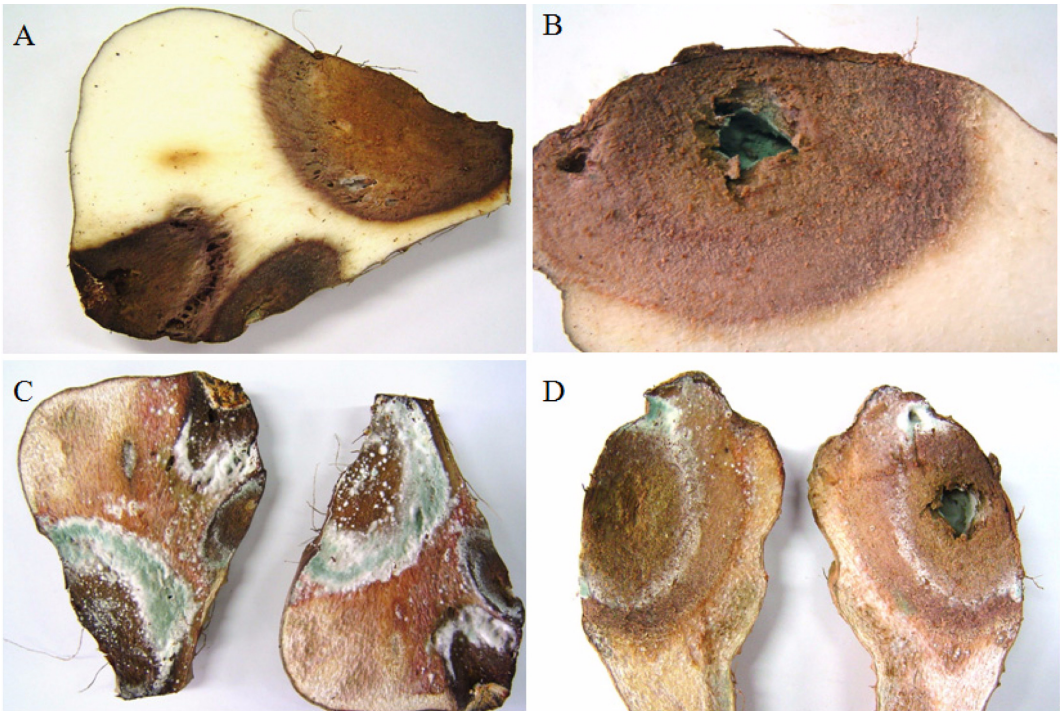

Fig. 1. Brown to dark-brown lesions in the flesh of the yam tuber (A, B), and whitish mycelia and bluish or blue-green spores that developed on the infected tissues after 2 days of incubation at room temperature (C, D).

kit (Promega, Madison, WI, USA). Purified double-stranded PCR fragments were directly sequenced with a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. The same primer sets as were used in PCR amplification were used to sequence both DNA strands. Gel electrophoresis and data collection were performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The sequences were proofread, edited, and merged into comparable sequences using the PHYDIT program version 3.2 (Chun, 1995). Sequences generated from materials in this study and retrieved from GenBank were initially aligned using the CLUSTAL X program (Thompson *et al*., 1997), and then alignment was refined manually using the PHYDIT program version 3.2. Ambiguously aligned regions were excluded from the following analyses.

Results and Discussion

Sequence analysis of the β-tubulin gene. The partial β-tubulin gene from four *Penicillium* isolates obtained from blue mold infected yam during a 2007 survey in Korea was amplified. Amplification with primers Bt2a and Bt2b yielded a β-tubulin gene fragment of approximately 500 bp. BLAST database searches were performed with the determined nucleotide sequences of the partial β-tubulin gene as queries to reveal relationship to published sequences. In a distance analysis with neighbor-joining method, sequences of three isolates including CNU-079938 were 100% identical to those of *P. sclerotigenum* CBS 101033, with a bootstrap value of 100% (Fig. 2 and Table 1). Isolate CNU-079937 and *P. polonicum* CBS 690.77 were determined to belong to the same group. Sequence similarity among them was 100% (Fig. 2 and Table 1).

The use of the β-tubulin gene for phylogenetic analysis in some fungi is both popular and appropriate. The amount of variation is suitable for studying phylogentic relationships among closely related species of *Penicillium* (Seifert and Louis-Seize, 2000; 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. Taxonomic description, photos of colonies, and fungal structures of *P. sclerotigenum*, which is novel to Korea are given below and in Fig. 3.

***Penicillium sclerotigenum* Yamamoto** (Scient. Rep. Hyogo Univ. Agric., Agric. Biol. Ser. 2, 1: 69, 1955)
Isolates that were examined on yam tuber were CNU 079938, CNU 079940, CNU 079941 (7 June, 2007). Colonies on CYA were 45~65 mm in diameter, slightly radially sulcate, veltinous in surface texture; devoid of exudates; possessed conidiogenesis moderate to heavy, dull green; and the reverse of the colonies was light

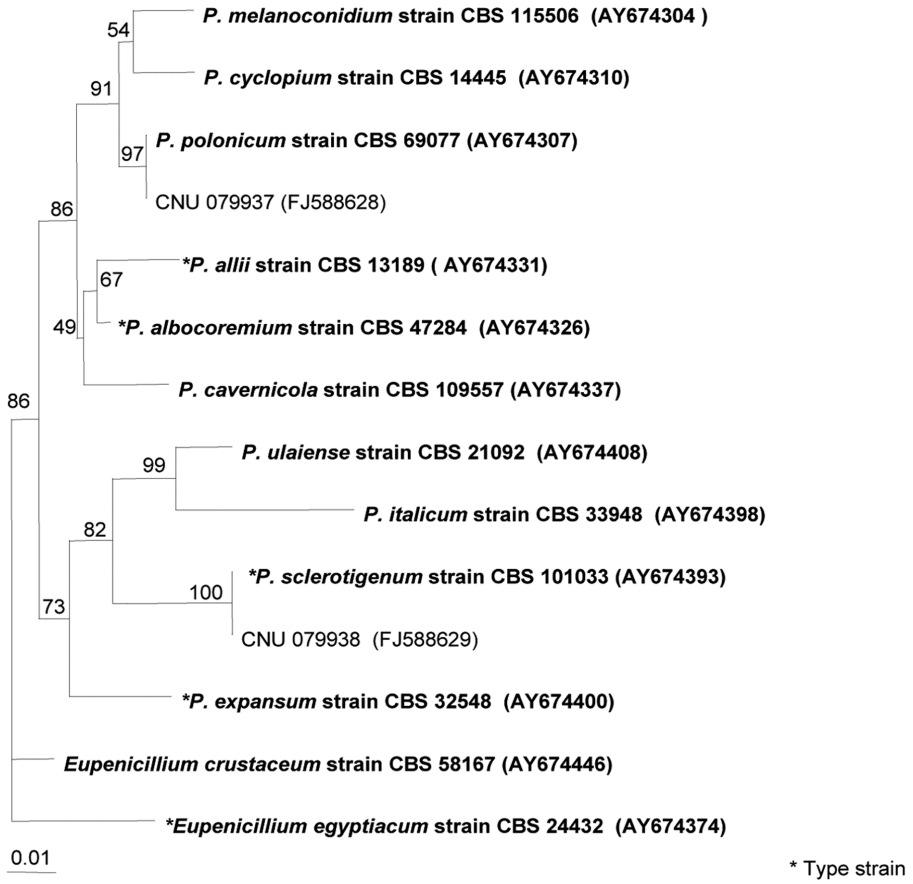

Fig. 2. Neighbor-joining tree based on phylogenetic analysis of β-tubulin gene sequence. The number above each branch indicates bootstrap values of distance. The bootstrap values were obtained after a bootstrap test of 1000 replications. Numbers in parentheses are GenBank accession numbers.

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

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	–													
2	96.3	–												
3	93.8	95.3	–											
4	93.3	94.8	94.1	–										
5	94.0	95.8	95.1	93.6	–									
6	93.8	95.6	94.6	93.3	96.3	–								
7	95.8	97.1	95.8	94.6	97.8	98.1	–							
8	93.5	95.8	94.6	93.6	96.1	95.8	96.8	–						
9	93.2	96.0	94.8	93.0	95.8	95.6	96.6	97.5	–					
10	90.8	92.8	92.9	95.3	91.6	91.1	92.1	90.6	90.8	–				
11	93.5	95.3	94.8	95.1	93.6	93.6	94.8	92.8	92.8	93.1	–			
12	93.5	95.3	94.8	95.1	93.6	93.6	94.8	92.8	92.8	93.1	100.0	–		
13	94.5	96.3	95.3	94.3	96.6	96.8	97.8	97.6	98.3	91.6	93.8	93.8	–	
14	94.5	96.3	95.3	94.3	96.6	96.8	97.8	97.6	98.3	91.6	93.8	93.8	100.0	–

1, *Eupenicillium egyptiacum* CBS 24432; 2, *E. crustaceum* CBS 58167; 3, *Penicillium expansum* CBS 32548; 4, *P. ulaiense* CBS 21092; 5, *P. cavernicola* CBS 109557; 6, *P. allii* CBS 13189; 7, *P. albocoremium* CBS 47284; 8. *P. melanoconidium* CBS 115506; 9, *P. cyclopium* CBS 14445; 10, *P. italicum* CBS 33948; 11, *P. sclerotigenum* CBS 101033; 12, CNU 079938, 13, *P. polonicum* CBS 69077; 14, CNU-079937.

brown with a darker-brown center. Colonies on MEA were 40~50 mm in diameter, planar, floccose in texture, devoid of exudates; conidiogenesis light to moderate, green; and the reverse of the colonies was brownish-orange to reddish-orange. Colonies on YES were 53~80 mm in diameter, planar, floccose in texture, devoid of exudates; conidiogenesis moderate to heavy, dull green; and the reverse of the colonies was light brown with a

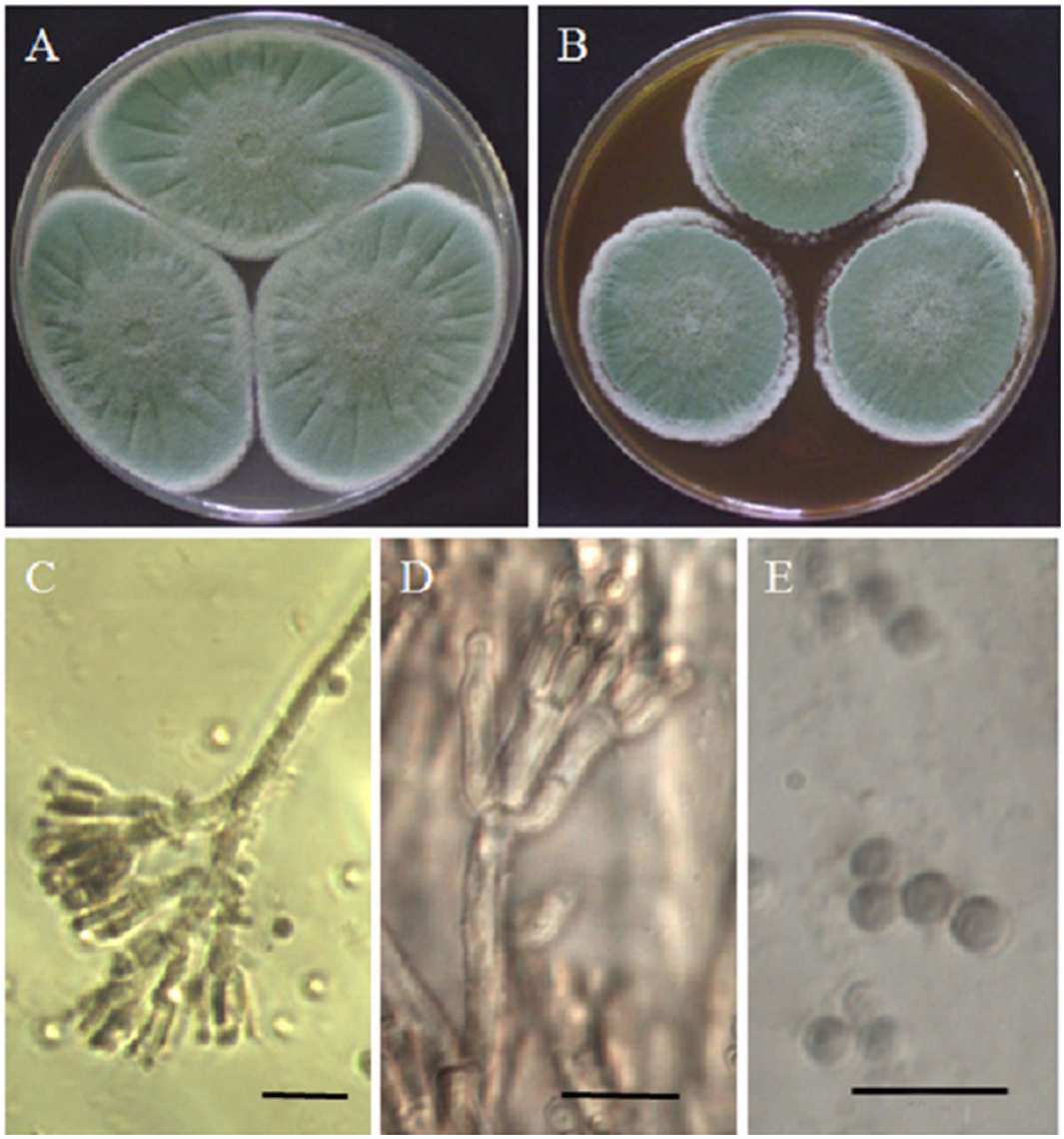

Fig. 3. *Penicillium sclerotigenum*. Colonies on CYA (A) and MEA (B) after 7 days of incubation. (C, D). Photomicrographs of conidiophores and conidia. (E) Photomicrograph of conidia. Scale bar = 20 μm (C), 15 μm (D), 10 μm (E).

darker-brown center. Conidiophores were borne from surface hyphae, stipes were 200~650 μm long, rough-walled, biverticillate, and terverticillate; rami were 17~25 μm long, 3~4 μm wide; metulae were cylindrical, 15~25 μm long; phialides were 8~12 μm long, smooth-walled with ellipsoidal conidia ellipsoidal, and dimensions of 4~5 μm × 2.5~3.5 μm. Colony characteristics and micromorphology of the species agrees well with the previous description of *P. sclerotigenum* (Frisvad and Samson, 2004). This species is most closely related to *P. expansum*, but differs in terms of poor growth on CREA and presence of numerous biverticillate penicilli (Frisvad and Samson, 2004). The species has been reported as the cause of blue mold of yam tubers in Japan (Anonymous, 2000), and from yam tubers and yam products in Philippines, Taiwan, Russia, and Jamaica (Frisvad and Samson, 2004). This is the first record of *P. sclerotigenum* in Korea.

***Penicillium polonicum* K. Zaleski** (Bull. Int. Acad. Pol. Sci. Lett., Ser. B. 1927: 445)

The isolate examined on yam tuber was CNU 079937 (7 June, 2007). Colony characteristics and micromorphology of this fungus agree well with a previous description of *P. polonicum* (Frisvad and Samson, 2004; Yu, 2006). This is the first record of *P. polonicum* from a yam tuber in Korea, although the fungus has been reported from citrus fruits in Korea (Yu, 2006). Whether the fungus is the cause of the disease remains unclear.

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