

Six Species of *Penicillium* Associated with Blue Mold of Grape

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Grape fruits with blue mold symptoms were collected from house storages in different locations in Korea and were investigated for their association with *Penicillium* species. A total of 12 isolates of *Penicillium* were isolated from the collected fruits. Based on morphological and cultural characteristics and β -tubulin gene sequence data analysis, they were identified as *P. bialowiezense*, *P. citrinum*, *P. echinulatum*, *P. expansum*, *P. solitum* and unidentified *Penicillium* species. *P. solitum* was the predominant followed by *P. expansum*. *P. bialowiezense* and *P. echinulatum* were newly recorded in Korea. β -Tubulin gene sequences could be used to distinguish each species of *Penicillium* and the molecular groups were correlated well with the morphological species. The unidentified species was supposed to be a new species, not previously reported in literature.

KEYWORDS: Blue mold, Colony characteristics, Grape, Morphological characteristics, *Penicillium*, β -Tubulin gene

During the storage period, fruits of grape may undergo various attacks from insects and microorganisms like fungi, yeasts and bacteria. *Penicillium* species are a major cause of deterioration and decay amongst a wide range of post harvest plant products, particularly fruits such as grape, oriental pear and citrus.

Penicillium of Korea are insufficiently known. The published Korean literature on the genus is scattered and fragmentary and pertains to about 60 species (Anonymous, 2004; Cho *et al.*, 2005; Lee *et al.*, 2003). Most of them are soil-borne and only 10 species have been reported to be associated with storage diseases of plant products (Anonymous, 2004; Kim *et al.*, 2002; Oh *et al.*, 1999).

Identification of *Penicillium* is not easy. It is a large genus, and many common species look alike to the uninitiated. At the same time there is a great deal of variability within the species, therefore, unambiguous identification of the species requires molecular identification (Guerche *et al.*, 2004). Among the molecular tools available, β -tubulin gene has proven useful for identification of closely related *Penicillium* species (Kim *et al.*, 2006; Seifert and Louis-Seize, 2000; Samson *et al.*, 2004).

The objectives of this study were to clearly identify *Penicillium* isolates from blue mold of grapes, by using two approaches; the analysis of morphological and cultural characteristics and the analysis of their β -tubulin gene sequences.

Materials and Methods

Isolation. Grape fruits with blue mold were collected from store houses in Daejeon, Naju and Suwon in Korea

during the period of December 2004 and February 2006. The conidia assumed to be *Penicillium* were picked up from blue molds of grapes 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 inoculated onto Czapek yeast extract agar (CYA; K₂HPO₄ 1.0 g, Czapek concentrate 10 mg, yeast extract 5 g, sucrose 30 g, agar 15 g, distilled water 1 liter) and malt extract agar (MEA) media in 3 points in a petridish. Colony appearance, exudates production, pigmentation and reverse coloration were assessed and colony diameters were measured and recorded after one week incubation at 25°C.

Morphological observations. *Penicillium* isolates were identified with the help of keys developed by Pitt (1979, 2000) and Frisvad and Samson (2004). Cultures were inoculated on CYA and 2% MEA media in three-points of 9 cm plastic Petri dishes. Petri dishes were incubated at 25°C in a dark condition. The cultures were examined after 7 days of incubation. All morphological data were examined on cultures grown on 2% MEA for 7 days at 25°C. The examination and measurements of conidiophores and conidia were made from slide preparations stained with 3% KOH. Differential interference contrast microscopy was used for the observation and 30 units of each morphological character were measured.

DNA extraction and PCR amplification. Isolates were grown on liquid shake culture of 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

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tubes. These samples were frozen at -70°C before use. DNA was extracted by the method of Cubero *et al.* (1999). For the amplification of β -tubulin gene, primers Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') (Glass and Donaldson, 1995) 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 MgCl₂, 2.5 U *Taq* polymerase and 15 ng of template DNA. PCR amplification was carried out in a *i*-cyclor (BIO-RAD, USA) for 30 cycles of 94°C for 40 s denaturing, 55°C for 40 s annealing and 72°C for 1 min extension. Initial denaturing at 94°C was extended to 3 min and the final extension was at 72°C for 15 min.

The PCR products were purified by using a Wizard PCR prep. kit (Promega, Madison, WI, USA). Purified double stranded PCR fragments were directly sequenced with BigDye terminator cycle sequencing kits (Applied Biosystems, Forster City, CA, USA) by following 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, USA).

Sequencing and phylogenetic analysis. The sequences were compared with β -tubulin gene sequence available in the GenBank by the BLAST search analysis. 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 the alignment was refined manually using the PHYDIT program version 3.0 (Chun, 1995; available at <http://plaza.sun.ac.kr/~jchun/phydit>).

Ambiguously aligned regions were excluded from the subsequent analyses. A neighbor-joining tree was reconstructed with Kimura's 2-parameter distance model (Kimura, 1980) using the PHYDIT 3.57c package (Felsenstein, 1995). The bootstrap analysis using 1000 replications were performed to assess the relative stability of the branches.

Results and Discussion

Grape fruits with blue mold were collected from different store houses in Korea. Twelve isolates were selected as *Penicillium* on the basis of their morphological structure. According to cultural and morphological characteristics, the *Penicillium* species were divided into 6 groups. On the basis of previous description by Pitt (1979, 2000) and Frisvad and Samson (2004), each group was identified as *P. bialowiezense*, *P. citrinum*, *P. echinulatum*, *P. expansum*, *P. solitum* and unidentified *Penicillium* sp. Taxonomic descriptions, photos of colonies and drawings of fungal structures of each species are given below.

***Penicillium bialowiezense* K. Zaleski** Figs. 1(A, B), 3(A)
Bul. Int. Acad. Pol. Sci. Lett., Ser. B 1927: 462, 1927

Colonies on CYA 20~28 mm diam, radially sulcate, surface texture vultinous; conidiogenesis moderate to heavy, dark green to grey green; exudate often present as pale to reddish brown droplets; reverse beige to yellowish cream.

Colonies on MEA 15~23 mm diam, pale or slightly sulcate; surface texture velutinous, floccose in center; conidiogenesis light to moderate, pale green to pale yellow green; exudates absent; reverse pale yellow to yellow orange.

Conidiophores long, appressed, stipes 250~450 μm long, smooth-walled, terverticillate; rami 20~25 μm long; metulae cylindrical but apically inflated, 10~15 μm long, phialides cylindrical with gradually tapering collula, 6~10 \times 2.5~3 μm ; conidia subglobose to ellipsoidal, finely roughened, 2.5~3.8 \times 2~3 μm .

Isolate examined: CNU 060156.

Notes: Colony characteristics and micromorphology of the fungus agreed well with the description of *P. bialowiezense* (Frisvad and Samson, 2004). The species is most closely related to *P. bravipectum*. *P. bialowiezense* has shorter and less wide stipes than *P. bravipectum* which differentiate it from *P. bravipectum*. The fungus has been reported from forest soil, wheat bread, grapes etc. in Denmark, Poland, Italy, Slovenia, Chile, Wyoming, USA, Canada, Saudi Arabia etc. (Frisvad and Samson, 2004).

***Penicillium citrinum* Thom** Figs. 1(C, D), 3(A)
Bull. Bur. Anim. Ind. US Dep. Agric. 118: 61, 1910

Colonies on CYA 26~32 mm diam, radially sulcate, surface texture floccose in center and vultinous at margins; conidiogenous light to moderate, greyish green; exudates clear, pale yellow or pale brown to reddish brown; reverse yellow, yellow brown to reddish brown.

Colonies on MEA 20~22 mm diam, plane or radially sulcate; surface texture floccose; conidiogenesis moderate to heavy, grey blue at the margin, elsewhere dull green; exudates absent; reverse pale brown to yellow brown.

Conidiophores arising from subsurface or surface hyphae, stipes 100~300 μm long, smooth walled, biverticillate with 3~5 divergent metulae in a whorl; metulae usually uniform, 15~20 \times 2~5 μm , bearing 6~10 phialids; phialids ampulliform, 7~10 \times 2~2.5 μm ; conidia globose to subglobose, 2.5~3.0 μm diam, smooth-walled or finely rough-walled, produced in long columns.

Isolates examined: CNU 04245, CNU 04246.

Notes: The principle distinguishing character of this species is the penicillus, which consists of 3~5 divergent and usually vesiculate metulae, bearing long well defined columns of conidia (Pitt, 1979). Colonies on CYA at 25°C were dominated by copious clear to yeallow or brown exudates at center; on MEA, growth was slow and dense, with heavy conidiogenesis (Pitt, 1979). This fungus has

been reported from soils in Korea (Kim, 1979; Kim and Lee, 1980; Min *et al.*, 1980).

***Penicillium echinulatum* Fassati** **Figs. 1(E, F), 3(C)**
Acta Univ. Carol. Biol. 12: 326, 1977

Colonies on CYA 32–40 mm diam, deeply radially sulcate, dense, texture velutinous to weakly fasciculate, floccose in center; conidiogenesis heavy, dull green to dark green; exudate absent or clear droplets; reverse light bluish grey or cream coloured.

Colonies on MEA 20–30 mm diam, lightly radially sulcate, texture velutinous to granular, occasionally crustose centrally; conidiogenesis moderate to heavy, dull green to dark green; exudates clear; reverse yellow orange to orange brown.

Conidiophores borne from subsurface hyphae, singly or in fascicles, appressed, stipes 250–500 × 3.0–4.0 μm long, rough-walled, terverticillate; rami cylindrical 12–20 × 3.5–4.0 μm long; metulae cylindrical, 10–15 × 2–3 μm; phialides ampulliform tapering to a distinct column, 8–11 × 2–3 μm; conidia globose to subglobose, rough-walled, 3.5–4.5 μm.

Isolate examined: CNU 04225.

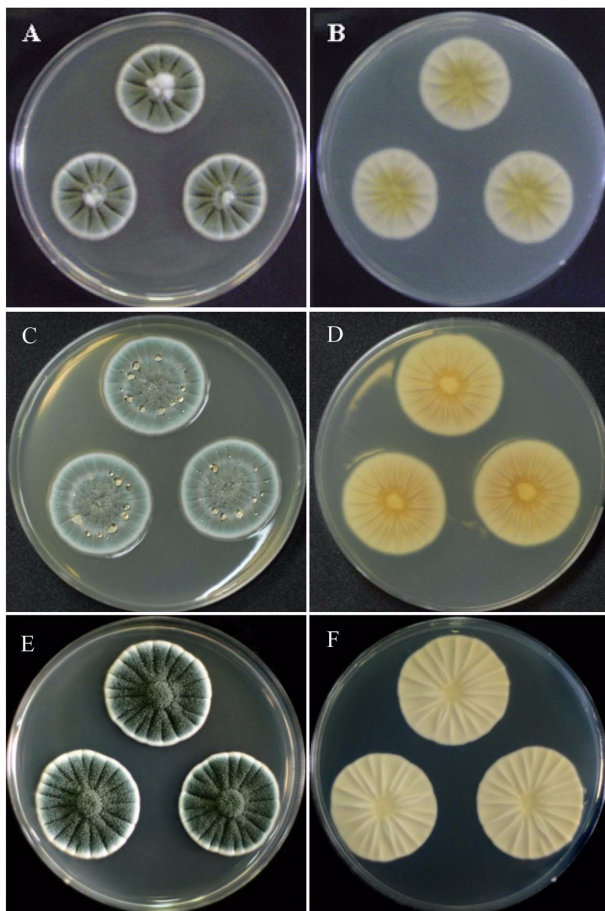


Fig. 1. *Penicillium* colonies on CYA after 7 days of incubation: (A, B) *P. bialowiezense*; (C, D) *P. citrinum*; (E, F) *P. echinulatum*: (A, C, E) obverse; (B, D, F) reverse.

Notes: Colony characteristics and micromorphology of the fungus agreed well with the description of *P. echinulatum* (Frisvad and Samson, 2004). The species is closely related to *P. solitum* and *P. discolor*, but differs from *P. solitum* by producing rough-walled conidia and from *P. discolor* by inability to produce a diffusible red pigment (Frisvad and Samson, 2004). The fungus has been reported from food (margarine, chesse and other lipid containing substrate) in Japan, Denmark, Sweden, Norway, the Netherlands, Germany, France, Russia, USA, Wyoming, Thailand, South Africa and Canada (Frisvad and Samson, 2004). This is the first record of *P. echinulatum* from grape in Korea.

***Penicillium expansum* Link** **Figs. 2(A, B), 3(D)**
Obs. Mycol. 1: 16, 1809

Colonies on CYA 35–50 mm diam, radially sulcate, moderately deep, texture floccose to weakly fasciculate; conidiogenesis moderate, blue green to moderate bluish green; exudate clear to pale orange brown; reverse light to vivid yellow.

Colonies on MEA 25–35 mm diam, plane, texture velu-

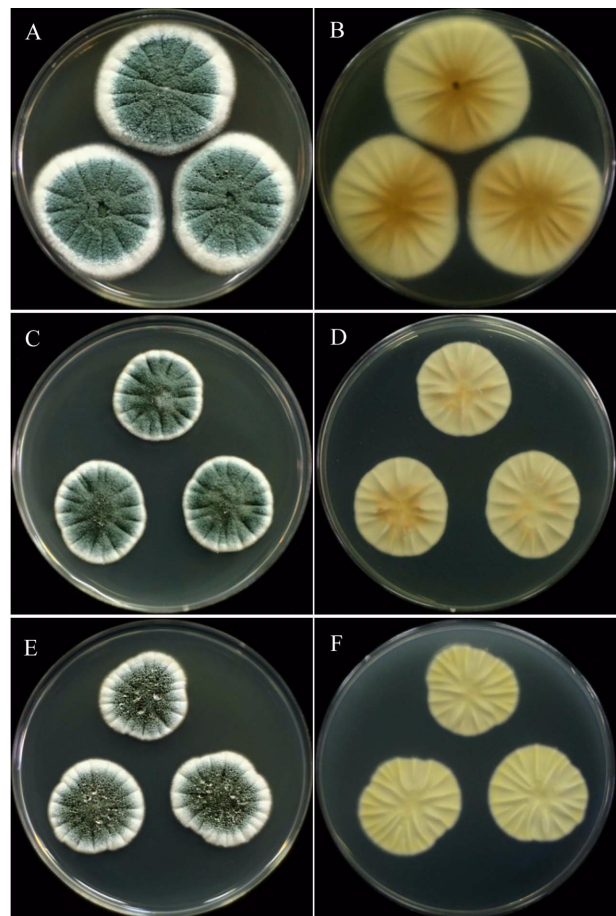


Fig. 2. *Penicillium* colonies on CYA after 7 days of incubation: (A, B) *P. expansum*; (C, D) *P. solitum*; (E, F) unidentified *Penicillium* sp.: (A, C, E) obverse; (B, D, F) reverse.

tinose or coremia, sometimes becoming crustose; conidiogenesis heavy, yellow green to blue green; reverse pale to orange brown.

Conidiophores borne from subsurface or aerial hyphae, single or in fascicles, appressed, stipes usually smooth-walled, occasionally very finely rough-walled, terverticillate, less commonly biverticillate; rami cylindrical, $15\text{--}25 \times 3\text{--}4 \mu\text{m}$; metulae more or less cylindrical, $10\text{--}17 \times 3\text{--}4 \mu\text{m}$; phialids ampulliform, $9\text{--}12 \times 2\text{--}4 \mu\text{m}$; conidia ellipsoidal to subglobose, smooth-walled, $3\text{--}3.5 \times 2.5\text{--}3.0 \mu\text{m}$ diam.

Isolates examined: CNU 04209, CNU 04210.

Notes: Colony characteristics and micromorphology of the fungus agreed well with the description of *P. expansum* (Frisvad and Samson, 2004). The species is similar to *P. crustosum* and *P. viridicatum* in colony morphology and colouration. The fungus, however, has ellipsoidal conidia and usually smooth stipes. The fungus has been reported from apples, pears and other pomaceous fruits, cherries, peaches etc. in Denmark, United Kingdom, Sweden, Norway and many other countries (Frisvad and Samson, 2004).

***Penicillium solitum* Wrestling** Figs. 2(C, D), 3(E)
Ark. Bot. 11: 52, 1911

Colonies on CYA 20–30 mm diam, radially sulcate, surface texture vultinuous, less commonly fasciculate; conidiogenesis very heavy, dark bluish green to dark green; exudate often present, clear to light yellow droplets on the surface; reverse pale or cream to light beige.

Colonies on MEA 15–27 mm diam, radially sulcate; surface texture velutinuous; conidiogenesis heavy, pale bluish green to greyish blue green; exudates rarely present, clear to light yellow; reverse light to moderate orange yellow.

Conidiophores arising from subsurface hyphae, stipes 200–400 μm long, rough-walled, terverticillate; rami cylindrical 10–18 μm long; metulae cylindrical, 10–15 μm long; phialides ampuliform, $9\text{--}11 \times 2\text{--}3 \mu\text{m}$; conidia globose to subglobose, less commonly broadly ellipsoidal, smooth to finely rough-walled, 3–4.5 μm .

Isolates examined: CNU 04211, CNU 04213, CNU 04214.

Notes: Colony characteristics and micromorphology of the fungus agreed well with the description of *P. solitum* (Frisvad and Samson, 2004). The species is most closely related to *P. echinulatum* and *P. discolor*, but differs from *P. echinulatum* by producing smooth-walled conidia and from *P. discolor* by inability to produce a diffusible red pigment (Frisvad and Samson, 2004). The fungus has been reported from apples, oriental pear, grape, citrus, processed meat in Japan, Denmark, Greenland, United Kingdom, Sweden, Norway, the Netherlands, Germany, France, Russia, USA and Canada (Frisvad and Samson, 2004).

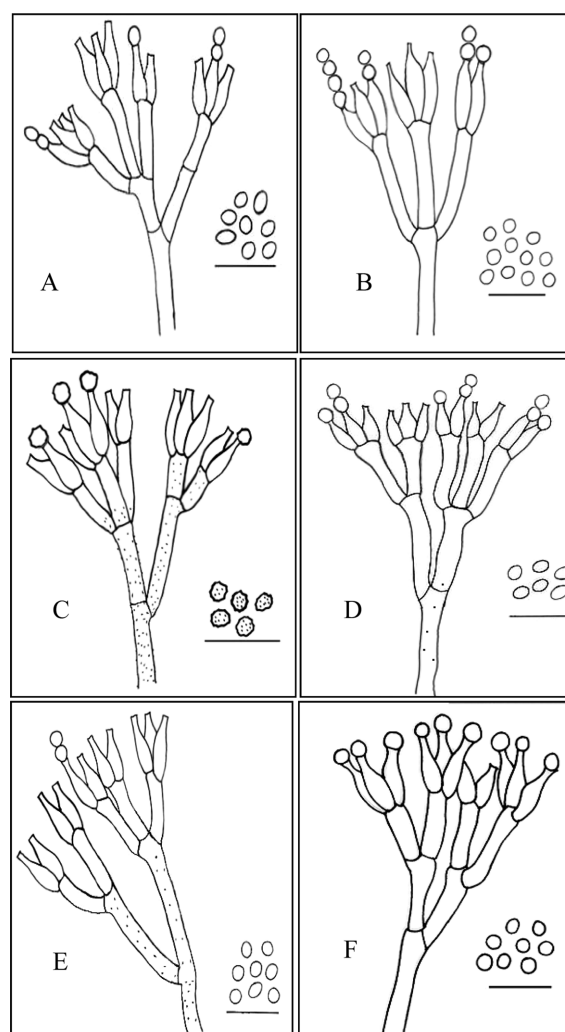


Fig. 3. Conidiophores and conidia of (A) *P. bialowiezense*, (B) *P. citrinum*, (C) *P. echinulatum*, (D) *P. expansum*, (E) *P. solitum* and (F) unidentified *Penicillium* sp. Scale bar = 12 μm .

***Penicillium* taxon** Figs. 2(E, F), 3(F)

Colonies on CYA 20–30 mm diam, radially sulcate, surface texture vultinuous; conidiogenesis moderate to heavy, green to dull green; exudate present as clear or colourless droplets on surface; reverse pale greenish yellow to greyish yellow green.

Colonies on MEA 15–25 mm diam, plane or lightly sulcate; surface texture velutinuous; conidiogenesis light to moderate, pale green; exudates absent, reverse light yellow to vivid yellow.

Conidiophores arising from submerged or aerial hyphae, stipes 150–300 μm long, smooth or rough-walled, usually terverticillate, sometime biverticillate; rami cylindrical 15–25 μm long; metulae cylindrical, 8–14 μm long, phialides ampuliform, flask-shaped with short necks, 10–13 mm; conidia globose to subglobose, smooth-walled, 2.5–4.0 μm diam.

Isolate examined: CNU 060161.

Notes: This taxon can be distinguished from *P. bialowiezense* by its globose conidia.

Sequence analysis of β -tubulin gene. The partial β -tubulin gene from 12 isolates of *Penicillium* from blue mold fruits of grape was amplified. Amplification of β -tubulin gene with primers Bt2a and Bt2b yields fragments of approximately 500 bp. BLAST database searches were performed with partial β -tubulin gene as queries to reveal relationship to published sequences.

In a distance analysis with neighbor-joining method, sequence of isolates CNU 04211, CNU 04213 and CNU 04214 was 100% identical to that of *P. solitum* CBS 14686, with a bootstrap value of 99% (Fig. 4, Table 1). Sequence of CNU 04225 isolate was 100% identical to that of *P. echinulatum* CBS 19067, with a bootstrap value of 100%. Isolates CNU 04209 and CNU 04210 and *P. expansum* CBS 28197 were belonged to the same group. Sequence similarity among them was 100%, which was supported by a bootstrap value of 100%. β -tubulin sequence of CNU 060156 and CNU 060161 were 100% and 98% identical to *P. bialowiezense* CBS 112882, respectively. Sequence of isolates CNU 04245 and CNU 04246 was 99.5% identical to that of *P. citrinum* DAOM 216702 (Table 1). Sequence of CNU 04206 isolate matched well with that of *P. camemberti* CBS 19067 (Fig. 3, Table 1).

The β -tubulin gene sequence has been used extensively to characterize a wide range of fungi, specially

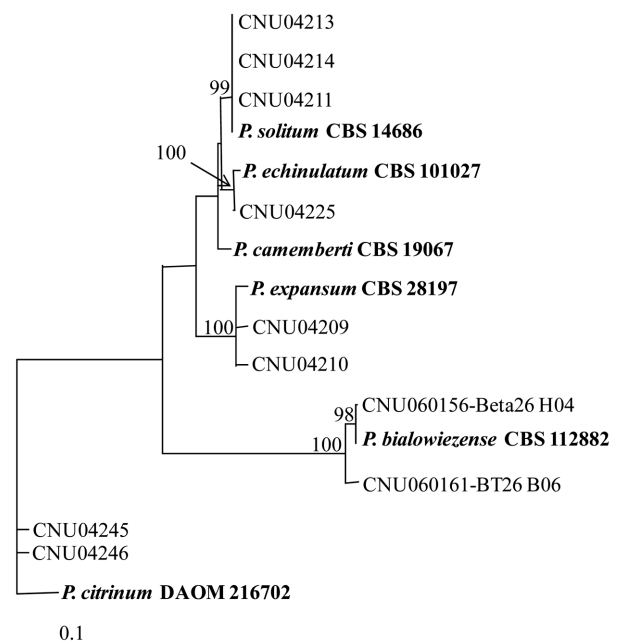


Fig. 4. Neighbor-joining tree based on phylogenetic analysis of β -tubulin gene sequences. The number below each branch indicates bootstrap values of distance. The bootstrap values were obtained after a bootstrap test of 1000 replications. References, which were taken from GenBank database, are in bold type.

Penicillium. The amount of variation is suitable for studying phylogenetic relationship among closely related species of *Penicillium* (Samson *et al.*, 2004) and filamentous

Table 1. DNA similarity matrix for $\hat{\alpha}$ -tubulin gene sequences of *P. bialowiezense*, *P. citrinum*, *P. echinulatum*, *P. expansum*, *P. solitum* and unidentified *Penicillium* species and their related species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1																
2	80.5															
3	80.5	100														
4	80.5	100	100													
5	80.3	94.4	94.4	94.4												
6	80.9	94.7	94.7	94.7	99.5											
7	80.9	94.7	94.7	94.7	96.8	97.1										
8	80.9	94.7	94.7	94.7	96.8	97.1	100									
9	99.5	80.9	80.9	80.9	80.7	81.2	80.9	80.9								
10	99.5	80.9	80.9	80.9	80.7	81.2	80.9	80.9	100							
11	81.3	93.7	93.7	93.7	97.6	98.2	97.9	97.9	81.7	81.7						
12	81.3	93.7	93.7	93.7	97.6	98.2	97.9	97.9	81.7	81.7	100					
13	81.3	93.7	93.7	93.7	97.6	98.2	97.9	97.9	81.7	81.7	100	100				
14	81.3	93.7	93.7	93.7	97.6	98.2	97.9	97.9	81.7	81.7	100	100	100			
15	74	81.6	81.6	81.6	81.0	81.3	81.0	81.0	74	74	81.6	81.6	81.6	81.6		
16	74.4	81.6	81.6	81.6	81.1	81.4	81.1	81.1	74.4	74.4	81.6	81.6	81.6	81.6	81.6	98.1
17	74.4	81.7	81.7	81.7	81.1	81.4	81.1	81.1	74.4	74.4	81.7	81.7	81.7	81.7	98.1	100

1. *P. citrinum* DAOM216702 (GenBank database); 2. *P. expansum* CBS28197 (GenBank database); 3. *P. expansum* CNU04209; 4. *P. expansum* CNU04210; 5. *P. echinulatum* 101027(GenBank database); 6. *P. echinulatum* CNU04225; 7. *P. camemberti* CBS19067 (GenBank database); 8. *P. sp.* CNU04206; 9. *P. citrinum* CNU04245; 10. *P. citrinum* CNU04246; 11. *P. solitum* CBS14686 (GenBank database); 12. *P. solitum* CNU04211; 13. *P. solitum* CNU04213; 14. *P. solitum* CNU04214; 15. *P. bialowiezense* CNU060161; 16. *P. bialowiezense* CNU060156; 17. *P. bialowiezense* CBS112882 (GenBank database).

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 except CNU 04206 and *P. camemberti*. The β -tubulin gene sequence evidenced that isolate CNU 04206 was identical to the GenBank sequence of *P. camemberti* CBS 19067 (Table 1), but morphological and cultural characteristics of our isolates did not agree with the description of *P. camemberti* (Frisvad and Samson, 2004). The species was not identified and was supposed to a new species, not previously reported in literature.

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References

- Anonymous. 2004. List of plant diseases in Korea. 4th ed. The Korean Society of Plant Pathology.
- Cho, H. S., Hong, S. B. and Go, S. J. 2005. First report of *Penicillium brasilianum* and *P. daleae* isolated from soil in Korea. *Mycobiol.* **33**: 113-117.
- Chun, J. 1995. Computer-assisted classification and identification of actinomycetes. PhD thesis, University of Newcastle, Newcastle upon Tyne, UK.
- Cubero, O. F., Crespo, A. N. A., Fatehi, F. and Bridge, P. D. 1999. DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Pl. Syst. Evol.* **216**: 243-249.
- Felsenstein, J. 1995. PHYLIP (Phylogeny Inference Package) Version 3.57C. Seattle, WA: University of Washington.
- Frisvad, J. C. and Samson, R. A. 2004. Phylogenetic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate penicilla and their mycotoxins. *Stud. Mycol.* **49**: 1-173.
- Glass, N. L. and Donaldson, G. C. 1995. Development of primer sets designed for use with the PCR to amplify conserved gene from filamentous Ascomycetes. *Appl. Environ. Microbiol.* **61**: 1323-1330.
- Guerche, S. L., Garcia, C., Darriet, P., Dubourdiou, D. and Labarere, J. 2004. Characterization of *Penicillium* species isolated from grape berries by their internal transcribed spacer (ITS) sequences and by gas chromatography-mass spectrometry analysis of geomsin production. *Curr. Microbiol.* **48**: 405-411.
- Kim, J. H., Lee, W. H., Cheong, S. S., Choi, J. S., Ryu, J. and Choi, Y. G. 2002. Identification and characteristics of *Penicillium* spp. isolated from post-harvest decay of pear. *Res. Plant Dis.* **82**: 113-116.
- Kim, W. K., Park, M. S., Hahm, S. S. and Yu, S. H. 2006. Two new records of *Penicillium* with blue moldy bulbs of lily in Korea. *Mycobiol.* **34**: 176-179.
- Kim, K. S. 1979. Soil-borne fungi of *Phyllosticta reticulata* forests soil in Korea (I). *Kor. J. Mycol.* **7**: 91-116.
- Kim, K. S. and Lee, J. Y. 1980. Soil-borne fungi of *Phyllosticta reticulata* forests soil in Korea (II). *Kor. J. Mycol.* **8**: 45-51.
- Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequence. *J. Mol. Evol.* **16**: 111-120.
- Lee, H. K., Hong, S. B. and Kim, C. Y. 2003. Contribution to the checklist of soil-inhabiting fungi in Korea. *Mycobiol.* **31**: 9-18.
- Min, K. H., Hong, S. W. and Yokoyama, T. 1980. Hyphomycetes from Korean soil. The genus *Penicillium* with a teleomorphic state *Eupenicillium javanicum*. *Kor. J. Microbiol.* **18**: 91-103.
- Oh, S. Y., Chung, I. M., Paik, S. B. and Yu, S. H. 1999. Survey and control of the occurrence of mycotoxins from post-harvest fruits. 1. Mycotoxins produced by *Penicillium* isolates from apple, pear, citrus and grape. *Plant Dis. Agric.* **5**: 100-104.
- Pitt, J. I. 1991. Distribution of ubiquinones in *Penicillium* and related genera. *Mycol. Res.* **95**: 705-711.
- Pitt, J. I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic press, London, UK, pp. 634.
- Samson, R. A., Seifert, K. A., Kuijpers, A. F. A., Houbraken, J. A. M. P. and Frisvad, J. C. 2004. Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial β -tubulin sequences. *Stud. Mycol.* **49**: 175-200.
- Seifert, K. A. and Louis-Seize, G. 2000. Phylogeny and species concepts in the *Penicillium aurantiogriseum* complex as inferred from partial β -tubulin gene DNA sequences. pp. 189-198. In: Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification. Harwood Academic Publishers, Amsterdam, the Netherlands. 510 pp.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The CLUSTALX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* **25**: 4876-4878.