Penicillium from Rhizosphere Soil in Terrestrial and Coastal Environments in South Korea

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ABSTRACT
Penicillium, the most common genus plays an important ecological role in various terrestrial and marine environments. However, only a few species have been reported from rhizosphere soil. As part of a project to excavate Korean indigenous fungi, we investigated rhizosphere soil of six plants in the forest (terrestrial habitat) and sand dunes (coastal habitat) and focused on discovering Penicillium species. A total of 64 strains were isolated and identified as 26 Penicillium species in nine sections based on morphological characteristics and the sequence analysis of β-tubulin and calmodulin. Although this is a small-scale study in a limited rhizosphere soil, eight unrecorded species and four potential new species have been identified. In addition, most Penicillium species from rhizosphere soil were unique to each plant. Penicillium halotolerans, P. scabrosum, P. samsonianum, P. jejuense, and P. janczewskii were commonly isolated from rhizosphere soil. Eight Penicillium species, P. aurantioviolaceum, P. bissettii, P. cairnsense, P. halotolerans, P. kananaskense, P. ortum, P. radiatolobatum, and P. verhagenii were recorded for the first time in Korea. Here, we provide the detailed morphological description of these unrecorded species.

1. Introduction
Fungi play an important role in rhizosphere soil. They affect plant fitness and adaptability in ecosystems through various biological processes [1,2]. The genus Penicillium is one of the most common fungi found in rhizosphere soil [3]. Some Penicillium species produce solubilized phosphorus, siderosphere, and phytohormones such as indole acetic acid and gibberellic acid, which are important for plant health [4–6]. Although the importance of Penicillium isolated in rhizosphere soil has been reported, many Penicillium species were only identified at the genus level [7,8]. There are two possible reasons for this; one being that studies conducted previously have focused on simply screening for useful enzymes and bioactive compounds, and two that it is naturally difficult to identify Penicillium species.

The members of the Penicillium genus are easily identified at the genus level based on their microscopic features in culture condition, whereas they are difficult to identify at the species level due to their morphological plasticity under different growth conditions [9]. Recently, the identification of Penicillium species has become much easier since standardized methods including morphology and multigene phylogenetic analysis have been proposed [9]. To date, Penicillium has been reported in 25 sections of 345 species worldwide through the use of standardized methods [9]. In Korea, approximately 102 Penicillium species from terrestrial environments [10,11] and 100 Penicillium species from marine environments [12–17] have been reported. It was confirmed that many Penicillium species from marine environments are different from those found in terrestrial environments. So far, a total of 152 Penicillium species have been reported in Korea. However, there are only a few studies on Penicillium from rhizosphere soil [18,19], and since rhizosphere soil is unique for each plant species, it can be assumed that different Penicillium species may exist for each plant.

As part of the projects organized by the National Institute of Biological Resources (NIBR) and the Ministry of Ocean and Fisheries established the Marine Fungal Resource Bank (MFRB) to excavate Korean indigenous fungi, we investigated rhizosphere soil to discover Penicillium species. The main objective of this study was to isolate Penicillium...
species from rhizosphere soil of six plants: *Rhododendron brachycarpum*, *Sorbus commixta*, and *Taxus cuspidate* from a forest (terrestrial habitat) and *Calystegia soldanella*, *Lathyrus japonicus*, and *Orobanche coerulescens* from a sand dune (coastal habitat). All *Penicillium* isolates were identified at the species level using the sequence analysis of the β-tubulin (BenA) and calmodulin (CaM) loci. We identified eight unrecorded species and four new species candidates of *Penicillium* in Korea.

2. Materials and methods

2.1. Sample collections and isolation

Rhizosphere soil from six plants were collected from six sites in Korea in 2019 (Figure 1, Table 1). *Rhododendron brachycarpum*, *Sorbus commixta*, and *Taxus cuspidate* were collected from a forest (terrestrial habitat) and *Calystegia soldanella*, *Lathyrus japonicus*, and *Orobanche coerulescens* were collected from a sand dune (coastal habitat). All samples were stored at 4°C before fungal isolation. For each sample, 5g of rhizosphere soil was diluted ten-fold with sterile water. Next, 100μL of each dilution was plated on dichloran rose bengal chloramphenicol agar (Difco, Becton Dickinson, Sparks, MD). All plates were incubated at 25°C for seven days. *Penicillium* strains were transferred to a potato dextrose agar (PDA; Difco, Becton Dickinson) plate. Each strain was then stored in 20% glycerol at –80°C at the Seoul National University Fungus Collection (SFC) (Table 1).

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from *Penicillium* using a modified cetyltrimethylammonium bromide extraction protocol [20] with respect to the amount of sample tissue. The primer sets, Bt2a/Bt2b [21] and CF1/CF4 [22] were used to amplify BenA and CaM, respectively. Each PCR was performed in a C1000 thermal cycler (Bio-Rad, Richmond, CA) using previously described methods [14]. The PCR products were purified with an Expin™ PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea), according to the manufacturer’s instructions. DNA sequencing was performed on an ABI Prism 3700 genetic analyzer (Life Technologies, Gaithersburg, MD) at Macrogen (Seoul, Korea), in both directions using the PCR primers.

2.3. Phylogenetic analysis

The sequences were assembled and proofread using MEGA5 [23] and were deposited in GenBank (accession Nos. are shown in Table 1). Molecular identification was performed in two steps. First, the sectional position of the strains was determined by comparison to the BenA sequences with database containing the sequence of the type strains. Next,
### Table 1. Summary and GenBank accession numbers for *Penicillium* strains isolated from the rhizosphere soil of six different plants. The unrecorded *Penicillium* species in Korea are represented in bold.

| Species                | Section         | Strain                        | Location                          | Substrate                  | GenBank accession numbers |
|------------------------|-----------------|-------------------------------|-----------------------------------|----------------------------|----------------------------|
| *P. aurantioclavatum*  | Aspergilloides  | SFCP02.20, ZRSFG0000000005    | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Sorbus commixta            | MT453816                  |
| *P. bissettii*         | Lanata-Divaricata| SFCP02.18                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Sorbus commixta            | MT453814                  |
|                        |                 | SFCP02.19                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Sorbus commixta            | MT453815                  |
|                        |                 | SFCP02.29, ZRSFG0000000006    | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Rhododendron bradycarpum   | MT453824                  |
|                        |                 | SFCP02.59                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Rhododendron bradycarpum   | MT453838                  |
| *P. brasiliense*       | Citrina          | SFCP01.98                     | Yongho-dong, Nam-gu, Busan         | Lathyrus japonicus         | MT453805                  |
|                        |                 | SFCP01.168, NIBRF0000000000    | Sannae-myeon, Miryang-si, Gyeongsangnam-do | Sorbus commixta            | MT453776                  |
| *P. citrinum*          | Citrina          | SFCP01.69                     | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453846                  |
| *P. concentricum*      | Citrina          | SFCP01.70                     | Sannae-myeon, Miryang-si, Gyeongsangnam-do | Sorbus commixta            | MT453777                  |
|                        |                 | SFCP02.31                     | Hwagee-myeon, Hadang-gun, Gyeongsangnam-do | Sorbus commixta            | MT453826                  |
| *P. cosmopolitanum*    | Citrina          | SFCP02.26                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Rhododendron bradycarpum   | MT453831                  |
| *P. crustosum*         | Fasciculata      | SFCP05.25                     | Yongho-dong, Nam-gu, Busan         | Calystegia soldanella      | MT453811                  |
|                        |                 | SFCP05.25                     | Yongho-dong, Nam-gu, Busan         | Calystegia soldanella      | MT453828                  |
|                        |                 | SFCP05.23                     | Yongho-dong, Nam-gu, Busan         | Calystegia soldanella      | MT453834                  |
| *P. daesee*            | Lanata-Divaricata| SFCP02.25                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453821                  |
|                        |                 | SFCP02.26                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453822                  |
| *P. halotolerans*      | Chrysogena       | SFCP01.71, NIBRF0000000000    | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453779                  |
|                        |                 | SFCP01.72                     | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453780                  |
|                        |                 | SFCP01.73                     | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453781                  |
|                        |                 | SFCP01.74                     | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453782                  |
|                        |                 | SFCP01.75                     | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453783                  |
| *P. heteringtonii*     | Citrina          | SFCP09.98                     | Yongho-dong, Nam-gu, Busan         | Lathyrus japonicus         | MT453804                  |
|                        |                 | SFCP09.17                     | Yongho-dong, Nam-gu, Busan         | Lathyrus japonicus         | MT453807                  |
| *P. janczewskii*       | Canescentia      | SFCP02.00                     | Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do | Lathyrus japonicus         | MT453839                  |
|                        |                 | SFCP02.22                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Sorbus commixta            | MT453817                  |
|                        |                 | SFCP02.23                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453819                  |
|                        |                 | SFCP02.24                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453820                  |
|                        |                 | SFCP05.26                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453830                  |
|                        |                 | SFCP05.25                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453835                  |
| *P. javanicum*         | Lanata-Divaricata| SFCP02.14                     | Yongho-dong, Nam-gu, Busan         | Calystegia soldanella      | MT453810                  |
| *P. jejuense*          | Aspergilloides   | SFCP02.22                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Sorbus commixta            | MT453818                  |
|                        |                 | SFCP02.27                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453823                  |
|                        |                 | SFCP02.34                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453829                  |
|                        |                 | SFCP05.27                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453836                  |
|                        |                 | SFCP05.28                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Taxus cuspidata            | MT453837                  |
| *P. kanaanakense*      | Aspergilloides   | SFCP01.76                     | Sannae-myeon, Miryang-si, Gyeongsangnam-do | Sorbus commixta            | MT453784                  |
|                        |                 | SFCP01.77                     | Sannae-myeon, Miryang-si, Gyeongsangnam-do | Sorbus commixta            | MT453785                  |
|                        |                 | SFCP02.32, NIBRF0000000013    | Hwagee-myeon, Hadang-gun, Gyeongsangnam-do | Taxus cuspidate            | MT453827                  |
| *P. ochrochloron*      | Lanata-Divaricata| SFCP02.16                     | See-myeon, Ulleung-gun, Gyeongsangbuk-do | Sorbus commixta            | MT453812                  |
| *P. ortum*             | Lanata-Divaricata| SFCP01.99, NIBRF0000000015    | Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do | Lathyrus japonicus         | MT453806                  |
| *P. pasqualense*       | Citrina          | SFCP02.13                     | Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do | Lathyrus japonicus         | MT453876                  |
| *P. radiolobatum*      | Canescentia      | SFCP01.78                     | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453809                  |
| *P. samsonianum*       | Osmophila        | SFCP01.82, NIBRF0000000018    | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453838                  |
|                        |                 | SFCP01.83                     | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | Orobanchae aereulescens   | MT453839                  |
|                        |                 | SFCP01.84                     | Sannae-myeon, Miryang-si, Gyeongsangnam-do | Sorbus commixta            | MT453790                  |
|                        |                 | SFCP01.85                     | Sannae-myeon, Miryang-si, Gyeongsangnam-do | Sorbus commixta            | MT453860                  |
| Species | Strain | Location | Substrate | GenBank accession numbers |
|---------|--------|----------|-----------|--------------------------|
| *Penicillium* sp. 04 | SFCP0186 | Sannae-myeon, Miryang-si, Gyeongsangnam-do | *Sorbus commixta* | MT843793 |
| *Penicillium* sp. 04 | SFCP0187 | Sannae-myeon, Miryang-si, Gyeongsangnam-do | *Sorbus commixta* | MT843794 |
| *Penicillium* sp. 04 | SFCP0188 | Sannae-myeon, Miryang-si, Gyeongsangnam-do | *Sorbus commixta* | MT843795 |
| *Penicillium* sp. 04 | SFCP0189 | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | *P. scabrosum* | MT843796, MT843861 |
| *Penicillium* sp. 04 | SFCP0190 | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | *Orobanche coerulescens* | MT843797 |
| *Penicillium* sp. 04 | SFCP0191 | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | *Orobanche coerulescens* | MT843798 |
| *Penicillium* sp. 04 | SFCP0192 | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | *Orobanche coerulescens* | MT843799 |
| *Penicillium* sp. 04 | SFCP0193 | Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do | *Orobanche coerulescens* | MT843800 |
| *Penicillium* sp. 08 | SFCP0194 | Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do | *Calystegia soldanella* | MT843802, MT843863 |
| *Penicillium* sp. 24 | SFCP0195 | Seo-myeon, Ulleung-gun, Gyeongsangbuk-do | *Rhododendron brachycarpum* | MT843803, MT843864 |
| *Penicillium* sp. 26 | SFCP0196 | Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do | *Lathyrus japonicus* | MT843808, MT843868 |
| *Penicillium* sp. 08 | SFCP0201 | Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do | *Calystegia soldanella* | MT843833, MT843887 |
| *Penicillium* sp. 24 | SFCP0217 | Seo-myeon, Ulleung-gun, Gyeongsangbuk-do | *Sorbus commixta* | MT843813, MT843873 |
| *Penicillium* sp. 26 | SFCP0218 | Seo-myeon, Ulleung-gun, Gyeongsangbuk-do | *Calystegia soldanella* | MT843825, MT843882 |
| *Penicillium* sp. 26 | SFCP0230 | Seo-myeon, Ulleung-gun, Gyeongsangbuk-do | *Sorbus commixta* | MT843825, MT843882 |
| *Penicillium* sp. 26 | SFCP0237 | Seo-myeon, Ulleung-gun, Gyeongsangbuk-do | *Calystegia soldanella* | MT843832, MT843886 |

For section *Lanata-Divaricata* (7 *Penicillium* spp.) and *Citrina* (6) showed a relatively higher diversity compared to other sections (Table 1). Eight unrecorded species were detected in five sections. These were *Aspergilloides* (3 spp.), *Canescentia* (1 sp.), *Chrysogena* (1 sp.), *Citrina* (1 sp.), and *Lanata-Divaricata* (2 spp.). Four potential new species were identified in section *Canescentia*, *Chrysogena*, *Citrina*, and *Lanata-Divaricata* (Table 1). The detailed morphological descriptions for unrecorded species are presented in taxonomic part.

For section *Aspergilloides*, 10 strains were identified as *P. aurantioviolaceum* (1 strain), *P. jejuense* (5), *P. kankanaskense* (3), and *P. verhagenii* (1). Three species (*P. aurantioviolaceum, P. kankanaskense,* and *P. verhagenii*) were confirmed as unrecorded species in Korea. For section *Canescentia*, 11 strains were confirmed as *P. janczewskii* (5), *P. radiatolobatum* (2), and *Penicillium* sp. 04 (4). *P.
**Figure 2.** Maximum likelihood phylogenetic tree of the combined data set of BenA and CaM used to identify strains to the species level in *Penicillium* 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. Strains reported in the current study are represented in bold. The species labeled in red represent previously unrecorded species in Korea. The names in blue are potential new species.

**radiatolobatum** was an unrecorded species in Korea. *Penicillium* sp. 04 (SFCP0195, SFCP0196, SFCP0201, and SFCP523) formed a distinct clade from previously reported species. For section *Chrysogena*, seven strains were determined as *P. halotolerans* (5) and *Penicillium* sp. 24 (2). *P. halotolerans* was an unrecorded species in Korea. *Penicillium* sp. 24 was a potential new species. For section *Citrina*, 10 strains were identified as *P. cairnsense* (1), *P. citrinum* (1), *P. cosmopolitanum* (1), *P. hetheringtonii* (3), *P. pasqualense* (2), and *Penicillium* sp. 26 (2). *P. cairnsense* was unrecorded species in Korea and
Penicillium sp. 26 was a potential new species. Section Lanata-Divaricata contained seven species (11 strains); P. bissettii (4), P. brasiliannum (1), P. daleae (2), P. javanicum (1), P. ochrochloron (1), P. ortum (1), and Penicillium sp. 08 (1). Two species (P. bissettii and P. ortum) were new records to Korea. Penicillium sp. 08 (SFCP0217) was a potential new species. P. crustosum (3), P. samsonianum (5), P. concentricum (2), and P. scabrosum (5) included in Section Fasciculata, Osnomphila, Penicillium, and Ramasa were identified from rhizosphere soil, respectively (Table 1, Figure 2).

3.2. Penicillium composition

Penicillium halotolerans, P. scabrosum, P. samsonianum, P. jejuense, and P. janczewskii were the dominant species in rhizosphere soil (Table 1). A different number of species were found in the forest (15 spp.) and sand dune (12 spp.). Penicillium sp. 04 was the only species shared across both habitats. Most species were detected in their own sites: forest (14 species) and sand dune (11 species) (Figure 1(B)).

The different numbers (4–11 Penicillium spp.) of Penicillium species were found depending on the plant surveyed. Penicillium diversity was the highest in Sorbus commixta (11 spp.), followed by Orobanchae coerulescens (5) and Lathyrus japonicus (5) (Figure 1(C)). There were only a few Penicillium species commonly found between the plant hosts on the sand dune, whereas more species were found to overlap in the forest (Figure 1(C)).

4. Taxonomy

Penicillium aurantiiovaleum Biourge (1923)

Description: Colony diam, 7 d, in mm: CYA 60–65; CYA 15°C 28–33; CYA 30°C 28–33; CYA 37°C no growth; MEA 50–56; YES 60–65 (Figure 3(A)).

Colony characters: CYA, 25°C, 7d: Colonies low to moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (25D3); exudates clear; soluble pigments absent; reverse color greyish yellow (4B5). MEA, 25°C, 7d: Colonies low to moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (25D3); exudates clear; soluble pigments absent; reverse color greyish yellow with light yellow (3A5) at margin.

Conidiophores monoverticillate, rough walls, 2.4–4.0 μm wide, phialides ampulliform, 8.0–11.0 × 2.5–3.0 μm. Conidia smooth walls, ellipsoidal, 3.0–3.7 × 2.0–2.8 μm; Sclerotia white when young becoming orange (6B7) at age; Ascii and ascospores not observed.

Strain examined: SFCP0220 and SFC20190612-M12

Note: Penicillium aurantiiovaleum is phylogenetically similar to P. fusisporum. The former species can be distinguished from the latter by the shape of the conidia and growth rate on CYA at 25°C. P. aurantiiovaleum is characterized by ellipsoidal spores, whereas P. fusisporum has fusiform to oblong conidia. P. aurantiiovaleum grows faster than P. fusisporum on CYA at 25°C (60–65 vs. 50–53) [29].

Penicillium bissettii Visagie & Seifert (2016)

Description: Colony diam, 7 d, in mm: CYA 45–55; CYA 15°C 25–30; CYA 30°C 35–40; CYA 37°C no growth; MEA 50–60; YES 45–55 (Figure 3(B)).

Colony characters: CYA, 25°C, 7d: Colonies low to moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture floccose; sporulation absent to sparse; exudates clear; soluble pigments absent; reverse color greyish yellow (3B5) with pale yellow (3A3) at margin. MEA, 25°C, 7d: Colonies low, radially sulcate; margins low, wide, entire; mycelia white; texture floccose; sporulation absent to sparse; conidia greyish green (25B2); exudates clear; soluble pigments absent; reverse color deep yellow (4A8). YES, 25°C, 7d: Colonies low to moderately deep, randomly furrowed; margins low, narrow, entire; mycelia white; texture floccose; sporulation dense; conidia greyish green (25B2); exudates absent; soluble pigments absent; reverse color greyish yellow (4B4).

Conidiophores biverticillate; stipes rough walls, 2.0–3.0 μm wide, phialides ampulliform, 7.0–11.0 × 2.5–3.5 μm. Conidia smooth walled, globose to subglobose, 2.5–3.5 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0218, SFCP0219, SFCP0229, and SFCP0529

Note: Penicillium bissettii is similar to P. vasconiae and P. annulatum. This species is characterized by the roughened conidiophores and no growth at 37°C, whereas P. desertorum has smooth walled conidiophores. Penicillium annulatum can be distinguished from P. bissettii by good growth on CYA at 37°C [30]. Penicillium bissetti in Korea grows faster than type strain of Penicillium bissettii on CYA at 25°C [30]. The type strain of Penicillium bissettii showed pinkish red mycelia on MEA, whereas the Korean strains have white mycelia.
Penicillium cairnsense Houbraken, Frisvad & Samson (2011)

Description: Colony diam, 7 d, in mm: CYA 36–38; CYA 15°C 17–19; CYA 30°C 9–10; CYA 37°C no growth; MEA 30–33; YES 42–47 (Figure 3(C)).

Colony characters: CYA, 25°C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (27E3); exudates clear; soluble pigments absent; reverse color light yellow (4A4). MEA, 25°C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (27E3); exudates absent; soluble pigments absent; reverse color light brown (7D5) at center, orange white (5A2) elsewhere. YES, 25°C, 7d: Colonies low to moderately deep, radially sulcate, randomly furrowed as well, sunken in at center; margins low, narrow, entire; mycelia white;

Figure 3. The unrecorded Penicillium species in Korea. (A) P. aurantioviolaceum (SFCP0220), (B) P. bissettii (SFCP0229), (C) P. cairnsense (SFCP0168), (D) P. halotolerans (SFCP0171), (E) P. kananaskense (SFCP0232), (F) P. ortum (SFCP0199), (G) P. radiatolobatum (SFCP0182), (H) P. verhagenii (SFCP0194). (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).
texture velvety; sporulation dense; conidia dull green (27E3); exudates absent; soluble pigments brownish red (9C6); reverse color brownish gray (11E2) with grayish red (8B63) area present.

Conidiophores mostly biverticillate; stipes smooth walls, 2.7–3.7 μm wide, phialides ampulliform, 7.0–10 × 2.5–3.5 μm. Conidia smooth walls, subglobose to broadly ellipsoidal, 2.5–3.5 × 2.5–3.0 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0168 and SFC20200506-M03

Note: *Penicillium cairnsense* is morphologically similar to *P. quebecense*. This species (9–10 mm in length) can be distinguished from *P. quebecense* (16–20 mm) by slower growth on CYA at 30 °C [31].

**Penicillium halotolerans** Frisvad, Houbraken & Samson (2012)

Description: Colony diam, 7 d, in mm: CYA 30–35; CYA 15 °C 20–25; CYA 30 °C 30–35; CYA 37 °C 10–13; MEA 34–36; YES 50–54 (Figure 3(D)).

Colony characters: CYA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia grayish turquoise (24D3); exudates clear to pale yellow (3A3); soluble pigments absent; reverse color yellowish orange (4B7). MEA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety, slightly floccose in center;

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Figure 3. Continued
sporulation moderate; conidia grayish turquoise (24D3); exudates clear to pale yellow (3A3) at center; soluble pigments absent; reverse color brownish orange (5C6). YES, 25°C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture floccose; sporulation moderate; conidia grayish turquoise (24D3); pale turquoise (24A3) at margin; exudates absent; soluble absent; reverse color light yellow (4A4).

Conidiophores terverticillate; stipes smooth walls, 3.0–4.0 μm wide, phialides ampulliform, 6.0–11.0 × 2.5–3.5 μm. Conidia smooth walls, globose, 2.5–3.5 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0171, SFCP0174, and SFC20200506-M02

Note: *Penicillium cairnsense* is phylogenetically similar to *P. desertorum*. This species is characterized by radially sulcate colonies on YES, whereas *P. desertorum* has randomly furrowed colonies on YES. *Penicillium halotolerans* in Korea grows faster than the type strain of *P. halotolerans* on CYA at 30 and 37°C. (30–35 vs. 20–25 at 30°C and 10–13 vs. 0–2 at 37°C) [32].

**Penicillium kananaskense** Seifert, Frisvad & McLean (1994)

Description: Colony diam, 7 d, in mm: CYA 38–42; CYA 15°C 24–26; CYA 30°C 14–16; CYA 37°C no growth; MEA 42–47; YES 45–48 (Figure 3(E)).

Conidiophores mostly biverticillate, sometimes monoverticillate; stipes smooth walls, 2.0–3.2 μm wide, phialides ampulliform, 6.5–9.5 × 2.2–3.2 μm. Conidia smooth walls, globose to subglobose, 2.5–3.3 × 2.4–3.1 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0199 and SFC20200506-M55

Note: *Penicillium orincki* (1972) is phylogenetically similar to *P. cremeogriseum*. The former species can be distinguished from the latter by relatively fast growth on CYA at 30°C (38–44 mm) [34].

**Penicillium radiotolobatum** Lorinczi (1972)

Description: Colony diam, 7 d, in mm: CYA 35–40; CYA 15°C 18–21; CYA 30°C 28–33; CYA 37°C 10–13; MEA 30–35; YES 45–51 (Figure 3(G)).

Conidiophores monoverticillate; stipes smooth walls or rough walls, 3.0–4.5 μm wide, phialides ampulliform, 8.0–13.0 × 3.0–4.0 μm. Conidia rough walls, broadly ellipsoidal to ellipsoidal, 3.0–3.5 × 2.5–3.0 μm; Sclerotia absent; Asci and ascospores not observed.
at margin. MEA, 25°C, 7d: Colonies low, radially sulcate; margins low, narrow, entire; mycelia white; texture floccose; sporulation moderate; conidia dull green (28E3); exudates hyaline to orange white (5A2) droplets in central areas; soluble pigments absent; reverse color light brown (6D5). YES, 25°C, 7d: Colonies low, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety to floccose; sporulation moderate; conidia greenish gray (25D2); exudates absent; soluble pigments absent; reverse color brown (7E7).

Conidiophores biverticillate; stipes smooth walls, 2.8–4.0 μm wide, phialides ampulliform, 5.5–9.0 × 2.5–3.0 μm. Conidia smooth walls, globose to subglobose, 2.8–3.2 × 2.8–3.1 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0182, SFCP0183, SFC20171120-M01, and SFC20200506-M01

Note: *Penicillium radiatolobatum* is phylogenetically similar to *P. canescens*. This species can be distinguished from *P. canescens* by faster growth on CYA and MEA at 25°C [30,35].

**Penicillium verhagenii** Houbraken (2014)

Description: Colony diam, 7 d, in mm: CYA 27–30; CYA 15°C 24–26; CYA 30°C no growth; CYA 37°C no growth; MEA 28–31; YES 34–40 (Figure 3(H)).

Colony characters: CYA, 25°C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety to floccose; sporulation moderate; conidia grayish green (27C3); exudates absent; soluble pigments absent; reverse color pale yellow (4A3) to grayish yellow (4B5). MEA, 25°C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety to floccose; sporulation moderate; conidia dull green (25E4); exudates absent; soluble pigments absent; reverse color pale brownish orange (5C4). YES, 25°C, 7d: Colonies low to moderately deep, radially sulcate, random furrows also present; margins low, narrow, entire; mycelia white; texture velvety to floccose; sporulation moderate; conidia dull green (27E3) at the center of colony, but greenish gray (27B2) at margin; exudates absent; soluble pigments absent; reverse color grayish yellow (4B3).

Conidiophores biverticillate; stipes finely rough-walled, 2.8–3.8 μm wide, phialides ampulliform, 8.0–11.0 × 2.8–3.5 μm. Conidia roughened walls, broadly ellipsoid to ellipsoidal, 3.0–4.0 × 2.5–3.5 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0194

Note: *Penicillium verhagenii* is phylogenetically similar to *P. ranomafanaense*. *P. verhagenii* is characterized by yellow reverse colors on CYA and YES, whereas *P. ranomafanaense* has orange or reddish [33].

### 5. Discussion

The rhizosphere is known as the most dynamic environment that provides a close association between plant root and fungi [1]. We isolated 64 *Penicillium* strains from rhizosphere soil of six plants. They were identified accurately by sequence-based identification as 26 species in nine sections. Four species could not be identified due to an unclear phylogenetic relationship. They were designated as *Penicillium* sp. We might be able to report them as new species in the future after a more detailed morphological comparison with phylogenetically similar species.

Eight species were records for the first time in Korea. These were *P. aurantioviolaceum*, *P. bissettii*, *P. cairnsense*, *P. halotolerans*, *P. kananaskense*, *P. ortum*, *P. radiatolobatum*, and *P. verhagenii*. The morphological characteristics of the unrecorded species were consistent with those of the respective type species, except for *P. bissettii* and *P. halotolerans*. The strains isolated from Korea of these two species grow faster compared to the type strains. Some fungi exhibit different growth rates or metabolism as they adapt to different environments [36,37]. *P. bissettii* and *P. halotolerans* were isolated from sand dune in Korea, whereas their type cultures were obtained from forest soil and salt marsh, respectively [30,32]. Although this is a small-scale study in a limited habitat, many unrecorded species and potential new species have been found. By analyzing a larger variety of hosts, it would be possible to discover more species.

Fungal diversity and composition were significantly correlated with habitat and plant communities [38,39]. Fungal diversity was significantly higher in terrestrial habitats than freshwater and mangrove habitats [38]. Similarly, the diversity of *Penicillium* species in rhizosphere soil was relatively higher in terrestrial habitats than coastal habitats. Most *Penicillium* species from rhizosphere soil were unique to each plant. The composition of the *Penicillium* species did not only differ by habitats but also by the plant host species. The *Penicillium* composition, in particular, is relatively much affected by plants in the marine environment. Despite the two host plants, *Calystegia soldanella* and *Lathyrus japonicus* having very similar environments, such as poor nutrient and abiotic stresses, fungal diversity varied depending on plants [40]. Recently, various *Penicillium* species have been reported by NGS-based studies in rhizosphere soil.
Although the role of these Penicillium in rhizosphere soil is unclear, they might have important effects on plants. Further studies are required to investigate the function of Penicillium in rhizosphere soil.

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