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Research Note

Three New Records of *Penicillium* Species Isolated from Insect Specimens in Korea

Kabir Lamsal[1], Sang Woo Kim[1], Shahram Naeimi[1], Mahesh Adhikari[1], Dil Raj Yadav[1], Changmu Kim[2], Hyang Burm Lee[3] and Youn Su Lee[1,*]

[1]*Department of Applied Plant Sciences, Kangwon National University, Chuncheon 200-701, Korea*
[2]*Microorganism Resources Division, National Institute of Biological Resources, Incheon 404-708, Korea*
[3]*Division of Applied Bioscience and Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea*

Abstract Three *Penicillium* species have been isolated from insect specimens in Korea; *Penicillium* sp., *P. steckii*, and *P. polonicum*. *Penicillium* sp. (KNU12-3-2) was isolated from *Lixus imperessiventris*, while *P. polonicum* (KNU12-1-8) and *Penicillium steckii* (KNU12-2-9) were isolated from *Muljarus japonicas* and *Meloe proscarabaeus,* respectively. The identification was based on the morphological characteristics of the fungi and in internal transcribed spacer analysis. This is the first report on the isolation of these three species of *Penicillium* from insects in Korea.

Keywords *Lixus imperessiventris*, *Meloe proscarabaeus*, *Muljarus japonicas*, *Penicillium* spp.

Penicillium species are usually regarded as soil fungi [1], but many species inhabit well-defined habitats other than soil. They function as decomposers of dead materials and are especially important postharvest, where they spoil food commodities [2-5]. Some reports have shown that *Penicillium* species can be isolated from insects and their body parts [6]. In this study, we report three species of *Penicillium* isolated from three different insect species in Korea.

Collection of insects and fungal isolation. Insect samples were collected from preserved specimens of the Insect Museum at Kangwon National University in Chuncheon (Gangwon Province, Korea). Collected insects were placed in a laboratory clean box for isolation of fungi. Collected insects were surface sterilized in a 2% sodium hypochlorite solution for 3 min, rinsed with plenty of sterile distilled water, then dried using filter paper. Surface sterilized cadavers were plated onto potato dextrose agar (PDA) containing 0.25 mg/mL chloramphenicol to inhibit bacterial growth and incubated at 25°C. Hyphae of the fungi growing and sporulating on cadavers (and on PDA medium) were cut, transferred to fresh PDA plates and incubated at 25°C.

ITS sequencing analysis. Fungal genomic DNA samples were extracted using InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA). The primers ITS1 primer (5'-TCCG-TAGGTGAACCTGCGG-3') and ITS5 (5'-GGAAGTAAAA-GTCGTAACAAGG-3') and ITS4 primer (5'-TCCTCCGC-TTATTGATATGC-3') were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 µL reaction mixture by using *EF-Taq* (SolGent, Daejeon, Korea) as follows: activation of Taq polymerase at 95°C for 2 min, 35 cycles of 95°C for 1 min, 55°C, and 72°C for 1 min each were performed, finishing with a 10-min step at 72°C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reactions were performed using the PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA, USA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then analyzed by the ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were compared using the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/

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***Corresponding author**
E-mail: younslee@kangwon.ac.kr

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Blast) for identification of the isolates. Sequences used to calculate phylogeny were first determined using BLAST results from databases [7] and a phylogenetic tree was subsequently prepared by the neighbor-joining method [8].

Morphological characteristics and identification.

***Penicillium polonicum*:** Conidiophores are observed as two-stage branched (terverticillate) with all elements adpressed and stipes rough-walled. Conidia were smooth, globose to subglobose, and 3~4 μm in diameter. Morphologically, conidia and conidiophores of our strain were similar to those of *Penicillium polonicum* (Fig. 1). When the internal transcribed spacer (ITS) sequences of the strain were compared with related species retrieved from GenBank, sequence analysis by BLAST indicated that KNU12-3-2 was highly related to *P. polonicum*, with a 99% sequence similarity (Table 1, Fig. 2).

***Penicillium steckii*:** Conidia observed were broadly ellipsoidal. Conidiophores from surface hyphae, symmetrically biverticillate, stipes smooth, and width 2.2~3.0 μm; metulae in whorls of 3~6, 13~18 × 2.5~3.3 μm; ampulliform phialides, 7.0~10 × 2.2~3.0 μm; conidia smooth-walled, broadly ellipsoidal, and slightly fusiform in some strains, 2.3~3.1 × 2.0~2.6 μm (Fig. 3). When the ITS sequences of the strain were compared with related species retrieved from GenBank, sequence analysis by BLAST indicated that KNU12-1-8

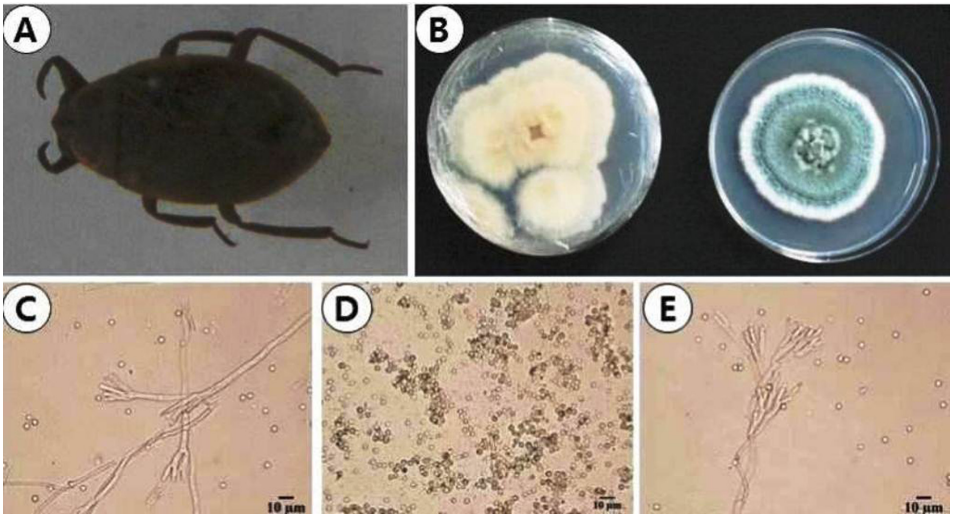

Fig. 1. Morphological characteristics of *Penicillium polonicum* isolated from *Muljarus japonicas*. A, Morphology of the host insect (*M. japonicas*); B, Colony on potato dextrose agar after 7 days of incubation; C, Mycelia of *P. polonicum*; D, Spores of *P. polonicum*; E, Conidia of *P. polonicum* (scale bars: C~E = 10 μm).

Table 1. ITS sequence analysis for the identification of *Penicillium* species

Isolate No.	Putative species	Related Genbank accession No.	Identity (%)
KNU12-1-8	*P. polonicum*	AF033475.1	568/570 (99)
KNU12-2-9	*P. steckii*	HM469415.1	577/580 (99)
KNU12-3-2	*Penicillium* sp.	HQ832995.1	574/574 (100)

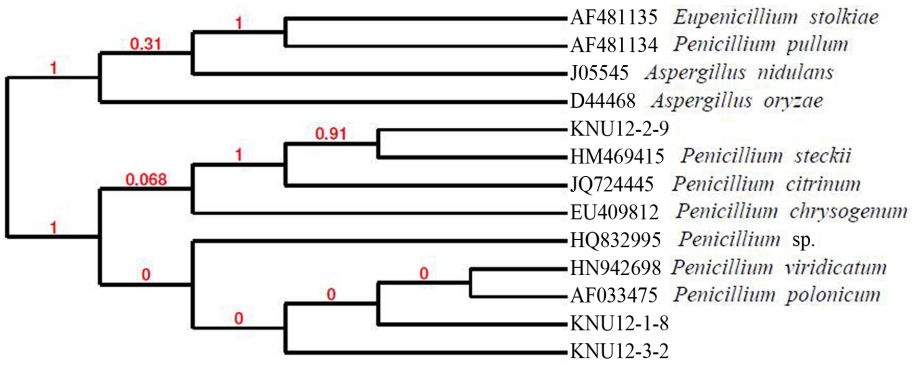

Fig. 2. Phylogenetic tree (using internal transcribed spacer sequences) showing the closest known relatives of newly reported *Penicillium* species in Korea. Numbers above the branches indicate bootstrap values of distance.

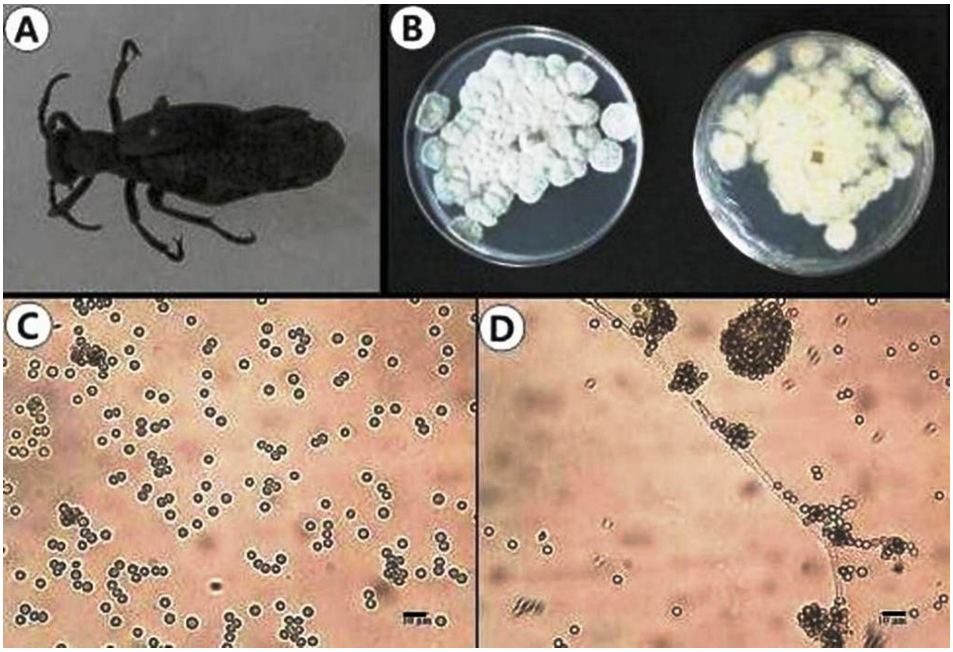

Fig. 3. Morphological characteristics of *Penicillium steckii* isolated from *Meloe proscarabaeus.* A, Morphology of the host insect (*M. proscarabaeus*); B, Morphology of *P. steckii* on potato dextrose agar; C, Spores of *P. steckii* under a microscope; D, Mycelia of *P. steckii* (scale bars: D, E = 10 μm).

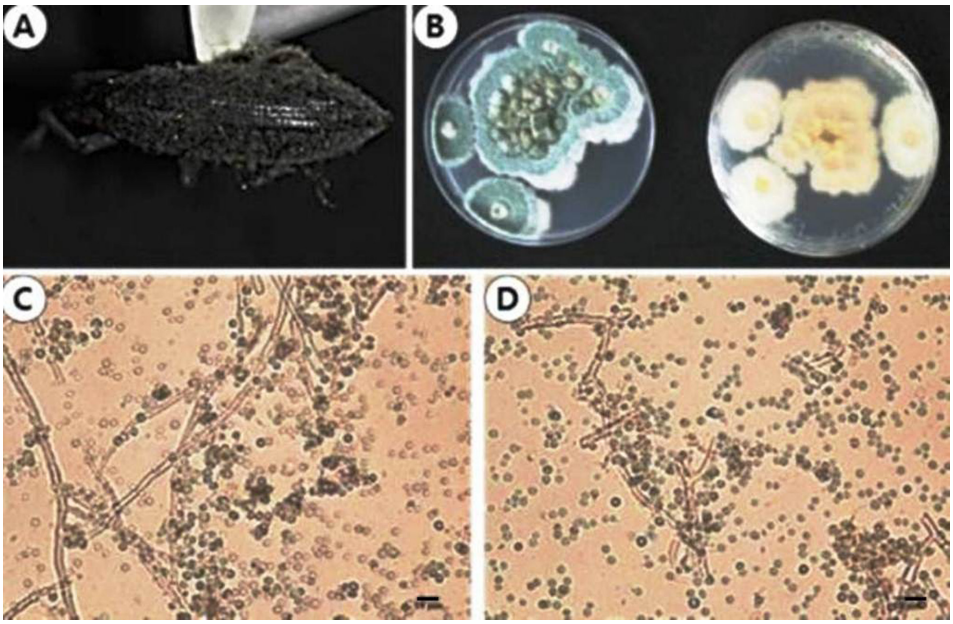

Fig. 4. Morphological characteristics of *Penicillium* sp. isolated from *Lixus imperessiventris.* A, Morphology of host insect (*L. imperessiventris*); B, Morphology of *Penicillium* sp. on potato dextrose agar; C, D, Mycelia and spores of *Penicillium* sp. under a microscope (scale bars: D, E = 10 μm).

was highly related to *P. steckii* with 99% sequence similarity (Table 1, Fig. 2).

***Penicillium* sp.:** The mycelium typically consists of a highly branched network of multinucleate, septate, and usually colorless hyphae. Many-branched conidiophores sprout on the mycelia, bearing individually constricted conidiospores. The conidiospores are the main dispersal route of the fungi, and are often greenish in color. Conidia are globose, ellipsoidal, cylindrical or fusiform, hyaline or greenish, and smooth or rough-walled (Fig. 4). When ITS sequences of the strain were compared with related species retrieved from GenBank, sequence analysis by BLAST indicated that

KNU12-2-9 was highly related to *Penicillium* sp. with 100% sequence similarity (Table 1, Fig. 2).

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REFERENCES

1. Pitt JI. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London: Academic Press; 1979.
2. Janisiewicz WJ. Postharvest biological control of blue mold on apples. Phytopathology 1987;77:481-5.
3. Pitt J, Hocking AD. Fungi and food spoilage. Cambridge; Cambridge University Press; 1997.
4. Holmes GJ, Eckert JW. Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. Phytopathology 1999;89:716-21.
5. Morales H, Marín S, Rovira A, Ramos AJ, Sanchis V. Patulin accumulation in apples by *Penicillium expansum* during postharvest stages. Lett Appl Microbiol 2007;44:30-5.
6. Seifert KA, Hoekstra ES, Frisvad JC, Louis-Seize G. *Penicillium cecidicola*, a new species on cynipid insect galls on *Quercus pacifica* in the western United States. Stud Mycol 2004;50: 517-23.
7. Dereeper A, Audic S, Claverie JM, Blanc G. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evol Biol 2010;10:8.
8. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, et al. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 2008;36:W465-9.