RESEARCH NOTE

Two Unrecorded *Penicillium* Species from Rhizosphere Soil in Korea

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ABSTRACT

Penicillium perform important ecological functions in the rhizosphere, however, only a relatively small number of *Penicillium* species have been reported from rhizosphere soils. During a study on *Penicillium* diversity in Korea, we isolated *Penicillium* strains from the rhizosphere soils of coniferous and broad-leaved trees. Based on phylogenetic analysis of the β-tubulin and calmodulin loci, we accordingly identified two *Penicillium* species previously unrecorded in Korea: *P. nucicola* and *P. yarmokense*. In this paper, we provide comprehensive morphological descriptions of these two species.

Keywords: *Penicillium nucicola*; *Penicillium yarmokense*, Rhizosphere soil, Unrecorded species

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The rhizosphere is colonized by a fungal community that plays pivotal roles in plant nutrient absorption and can confer resistance against phytopathogen invasion [1]. Species of the genus *Penicillium* are established to be among the most common fungi found in rhizosphere soils [2], wherein they influence the stable adaptation of plants to the ecosystem via the production of diverse secondary metabolites, such as solubilized phosphorus, indole acetic acid, siderophores, and extracellular enzymes [3,4]. During a study of *Penicillium* diversity in Korea, we isolated *Penicillium* strains from the rhizosphere soil of coniferous and broad-leaved trees. In this study, we identified two *Penicillium* species from rhizosphere soil previously unrecorded in Korea based on phylogenetic analysis of the β-tubulin (*BenA*) and calmodulin (*CaM*) genes and provided the detailed morphological descriptions.

Rhizosphere soils of coniferous and broad-leaved trees were collected in 2019 and 2020, and fungal strains were isolated on dichloran rose bengal chloramphenicol agar (DRBC; Difco, Becton Dickinson, MD, USA) using the serial dilution method. The isolated *Penicillium* strains were subsequently cultured on potato dextrose agar (PDA; Difco, Becton Dickinson, MD, USA).

For identification, genomic DNA was isolated from *Penicillium* strains using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). Each PCR was conducted using a C1000 thermal cycler (Bio-Rad, Richmond, CA), following previously described methods with Bt2a/Bt2b for *BenA* and CF1/CF4 or cmd5/cmd6 for *CaM* [4]. The sequencing was carried out using the PCR primers at Bioneer (Daejeon, Korea). Sequences were manually assembled, proofread, and edited using MEGA5 [5], and

have been deposited in the GenBank database (PQ310430-PQ310433 for *BenA*, PQ310434-PQ310437 for *CaM*). Sequence similarities for each species were calculated from the two assessed loci using MEGA5 [5]. Multiple sequence alignments were carried out using MAFFT v7 with default settings [6]. Maximum likelihood phylogenetic tree was constructed using RAxML [7] at the CIPRES web portal [8], utilizing the GTR+GAMMA model and 1,000 bootstrap replicates. Isolates AF13142 (NIBRFGC000509171) and AF13692 were accordingly found to cluster as a monophyletic group with the type strain (KAS 2203) of *P. nucicola*, supported by a 100% bootstrap value. The sequence similarities for *BenA* ranged from 99.8 to 100%, and was 100% for *CaM*. Isolates AF12432 (NIBRFGC000509176) and AF12471 were found to group with the type strain (CBS 410.69) of *P. yarmokense*, supported by a 100% bootstrap value. Sequence similarities for *BenA* ranged between 99.8 and 100%, and was 100% for *CaM* (Fig. 1).

The morphological features of the two previously unrecorded species were examined on three different media: Czapek yeast autolysate agar (CYA; Difco, Sparks, MD, USA), malt extract agar (MEA; Oxoid, Hampshire, UK), and yeast extract sucrose agar (YES) [4]. Microscopic characteristics were examined under a light microscope (DM2000; Leica, Germany) using colonies grown on MEA. Color descriptions and alphanumeric codes were based on reference to the Methuen Handbook of Color [9].

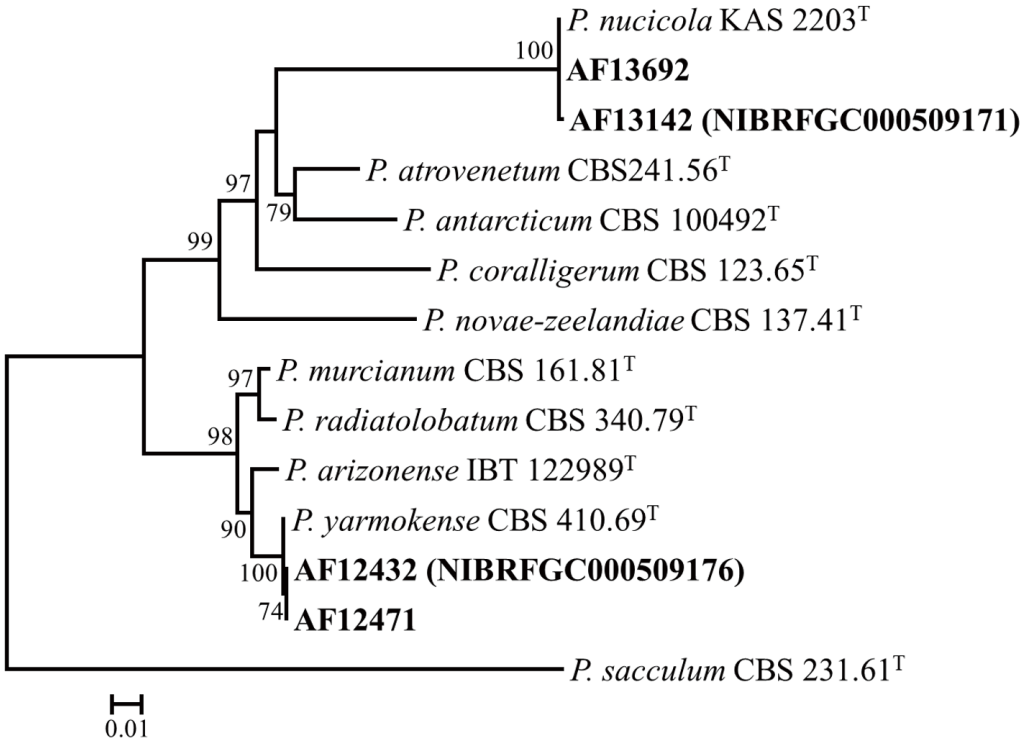

Fig. 1. A maximum likelihood phylogenetic tree based on the concatenated datasets (*BenA* and *CaM*) used to identify *Penicillium* strains isolated from rhizosphere soil. Bootstrap scores of >70 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. "T" indicates the ex-type strains. The strains described in the current study are represented in bold.

Penicillium nucicola Visagie, Malloc & Seifert, 2016

Colony diameter after 7 days (in mm): CYA at 25°C: 26-28; CYA at 30°C: no growth; CYA at 37°C: no growth; MEA at 25°C: 30-33; YES at 25°C: 50-51 (Fig. 2).

Colony characteristics: On CYA (25°C for 7 days): Colonies moderate, radially sulcate; entire, moderately deep, mycelia white at margins; grayish green (28B3) conidia; sporulation moderately dense; texture floccose, with exudates clear; reddish gray soluble pigments; dull green (26E3) to light yellow (4A4) reverse color. On MEA (25°C for 7 days): Colonies low, plane; entire, low deep at margins; grayish green (27C3) conidia; sporulation moderately dense; texture floccose, with clear exudates; soluble pigments absent; light yellow (3A5) reverse color. On YEA (25°C for 7 days): Colonies moderate, radially and concentrically sulcate; entire, low deep at margins; sporulation moderately dense; conidia grayish green (26C3); texture floccose, with exudates absent; soluble pigments absent; light yellow (1A4) reverse color.

Conidiophores biverticillate, rough-walled stipes; phialides ampulliform, 7.5–9.0 × 3.0–4.0 µm; rough-walled conidia, globose to subglobose, 2.8–3.3 µm diameter.

Strains examined: Buk-myeon, Ulleung-gun, Gyeongsangbuk-do, Korea, isolated from rhizosphere soil of broad-leaved trees, strains AF13142 (NIBRFGC000509171) and AF13692.

Note: *Penicillium nucicola* forms a group phylogenetically distinct from other species in the section *Canescentia*. On all assessed media at 25°C, the Korean strains of *P. nucicola* were characterized by a more rapid growth compared with that of the type strain [10].

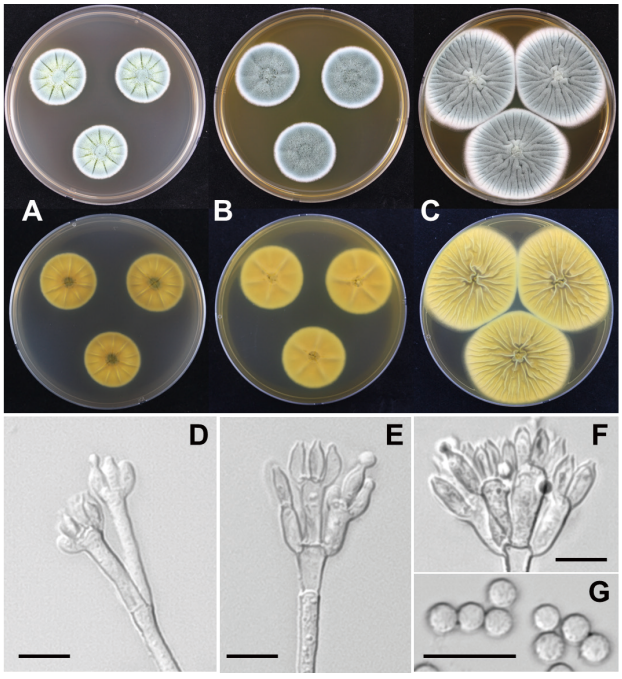

Fig. 2. *Penicillium nucicola* AF13142 (NIBRFGC000509171) in 7-day-old cultures grown at 25°C. A-C: Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top=obverse, bottom=reverse). D-F: Conidiophores; G: Conidia (scale bar: D-G=10 µm).

Penicillium yarmokense Baghd., 1968

Colony diameter after 7 days (in mm): CYA at 25°C: 27-32; CYA at 30°C: 10-11; CYA at 37°C: no growth; MEA at 25°C: 14-17; YES at 25°C: 23-25 (Fig. 3).

Colony characters: On CYA (25°C for 7 days): Colonies moderate, radially sulcate; entire, moderately deep, mycelia white at margins; greenish gray (28B2) conidia; sporulation absent to sparse; texture floccose, with clear exudates; soluble pigments absent; pale yellow (3A3) to grayish red (7B4) reverse color. On MEA (25°C for 7 days): Colonies low, plane; irregular, low at margins; turquoise white (24A2) conidia; sporulation moderately dense; texture floccose, with exudates absent; reddish brown soluble pigments; pale red (8A3) to reddish brown (9A6) reverse color. On YEA (25°C for 7 days): Colonies moderately deep, radially sulcate; entire, low deep at margins; greenish gray (28B2) conidia; sporulation absent to sparse; texture floccose, with exudates absent; soluble pigments absent; reddish brown (9E7) reverse color.

Conidiophores biverticillate, rough-walled stipes; phialides ampulliform, 7.0–9.0 × 2.5–3.0 μm; smooth rough-walled conidia, globose to subglobose, 2.5–3.0 × 2.4–3.0 μm diameter.

Strains examined: Naechon-myeon, Hongcheon-gun, Gangwon-do, Korea, isolated from rhizosphere soil of coniferous trees, strains AF12432 (NIBRFGC000509176) and AF12471.

Note: *Penicillium yarmokense* is phylogenetically similar to *P. arizonense* in section *Canescentia*. The former species can be distinguished from the latter by its relatively more rapid growth on MEA and YES [11].

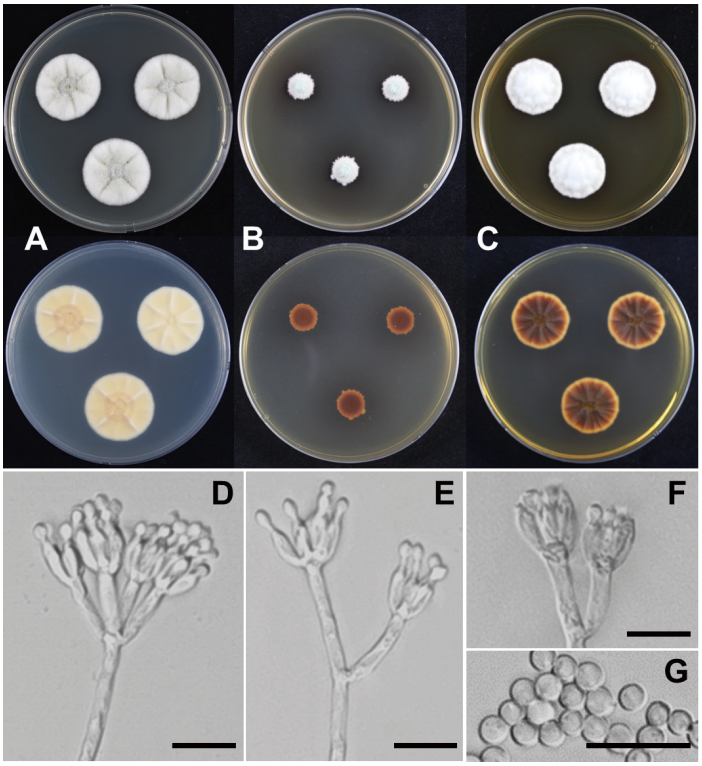

Fig. 3. *Penicillium yarmokense* AF12432 (NIBRFGC000509176) in 7-day-old cultures grown at 25°C. A-C: Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top=obverse, bottom=reverse). D-F: Conidiophores; G: Conidia (scale bar: D-G=10 μm).

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

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