

# Sixteen New Records of Ascomycetes from Crop Field Soil in Korea

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**ABSTRACT:** The present study reports 16 species of Ascomycota that were previously unknown in Korea, namely *Acremonium cellulolyticus* (KNU14-25), *Acremonium zonatum* (KNU14-15), *Chaetomium madrasense* (KNU14-9), *Cladosporium silenes* (KNU 14-18-1), *Humicolopsis cephalosporioides* (KNU15-3), *Leptosphaerulina chartarum* (KNU14-16), *Paecilomyces marquandii* (KNU14-8), *Paecilomyces tenuis* (KNU14-18-2), *Paraphaeosphaeria sporulosa* (KNU15-2), *Penicillium rubidurum* (KNU14-12), *Pochonia suchlasporia* (KNU15-6), *Sporothrix inflata* (KNU15-8), *Thielavia hyrcaniae* (KNU15-1), *Thielavia terricola* (KNU14-23-1), *Xylogone sphaerospora* (KNU15-7), and *Zopfiella longicaudata* (KNU15-5). These fungal species were isolated from soil samples collected from various regions of Korea and identified based on their morphological characteristics and rDNA internal transcribed spacer sequence data. Full descriptions and illustrations for each species are provided.

**KEYWORDS:** Ascomycota, Diversity, Morphology, rDNA

## Introduction

Ascomycota, the largest phylum of fungi, is morphologically diverse group covering from unicellular yeasts to complex cup fungi. During a survey on diversity of soil-inhibiting fungi we encountered sixteen ascomycetes' fungi, which have not been officially reported in Korea; *Acremonium cellulolyticus*, *Acremonium zonatum*, *Chaetomium madrasense*, *Cladosporium silenes*, *Humicolopsis cephalosporioides*, *Leptosphaerulina chartarum*, *Paecilomyces marquandii*, *Paecilomyces tenuis*, *Paraphaeosphaeria sporulosa*, *Penicillium rubidurum*, *Pochonia suchlasporia*, *Sporothrix inflata*, *Thielavia hyrcaniae*, *Thielavia terricola*, *Xylogone sphaerospora* and *Zopfiella longicaudata*.

The genus *Acremonium* consists of most simply struc-

ured among all filamentous anamorphic fungi with the morphological characteristics of septate hyphae, tapered, and mostly lateral phialides produced singly or in small groups [1]. *Acremonium zonatum* and *Acremonium cellulolyticus* belong to the family *Hypocreaceae*. The latter species produces cellulose which is mainly used for management of silage and has been shown to improve silage quality [2]. *Paecilomyces* is genus of the order Eurotiales. The genus *Paecilomyces* was first introduced by Bainier and he described that the genus is differentiated from the closely related genus *Penicillium* having long divergent phialides with colonies that are never typically green [3]. *Paecilomyces* consists of more than 30 recognized species [2]. *P. marquandii* is known for its ability to degrade herbicides like alachlor [4-5]. The synonymy of *P. marquandii* is *Metarhizium marquandii* [6]. *Paecilomyces* species are abundant on soils, indoor and outdoor air, food and water and have cosmopolitan distributions [4, 5, 7, 8]. *Paecilomyces tenuis* is characterized by the very slender phialides and fusiform to ellipsoidal conidia [9]. This species are thermophilic or thermotolerant [10].

Since *Paecilomyces* species are ubiquitous in nature, they are frequently cited in ecological studies. However, there may be many indigenous fungal species of *Paecilomyces* that have not been investigated yet. Thus, it is necessary to execute investigations regarding unreported indigenous fungal species.

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*Penicillium* is the member of the order Eurotiales and *Trichocomaceae* family recorded from soil and have a worldwide distribution. *Penicillium* is a genus of ascomycetous fungi which have greater importance in food and drug production as well as in natural environment [1]. *Penicillium rubidurum* is an anamorph, monoverticillate species of the genus *Penicillium* [8]. *Chaetomium madrasense*, *Thielavia terricola* and *Thielavia hyrcaniae* belongs to the family *Chaetomiaceae*. Members of the family *Chaetomiaceae* are ubiquitous ascospore-producing fungi commonly found in soil enriched manure or cellulosic materials [11]. *C. madrasense* is considered to be a rather common species and is known from several types of substrate in various regions of the world [12].

*Paraphaeosphaeria sporulosa* belongs to the family *Phaeosphaeriaceae*. Conidiogenous cells of *P. sporulosa* are globose to ampulliform, hyaline, discrete or positioned on aggregated clumps of cells that protrude into the cavity [13]. *Zopfiella longicaudata* and *Sporothrix inflata* belong to the family *Lasiophaeriaceae*. *Zopfiella* species consists of non-ostiolate ascomata, clavate to cylindrical usually evanescent asci lacking an apical ring [14]. The anamorphs of most *Zopfiella* species were unknown. *Zopfiella latipes* forms a Humicola-like anamorph in culture [15]. The anamorphic fungus *Sporothrix inflata*, known as a soil-borne fungus, can colonize living, dead as well as partly deteriorated roots [16].

Species of *Cladosporium* are cosmopolitan in distribution and many of them are agents of decay, deterioration, or a cause of allergy or even plant or animal disease [17]. The *Cladosporium* spp. are often of high environmental impact, the genus is of interest to researchers in a wide variety of disciplines [17]. *Cladosporium silenes* was named after the host plant, *Silene maritima*, on which it was collected [18].

Soil fungi play the crucial roles in terrestrial and microbial communities and in decomposition of organic matter and nutrient cycling [19]. For sixteen soil fungi newly isolated Ascomycetes from Korea, this work performed the morphological investigation and molecular phylogenetic analysis of the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) regions.

## Materials and Methods

### Soil sample collection and fungal isolation

Soil samples were collected in 2014 and 2015 from various places of Korea (Table 1). Soil samples were taken

from 0~15 cm depth after scrapping and removing the leaf litters and other plant debris on soil surface. Approximately 200~250 g soils were collected for each sample. The collected soil samples were prepared by drying at room temperature, grinding and sieving with an autoclave-sterilized brass sieve of 2 mm aperture size. The fungi were isolated using the dilution technique [20]. Briefly, 1 gram of soil sample was suspended in 9 mL of sterile distilled water and vigorously shaken for 2~3 min. Then, soil suspensions were diluted serially in sterile distilled water. One milliliter of each  $10^3$ ,  $10^4$ ,  $10^5$  and  $10^6$  dilution was pipetted out and poured into petri plates containing potato dextrose agar (PDA; Difco, Detroit, MI, USA); and plates were incubated for 5~7 days at 25°C until growth of fungal colony was observed. The morphologically distinct colonies were selected and further purified by sub-culturing on the plates containing PDA. The pure cultures were preserved on 20% glycerol stock at 4°C for further studies.

### Morphological characterization

Morphological characteristics of all the isolates were studied by growing on potato dextrose agar (PDA), oatmeal agar (OA), malt extract agar (MEA) and potato carrot agar (PCA). KNU14-8, KNU14-18-2 and KNU14-2 were additionally grown on czapek yeast extract agar (CYA) and yeast extract sucrose agar (YES). The strains were inoculated at three points on 9-cm petri dishes and incubated for 10 days at 26°C in dark place. All these media were prepared as described by Samson [21]. After incubation, the colony diameter on the various agar media was measured. Colony color (obverse and reverse sides) and the degree of sporulation were observed. Colony colors were described using Kornerup and Wanscher [22]. Microscopic pictures were taken with an HK 3.1 CMOS digital camera (KOPTIC, Seoul, Korea) connect to an Olympus BX50F-3 microscope (Olympus, Tokyo, Japan) and a scanning electron microscope (LEO Model 1450VP Variable Pressure Scanning Electron Microscope; Carl Zeiss, Oberkochen, Germany).

### Genomic DNA extraction, sequencing and data analysis

Total genomic DNA of the isolates were extracted using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. The internal transcribed spacer region (ITS) gene was amplified using primers ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [19]. The

**Table 1.** Place, GPS, accession numbers and sequence similarity of the studied fungal isolates

Place of collection	GPS of location	Isolates	NIBR deposition no.	GenBank accession no.	Gen region	Closest GenBank library strain	Similarity (%)
Pyeongchang, Kangwon-do	34°92'8.79" N 127°16'9.79" E	KNU14-25	NIBRFG0000142406			<i>Acremonium cellulolyticus</i>	99
Seongnam, Gyeonggi-do	37°43'0.44" N 127°13'7.26" E	KNU14-15	NIBRFG0000142398	KP055592		<i>Acremonium zonatum</i>	99
Yanggu, Kangwon-do	38°07'02.01" N 128°02'95.53" E	KNU14-9	NIBRFG0000135224	KP055593		<i>Chaetomium madrasense</i>	98
Hwaseong, Gyeonggi-do	37°11'7.96" N 126°80'2.43" E	KNU14-18-1	NIBRFG0000142400	KP055594		<i>Cladosporium silenes</i>	100
Pyeongchang, Kangwon-do	35°67'3.03" N 127°51'2.55" E	KNU15-3	NIBRFG0000142307	KY316383		<i>Humicolopsis cephalosporioides</i>	100
Cheongwon, Gyeongsangnam-do	36°76'70.42" N 127°49'79.36" E	KNU14-16	-	KP055597	ITS rDNA	<i>Leptosphaerulina chartarum</i>	99
Pyeongchang, Kangwon-do	37°36'6.21" N 128°28'34.24" E	KNU14-8	NIBRFG0000135221	KY316388		<i>Paecilomyces marquandii</i>	100
Hwaseong, Gyeonggi-do	37°24'27" N 126°80'7.56" E	KNU14-18-2	NIBRFG0000142401	KY316389		<i>Paecilomyces tenuis</i>	99
Boeun, Chungcheongbuk-do	36°46'8.85" N 127°73'91" E	KNU15-2	NIBRFG0000142228	KY316382		<i>Paraphaeosphaeria sporulosa</i>	100
Hwaseong, Gyeonggi-do	37°12'11.64" N 126°80'38.06" E	KNU14-12	NIBRFG000013521	KP055596		<i>Penicillium rubidurum</i>	99
Okcheon, Chungcheongbuk-do	36°31'6.09" N 127°60'9.19" E	KNU15-6	NIBRFG0000142316			<i>Pochonia suchlasporia</i>	98
Pyeongchang, Kangwon-do	36°28'5.94" N 127°55'1.37" E	KNU15-8	NIBRFG0000142323	KY316387		<i>Sporothrix inflata</i>	99
Okcheon, Chungcheongbuk-do	36°32'0.34" N 127°55'7.91" E	KNU15-1	NIBRFG0000142210	KY316381		<i>Thielavia hyrcaniae</i>	98
Seongnam, Gyeonggi-do	37°43'0.44" N 127°13'7.26" E	KNU14-23-1	NIBRFG0000142404	KP055605		<i>Thielavia terricola</i>	98
Pyeongchang, Kangwon-do	36°27'8.61" N 127°58'8.68" E	KNU15-7	NIBRFG0000142321	KY316386		<i>Xylogone sphaerospora</i>	98
Okcheon, Chungcheongbuk-do	36°28'014" N 127°55'9.79" E	KNU15-5	NIBRFG0000142312	KY316385		<i>Zopfiella longicaudata</i>	98

ITS, internal transcribed spacer.

amplified PCR products were sequenced with the ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). ITS sequences was implied to compare the sequences of GenBank at National Center for Biotechnology Information (NCBI) applying the basic local alignment search tool [23]. The nucleotide sequences were deposited at culture collection of National Institute of Biological Resources (NIBR). The culture numbers assigned by NIBR and accession numbers provided by GenBank were listed in Table 1. Phylogenetic relationships were analyzed using molecular evolutionary genetic analysis (MEGA 6) software [24]. Neighbor-joining phylogenetic

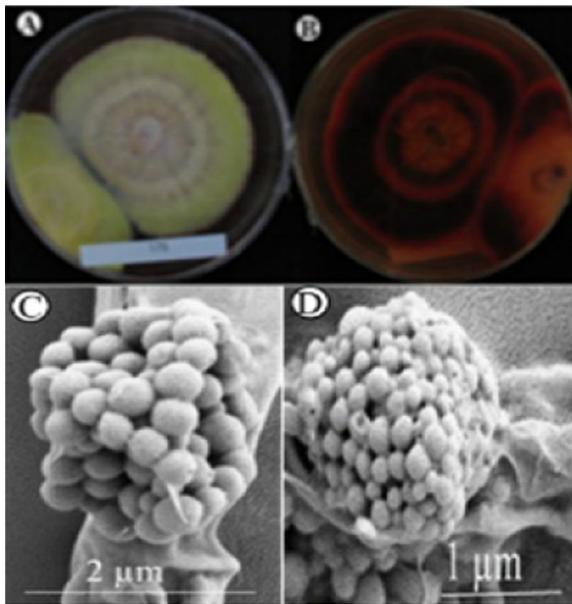
tree was constructed to infer the evolutionary relationship between the Korean isolates and the previously reported species with the type strains.

## Results

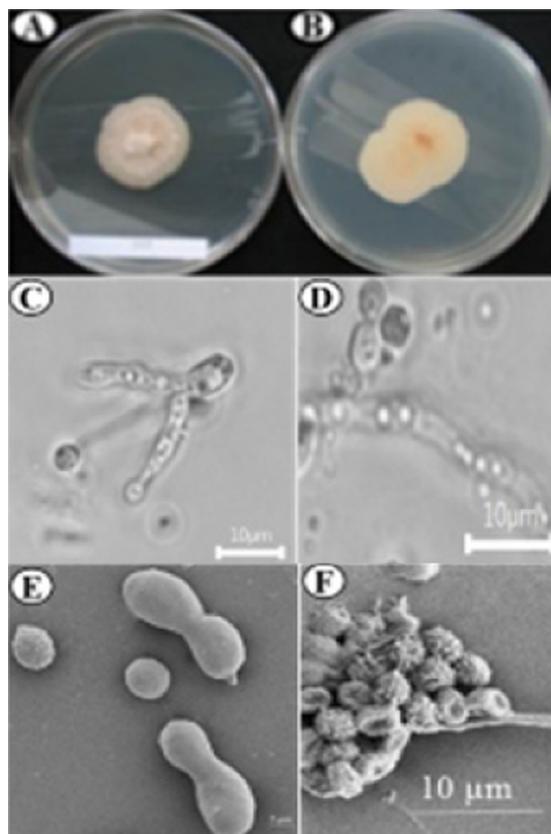
### Morphology of all the studied fungal isolates

*Acremonium cellulolyticus* (KNU14-25): *Talaromyces cellulolyticus* T. Fujii, FEMS Microbiology Letters 351: 39 (2013) [MB#805758]

Colony on PDA attains 55~60 mm in diameter. Front side of the colony was white at center and light green at



**Fig. 1.** Morphological characters of *Acremonium cellulolyticus* (KNU14-25) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, SEM of conidiophores.



**Fig. 2.** Morphological characters of *Acremonium zonatum* (KNU14-15) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, simple microscopic picture of conidiophores; E, F, SEM of conidiophores.

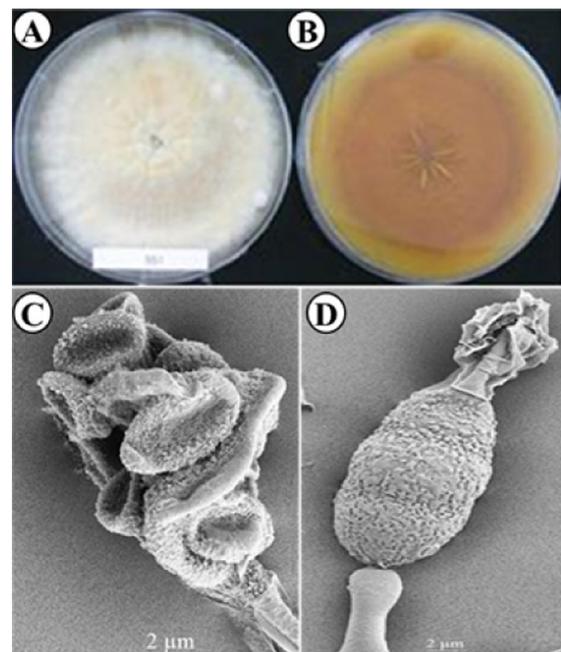
the margins and brown at back side (Fig. 1). Mycelium was white centrally and light green peripherally (Fig. 1). Exudate was absent. Irregular form and surface was rough. Sporulation was moderate to dense. Texture was woolly. Conidia was subglobose with the diameter of 0.4~2.1 µm.

***Acremonium zonatum* (KNU14-15): *Acremonium zonatum* (Sawada) W. Gams, *Cephalosporium-artige Schimmelpilze: 119* (1971) [MB#308213]**

Colonies attains 15~20 mm diameter of growth at 25°C on PDA with white to slightly pinkish in color. Conidiophores are very variable in length. Growth of colony was slow. Texture was woolly. Irregular form and surface was rough. The fungus has herbicidal properties and is used to control water hyacinth (Fig. 2). Exudate was absent. Sporulation was moderate to dense.

***Chaetomium madrasense* (KNU14-9): *Chaetomium madrasense* Natarajan, *Proc. Indian Acad. Sci. (Pl. Sci.): 255-264* (1971) [MB#310909]**

Colonies on PDA with aerial mycelium or sometimes partially with quite sparse white to yellowish aerial mycelium. Reverse light yellow to dark yellowish (Fig. 3). Colored exudates absent. Ascomata superficial, in reflected light olivaceous-green at first, then becoming black due to emerging ascospore masses from ascomatal ostioles, obo-



**Fig. 3.** Morphological characters of *Chaetomium madrasense* (KNU14-9) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, SEM of ascospores.

vate, spherical or nearly so, ostiolate, 198~273  $\mu\text{m}$  high, 149~198  $\mu\text{m}$  diam.; ascomatal wall brown, composed of textura intricata in surface view. Terminal hairs flexuous, undulate or slightly coiled, pale brown, indistinctly septate, verrucose, 2~3.5  $\mu\text{m}$  broad near the base. Asci fasciculate, clavate, stalked, without apical structures, 68~90  $\times$  11~16  $\mu\text{m}$ , 8-spored, evanescent. Ascospores limoniform, slightly biapiculate, bilaterally flattened, usually with a lateral bulge, olivaceous-brown or brown when mature, containing several droplets, 9~11.5 (~14)  $\times$  7.5 ~ 9 (~10)  $\times$  6~7.5  $\mu\text{m}$ , with an apical germ pore.

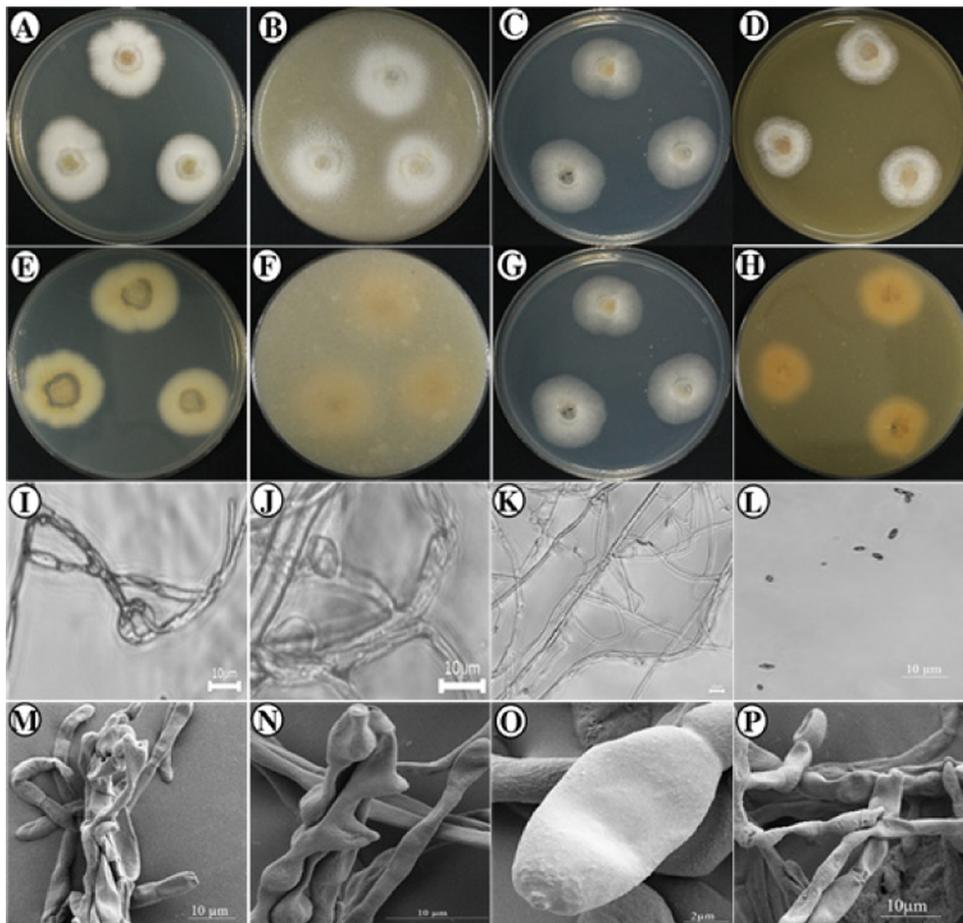
***Cladosporium silenes* (KNU14-18-1):** Crous, P.W; Tanaka, K; Summerell, B.A; Groenewald, J.Z. 2011. Additions to the *Mycosphaella* complex, IMA Fungus. 2(1): 49-64. [MB#560083]

Colonies of KNU 14-18-1 isolate cultured on different

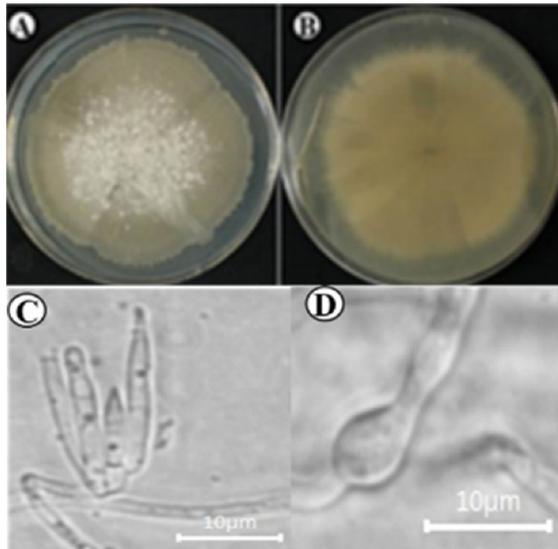
growth media viz., PDA, MEA, OA and PCA media at 25°C showed aerial mycelium, lobate margins and reached 24~25 mm, 22~25 mm, 21~25 mm and 16~18 mm diameter, respectively (Fig. 4A~4H). Colony surface was grey-olivaceous, reverse olivaceous-grey. Sporulation was moderate to dense. Exudate was absent.

**Taxonomy of the isolate KNU 14-18-1: Colony morphology of the present isolate was similar to one of *Clastoridium silenes* (Bensch et al., 2010)**

Mycelium of the KNU 14-18-1 isolate were septated, constricted, refractive, 2~4(~4.8)  $\mu\text{m}$  wide, branched with rare swellings and consisted of pale to light brown hyphae (Fig. 4I~4K). Conidiophores were arising terminally and laterally from ascending hyphae of diameter 14~50 (~150)  $\times$  2.5~4  $\mu\text{m}$ , (Fig. 4I~4K). Ramoconidia was aseptate, smooth, slightly curved, cylindrical of 12~22  $\times$  3~5 (~5.4)



**Fig. 4.** Morphological characters of *Cladosporium silenes* (KNU14-18-1) grown for 7 days on PDA, OA, MEA and PCA at 26°C. A~D, obverse colony from left to right grown on PDA, OA, MEA and PCA; E~H, reverse colony from left to right grown on PDA, OA, MEA and PCA; I~J, conidiophores and conidia produced on PDA and MEA; K, simple microscopic picture of conidiophore; L~N, SEM of conidiophore; O~P, SEM of conidia. PDA, potato dextrose agar; OA, oatmeal agar; MEA, malt extract agar; PCA, potato carrot agar.



**Fig. 5.** Morphological characters of *Humicolopsis cephalosporioides* (KNU15-3) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, SEM of conidia.

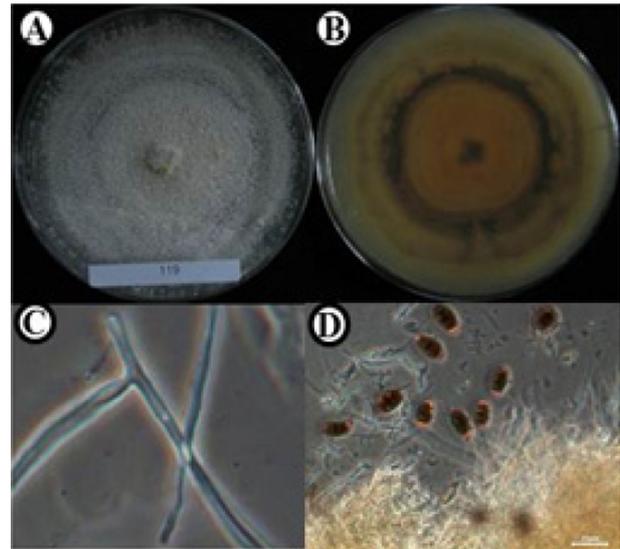
μm diameter. Conidia were aseptate, ovoid, with darkened and thickened hilum,  $4\sim 5 \times 3$  ( $\sim 3.5$ ) μm, abundant, connected in branched chains of up to 7 branches (Fig. 4I–4K).

***Humicolopsis cephalosporioides* (KNU15-3):** *Humicolopsis cephalosporioides* Cabral & Marchand, *Boletín de la Sociedad Argentina de Botánica* 17 (1-2): 70 (1976) [MB#315257]

Colonies on PDA was flat, smooth, umbonate and yellowish white. Colony growth was fast attaining a diameter of 70~75 mm in 7 days at 25°C. Exudate was absent. Sporulation was moderate to dense. Conidiophores were more or less differentiated and mostly unbranched, up to 90 μm long (Fig. 5).

***Leptosphaerulina chartarum* (KNU14-16):** *Leptosphaerulina chartarum* Cec. Roux, *Transactions of the British Mycological Society* 86 (2): 320 (1986) [MB#103530]

Colony growth was fast and attained 70~80 mm diameter in 7 days at 25°C, the front side of the colony was gray brown (Fig. 6) and the reverse side of it was tan. Mycelium was transparent, branched and septated. Vegetative and spore hypha was 2.0 to ~7.5 μm and 2.0 to 5.0 μm in diameter, respectively. Exudate was absent and sporulation was moderate. Conidia was dark brown in color and elliptic and obtuse on the apex. Three transverse septum and 0 to 1 longitudinal septa was usually observed in the



**Fig. 6.** Morphological characters of *Leptosphaerulina chartarum* (KNU14-16) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, SEM of conidia.

conidium and size is 10.0 to 19.0 μm.

***Paecilomyces marquandii* (KNU14-8):** *Paecilomyces marquandii* (Masse) S. Hughes, *Mycological Papers* 45: 30 (1951) [MB#302194]

Micrographs of morphological structures of the isolate are shown in Fig. 3. Colony grew moderately on PDA attaining a diameter of approximately 30~36 mm at 26°C after 14 days of inoculation. Front side of the colony color was whitish yellow and back side was light yellow (Fig. 7A, 7F). Exudates were absent, margin was irregular. Texture of colony was floccose. Colony margin was undulate. Sporulation was moderate to dense. On OA, colony was light brown at the center with white in margin in front side and yellow at back side. Colony was grown slowly reaching a diameter of 15~20 mm within 14 days at 26°C (Fig. 7B, 7G). Exudates absent, margin was slightly irregular. Texture of colony was floccose, and colony margin was undulate. Sporulation was moderate to dense. On CYEA, colony color was powdery white at front and yellowish brown at back side. Colony grew slowly attaining a diameter of 19~24 mm within 14 days at 26°C (Fig. 7C, 7H). Sporulation was moderate to dense. Exudates was absent. Texture of colony was floccose, and margin was undulate. On YES, colony grew moderately reaching a diameter of 21~25 mm within 14 days at 26°C (Fig. 7D, 7I). Front side of the colony color was white in color and back side was yellow in color. Colony was light brown at

the center. Exudates was absent, margin was slightly irregular, texture of the colony was floccose, and sporulation was moderate to dense. On MEA, colony grew moderately attaining a diameter of 22~26 mm within 14 days at 26°C (Fig. 7E, 7J). Front and back side of the colony color was light and yellowish brown respectively. Sporulation was moderate to dense. Exudates was absent, margin was slightly irregular, and texture of the colony was floccose.

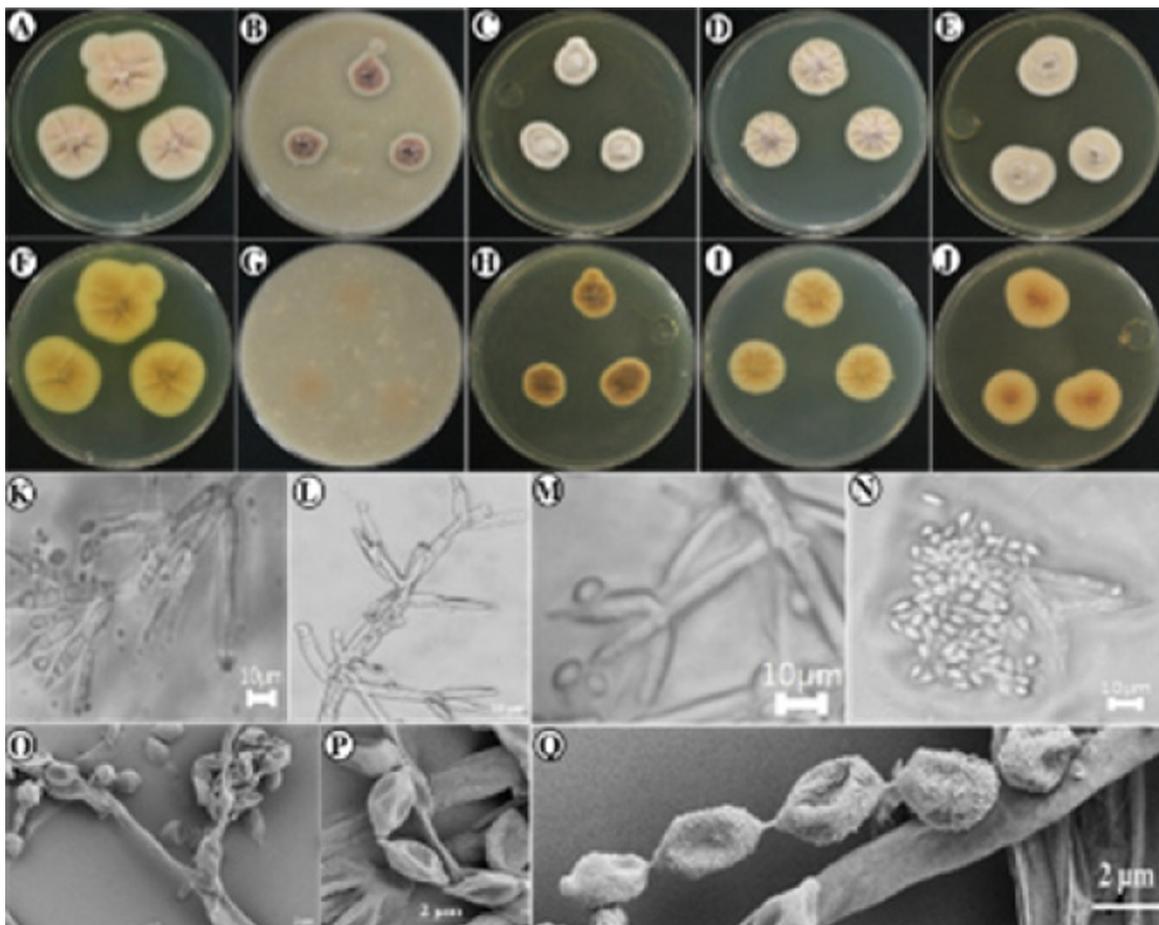
**Taxonomy of the isolate KNU14-8: *Paecilomyces marquandii* (Masse) S. Hughes, Mycological Papers 45: 30 (1951) [MB#302194] = *Metarhizium marquandii* (Masse) Kepler S.A Rehner & Humber. Comb. nov. Mycobank [MB806091]**

Conidiophores were hyaline, smooth walled.  $48\sim 292 \times 2.2\sim 2.7 \mu\text{m}$  in diameter (Fig. 7K~7M, 7O). Conidia were in chains, ellipsoidal and smooth walled of  $2.5\sim 2.9 \times 1.5\sim$

$2 \mu\text{m}$  diameter and dry chains or aggregating spore group (Fig. 7N, 7P, 7Q). Phialides were short cylindrical of  $7\sim 12 \times 1.5\sim 2 \mu\text{m}$  diameter. The phialides consist of a swollen basal part, tapering into thin and distinct necks. Chlamydospores-like structures were thin-walled, globose to ellipsoidal and  $2.9 \mu\text{m}$  in diameter.

***Paecilomyces tenuis* (KNU14-18-2): *Paecilomyces tenuis* Y. F. Han & Z.Q. Liang, Mycotaxon 102: 54 (2007) [MB#510919]**

Micrographs of morphological structures of the isolate are shown in Fig. 8. Colony on PDA, attaining a diameter of 34~40 mm within 14 days at 26°C. The front side of mycelium was milky white in color while back side was yellowish brown (Fig. 8A, 8F). Sporulation was dense, conidia were in mass. Irregular form, smooth surface. The color was initially white then became light yellow. Colony



**Fig. 7.** Morphological characters of *Paecilomyces marquandii* KNU14-8 grown for 7 days on PDA, OA, CYA and YES at 26°C. A~D, obverse colony from left to right grown on PDA, OA, CYA and YES; E~H, reverse colony from left to right grown on PDA, OA, CYA and YES; I~J, conidiophores and conidia produced on PDA and MEA; K, simple microscopic picture of conidiophore; L~N, SEM of conidiophore; O~Q, SEM of conidiophore and conidia. PDA, potato dextrose agar; OA, oatmeal agar; CYA, czapek yeast extract agar; YES, yeast extract sucrose agar; MEA, malt extract agar; PCA, potato carrot agar.

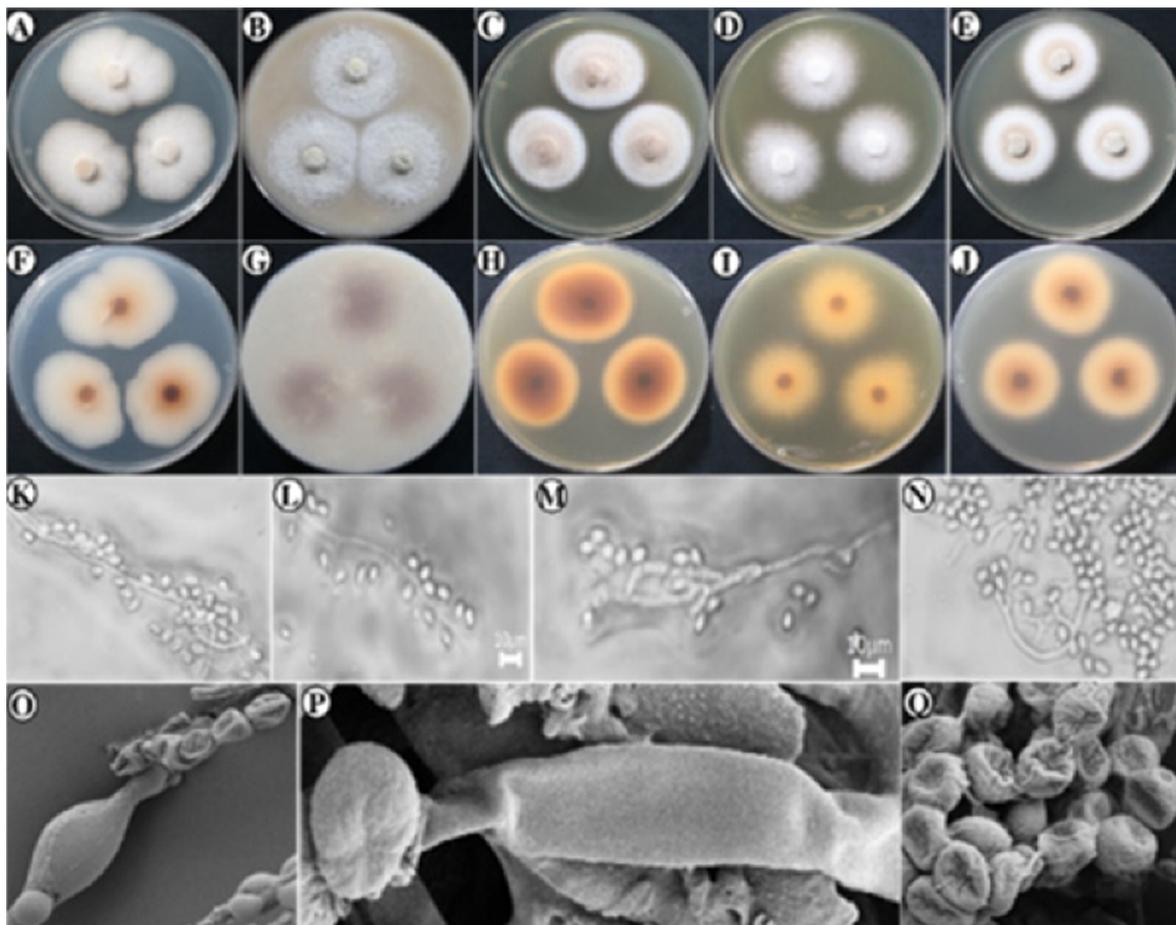
on OA, attaining a diameter of 33~38 mm within 14 days at 26°C. The front side of mycelium was wooly white in color while back side was light brown (Fig. 8B, 8G). Sporulation was moderate, conidia were in mass. Irregular form, rough surface. On CYA, attaining a diameter of 33~38 mm within 14 days at 26°C. The front side of mycelium was white and back side was reverse yellowish in color (Fig. 8C, 8H). Sporulation was moderate to dense. Conidia were in mass. Irregular form, smooth surface. On YES, attaining a diameter of 35~39 mm within 14 days at 26°C. The front side of mycelium was powdery white and back side was yellow in color (Fig. 8D, 8I). Sporulation was moderate to dense. Conidia were in mass. Irregular form, smooth surface. On MEA, attaining a diameter of 25~34 mm within 14 days at 26°C. The front side of mycelium was powdery white and back side was yellow in color (Fig. 8E, 8J).

**Taxonomy of the isolate KNU14-18-2. *Paecilomyces tenuis* Y. F. Han & Z.Q. Liang, Mycotaxon 102: 54 (2007) [MB#510919]**

Vegetative hyphae was hyaline, smooth-walled, 1.0~1.2 µm wide. Conidia was hyaline, smooth-walled, fusiform to long-ellipsoidal. Conidiophores were septate, 12~17.3 µm in diameter, forming a veticillate branches with phialides in whorls of 2~4. Conidia were in chains ellipsoidal and smooth walled of 2.3~2.6 × 1.5~1.7 µm diameter Phialides were divergent, very slender and consisting of a cylindrical or clavate basal portion.

***Paraphaeosphaeria sporulosa* (KNU15-2): *Paraphaeosphaeria sporulosa* (W. Gams & Domsch) Verkley, G Gkl & Stielow, Persoonia 32: 47 (2014) [MB#800768]**

Colonies on PDA: growing very fast and attains 80~90 mm diameter in 7 days at 25°C. The colony color is creamy

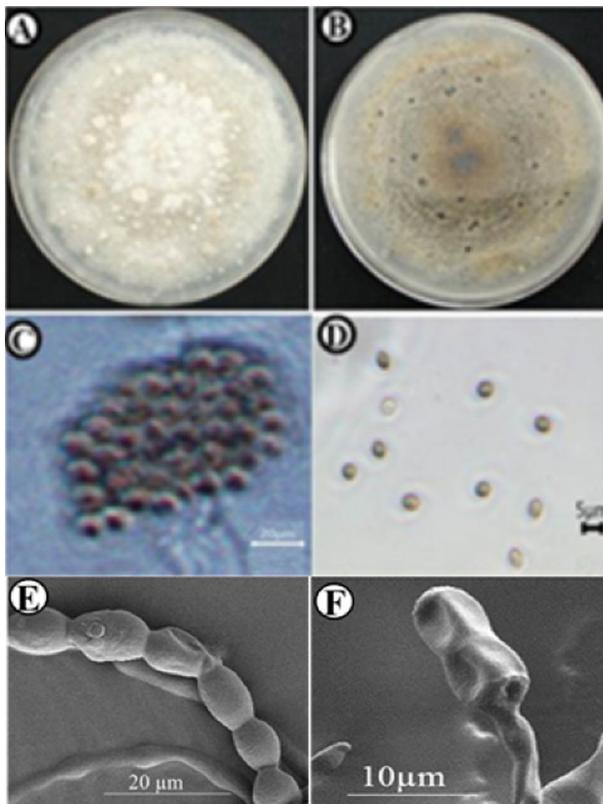


**Fig. 8.** Morphological characters of *Paecilomyces tenuis* KNU14-18-2 grown for 7 days on PDA, OA, CYA and YES at 26°C. A~D, obverse colony from left to right grown on PDA, OA, CYA and YES; E~H, reverse colony from left to right grown on PDA, OA, CYA and YES; I~J, conidiophores and conidia produced on PDA and MEA; K, simple microscopic picture of conidiophore; L~N, SEM of conidiophore; O~Q, SEM of conidiophore and conidia. PDA, potato dextrose agar; OA, oatmeal agar; CYA, czapek yeast extract agar; YES, yeast extract sucrose agar; MEA, malt extract agar; PCA, potato carrot agar.

white (Fig. 9). Exudate was absent. Irregular form and surface was rough. Sporulation was slightly moderate to dense. Conidia were globose to sub-globose and 1.0 to 2.5  $\mu\text{m}$  in diameter. Conidiogenous cells were globose to hyaline of  $4\text{--}6 \times 3.2\text{--}4.3 \mu\text{m}$  in size.

***Penicillium rubidurum* (KNU14-12): *Penicillium rubidurum* Udagawa & Y. Horie. Transactions of the Mycological Society of Japan 14: 381 (1973) [MB # 319295]**

Micrographs of morphological structures of the isolate are shown in Fig. 10. Colony on PDA, attaining a diameter of 19~21 mm within 7-days at 26°C. The front side of mycelium was milky white in color while back side was dark yellow (Fig. 10A, 10E). Sporulation was moderate, conidia were in mass, irregular form, smooth surface. The color of colony was initially white then became light yellow. Colony on OA, attaining a diameter of 22~25 mm within 14 days at 26°C. The front side of mycelium was white in color while back side was brown (Fig. 10B, 10F). Sporulation was moderate, conidia were in mass. Irregular form, rough surface. On CYA, attaining a diameter of 16



**Fig. 9.** Morphological characters of *Paraphaeosphaeria sporulosa* (KNU15-2) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, simple microscopic pictures of conidia; E, F, SEM of conidia.

~18 mm within 14 days at 26°C. The front side of mycelium was white and back side was light red in color (Fig. 10C, 10G). Sporulation was moderate to dense. Conidia were in mass, irregular form and smooth surface. On YES, attaining a diameter of 20~23 mm within 14 days at 26°C. The front side of mycelium was powdery white and back side was yellowish red in color (Fig. 10D, 10H). Sporulation was moderate to dense. Conidia were in mass, irregular form and smooth surface (Fig. 10K, 10O). Conidiophores were hyaline, smooth walled, 1.3~2.2  $\mu\text{m}$  in diameter (Fig. 10I). Conidiophore was monoverticillate, four phialides per metula. Phialides with terminal whorls. Phialides were in verticils of 3~5. Conidia in chains ellipsoidal and rough walled, 1.9~2.3  $\mu\text{m}$  (Fig. 10L). Phialides were short cylindrical to ellipsoidal chlamydospore-like structures, thin-walled and globose.

***Pochonia suchlasporia* (KNU15-6): *Pochonia suchlasporia* var. *suchlasporia*, Nova Hedwigia 73 (1-2): 67 (2001) [MB#485231]**

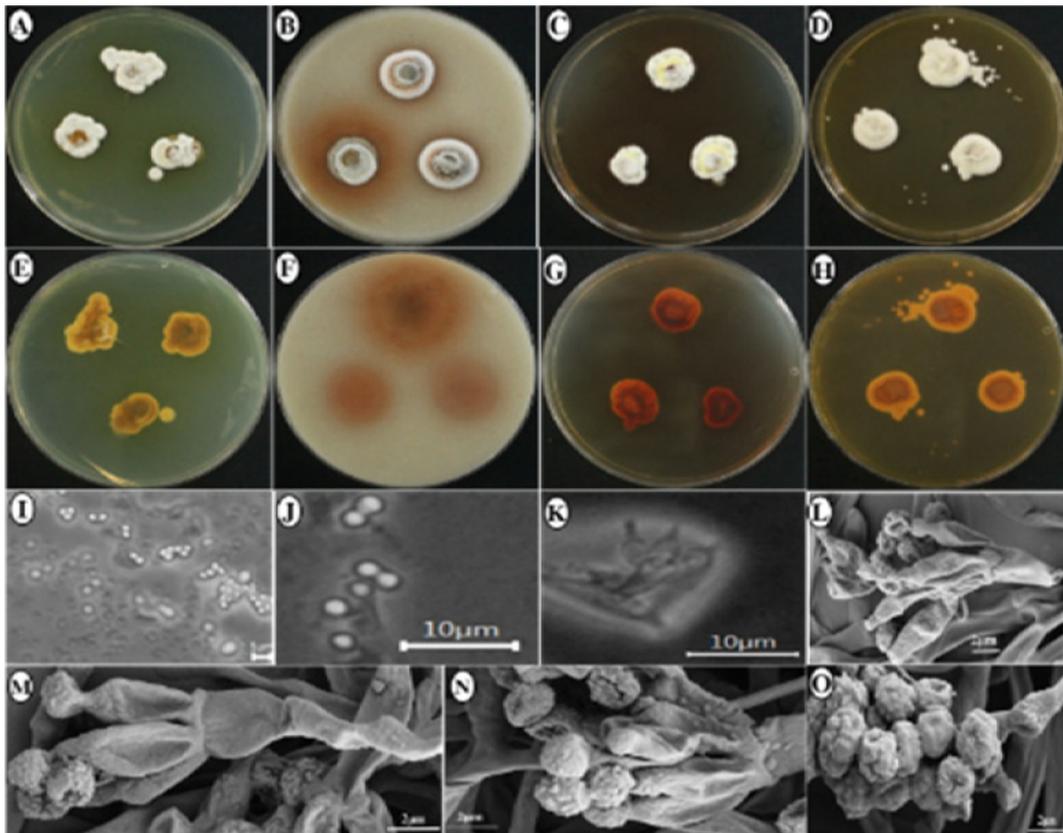
Colony on PDA grew fast reaching 60~70 mm diameter in 10 days 26°C. Colony color was woolly, white to yellowish; reverse yellow to brownish cream (Fig. 11). Exudate was absent. Sporulation was moderate. Texture was woolly. Irregular form and surface was rough. Conidiophores were long, erect and densely verticillate.

***Sporothrix inflata* (KNU15-8): *Sporothrix inflata* de Hoog, Studies in Mycology 7: 34 (1974) [MB#323935]**

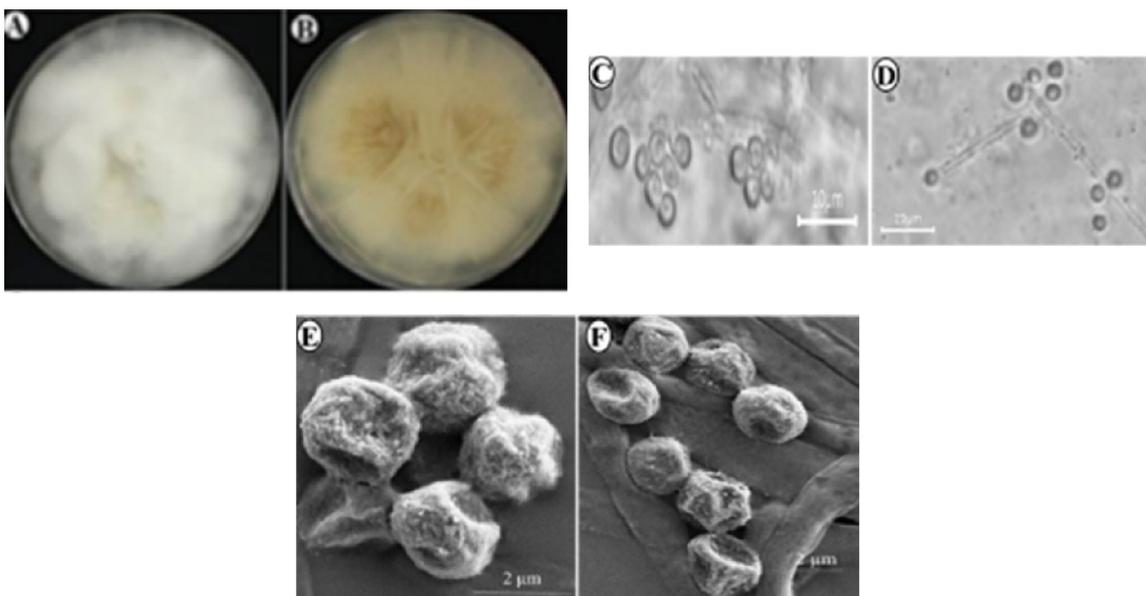
Colony pictures of the present isolate KNU 15-8 on four different culture media, namely PDA, MEA, OA and PCA at 26°C are indicated in Fig. 12A~12H. Sporulation was moderate. Irregular form and surface was rough. Colony was white, floccose and smooth and became floccose with few concentric rings at the edges and tufts at the center over time.

#### Taxonomy of the isolate KNU15-8

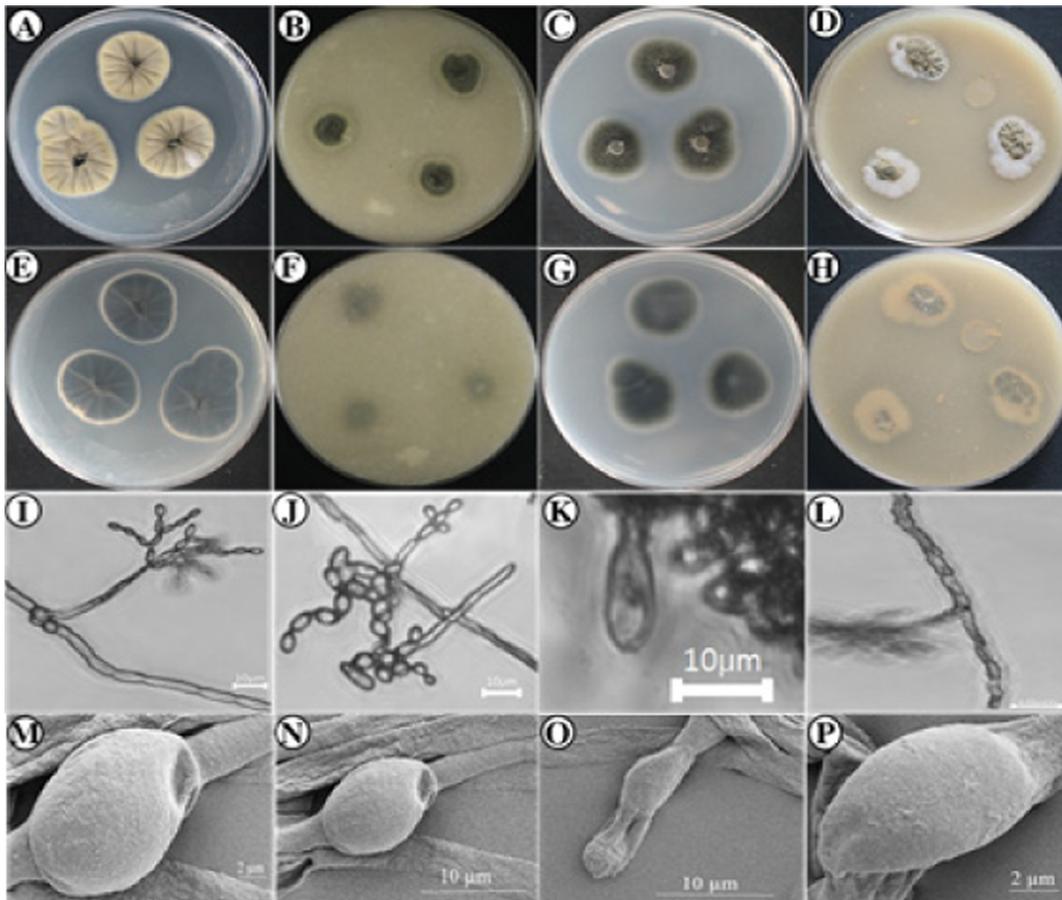
Colony size of the isolate on PDA, MEA, OA and PCA media was 21~30 mm, 24~29 mm, 21~25 mm and 25~30 mm in diameter, respectively. Vegetative hyphae of the isolate was hyaline, smooth-walled, many droplets, branched and 1~1.5  $\mu\text{m}$  wide with swellings (Fig. 12M~12P). Conidiogenous cells were dispersed, arising laterally and terminally on hyphae, 20~50  $\mu\text{m}$  long and 1~2.2  $\mu\text{m}$  wide at the base, swollen clusters formed from intercalary position (Fig. 12I, 12J, 12L). Two types conidia viz., hyaline ( $3\text{--}4 \times 1.5\text{--}3.0 \mu\text{m}$ ) and dark ( $3\text{--}4$  (~6.5)  $\times 2\text{--}4.0 \mu\text{m}$ )



**Fig. 10.** Morphological characters of *Penicillium rubidurum* (KNU14-12) grown for 7 days on PDA, OA, CYA and YES at 26°C. A~D, obverse colony from left to right grown on PDA, OA, CYA and YES; E~H, reverse colony from left to right grown on PDA, OA, CYA and YES; I~J, conidiophores and conidia produced on PDA and MEA; K, simple microscopic picture of conidiophore; L~N, SEM of conidiophore; O, SEM of conidia. PDA, potato dextrose agar; OA, oatmeal agar; CYA, czapek yeast extract agar; YES, yeast extract sucrose agar; MEA, malt extract agar; PCA, potato carrot agar.



**Fig. 11.** Morphological characters of *Pochonia suchlasporia* (KNU15-6) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, light microscopic pictures of conidiophores and conidia; E, F, scanning electronic pictures of conidia.



**Fig. 12.** Morphological characters of *Sporothrix inflata* (KNU15-20) grown for 7 days on PDA, OA, CYA and YES at 26°C. A~D, obverse colony from left to right grown on PDA, OA, MEA and PCA; E~H, reverse colony from left to right grown on PDA, OA, MEA and PCA; I~J, conidiophores and conidia produced on PDA and MEA; K, simple microscopic picture of conidiophore; L~N, SEM of conidiophore; O~P, SEM of conidia. PDA, potato dextrose agar; OA, oatmeal agar; CYA, czapek yeast extract agar; YES, yeast extract sucrose agar; MEA, malt extract agar; PCA, potato carrot agar.

conidia were observed and they were unicellular, smooth, obovate to ellipsoid, pointed at the base (Fig. 12J~12K).

***Thielavia hyrcaniae* (KNU15-1): *Thielavia hyrcaniae* Nicot, C. R. Acad. Sci. Paris: 304 (1961) [MB#340053]**

Colonies on PDA: fast growing and attains the size of 70~80 mm diameter in 7~10 days at 25°C. The front side of the colony is white in color and the reverse side of it is brownish white (Fig. 13). Sporulation was moderate to dense. Exudate was absent. Pycnidia were dark brown or black, globose to sub-globose, spores were elliptical, dark brown, apiculate at both ends, 12~16 × 8~11 μm.

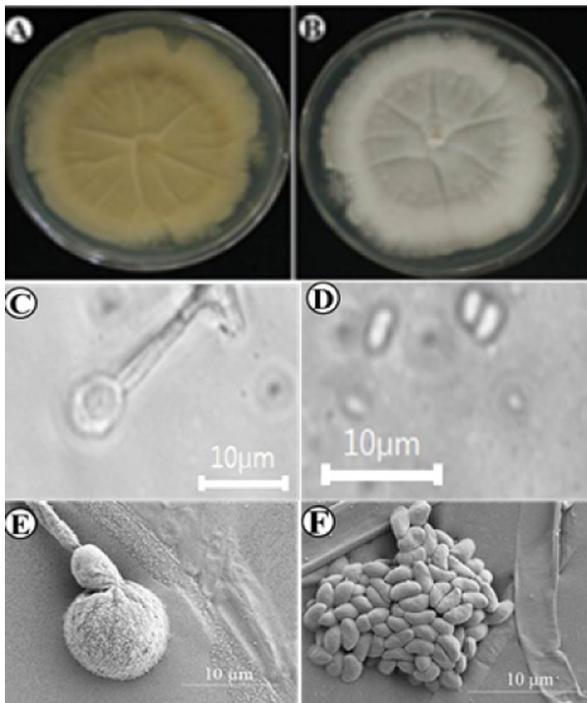
***Thielavia terricola* (KNU14-23-1): *Thielavia terricola* (J.C. Gilman & E.V. Abbott) C.W. Emmons, Bulletin of the Torrey Botanical Club 57: 123-126 (1930) [MB#255078]**

Colonies on PDA was medium to fast growing and att-

ained the size of 50~60 mm diameter in 7~10 days at 26°C. The front side of the colony was white in color and the reverse side of it was brownish white (Fig. 14). Exudate was absent. Irregular form and surface was rough. Pycnidia were dark brown or black, globose to sub-globose, mostly 100~200 μm in diameter, with small ostiolar papillae, spores are elliptical, dark brown, apiculate at both ends of 10~15 × 7~9 μm diameter.

***Xylogone sphaerospora* (KNU15-7): *Xylogone sphaerospora* Arx & T. Nilsson, Svensk Botanisk Tidskrift 63: 345 (1969) [MB#325626]**

Colonies fast growing. Initially orange-white, darkening to gray (Fig. 15) with development of abundant ascomata. Ascospores smooth, hyaline, subglobose, 3.9~5.2, 3, 2.3~4.1 μm.



**Fig. 13.** Morphological characters of *Thielavia hyrcanaiae* (KNU 15-1) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, light microscopic pictures of pycnidia; E, F, scanning electron pictures of pycnidia.

***Zopfiella longicaudata* (KNU15-5):** *Zopfiella longicaudata* (Cain) Arx, Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen Section C 76 (3): 291 (1973) [MB#325685]

Colonies on PDA was fast growing and attained a diameter of 60~70 mm in 7 days at 26°C, the front side of the colony was dark to olivaceous brownish white (Fig. 16), usually superficial cleistothecia with a peridium adorned with varying degree of hair. Sporulation was moderate. Exudate was absent. Asci were highly evanescent and are 8-spored, cylindrical to clavate of 73~110 µm.

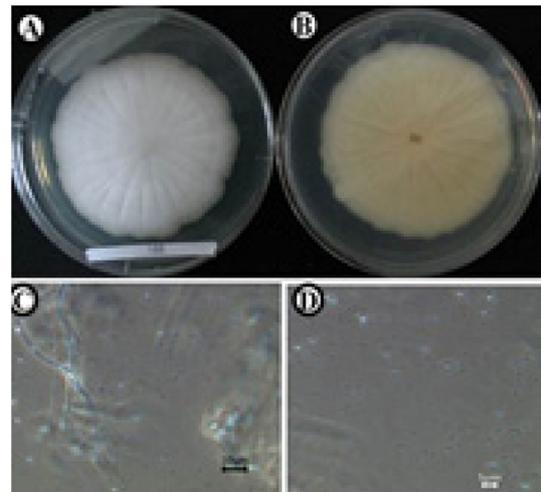
**Molecular phylogeny of all the studied fungal isolates**

***Acremonium cellulolyticus* (KNU14-25)**

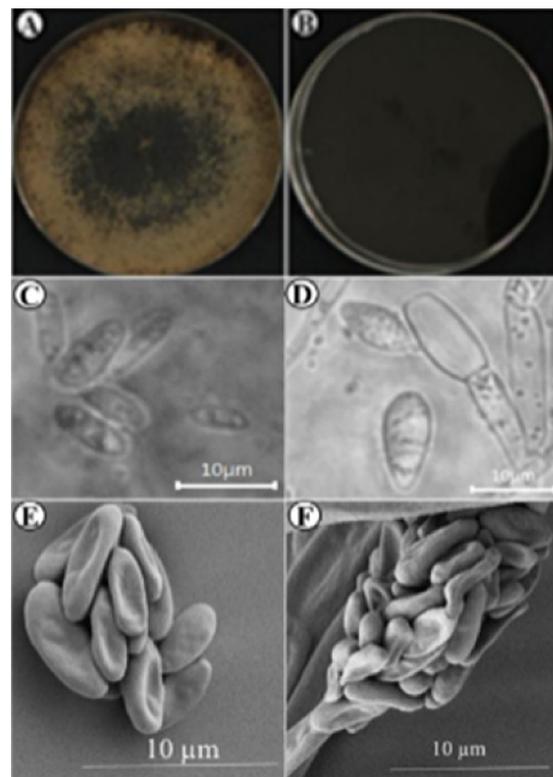
KNU14-25 was isolated from crop field soil in July 2014. ITS rDNA sequences were employed to determine the phylogenetic relationship between the isolate KNU14-25 and previously defined *Acremonium* species. The isolate was most closely related to *A. cellulolyticus* and formed a monophyletic group with a bootstrap value of 99% (Fig. 17).

***Acremonium zonatum* (KNU14-15)**

KNU14-15 was isolated from crop field soil in July 2014.

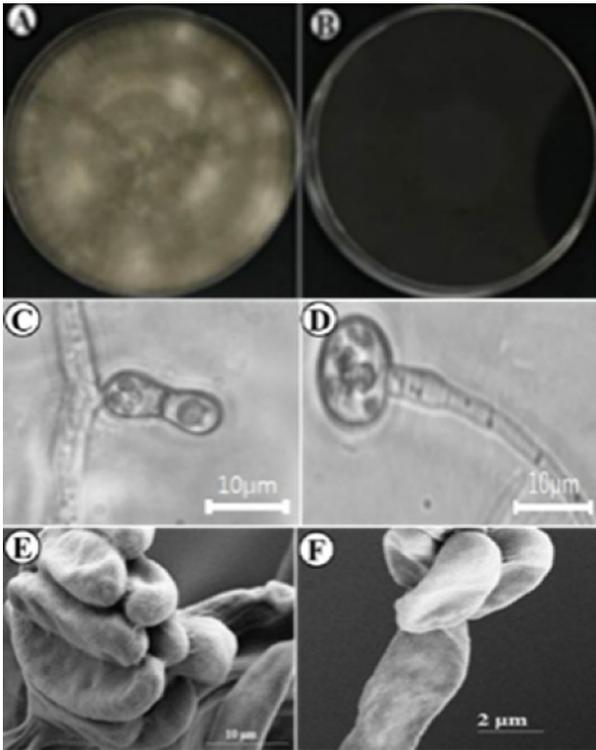


**Fig. 14.** Morphological characters of *Thielavia terricola* (KNU 14-23-1) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, light microscopic pictures of pycnidia.



**Fig. 15.** Morphological characters of *Xylogone sphaerospora* (KNU15-7) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, simple microscopic picture of ascospores; E, F, SEM of ascospores.

ITS rDNA sequence of the isolate KNU14-15 was most closely related to *A. zonatum* and formed monophyletic



**Fig. 16.** Morphological characters of *Zopfella longicaudata* (KNU15-5) grown for 7 days on potato dextrose agar. A, obverse colony; B, reverse colony; C, D, light microscopic pictures of conidiophores; E, F, scanning electronic pictures of ascospores.

group with bootstrap value of 99% (Fig. 17). The phylogenetic analysis showed that the isolate is *A. zonatum*. This is a common species found especially in soil though it is the first report from Korea.

#### *Chaetomium madrasense* (KNU14-9)

KNU14-9 was isolated from crop field soil in July 2014. The phylogenetic tree was inferred from the ITS rDNA sequence. Our study isolate supports the monophyly of *Chaetomium*. The strain has high similarity with *C. madrasense* with bootstrap value of 98% (Fig. 17). This suggests that our study isolate is *C. madrasense* which is not previously reported in Korea.

#### *Cladosporium silenes* (KNU14-18-1)

KNU14-18-1 was isolated from crop field soil in July 2014. The neighbor joining relationship tree of study isolate (KNU14-18-1) was carried out by using ITS rDNA sequence. According to the result, KNU14-18-1 showed close relation with the *C. silenes* (Fig. 17) with the similarity of 100% (Fig. 17).

#### *Humicolopsis cephalosporioides* (KNU15-3)

KNU15-3 was isolated from crop field soil in July 2014. ITS rDNA sequences were employed to determine the phylogenetic relationship between the isolate KNU14-15-3 and previously defined *Humicolopsis* species. The acquired ITS sequence showed 100% similarity with a *H. cephalosporioides* (accession no. KU516491) available in the GenBank database, suggesting the isolate is *H. cephalosporioides*. In addition, neighbor joining tree for the identification of the isolate for clarity of related taxa. Results revealed that the isolate was most closely related with *H. cephalosporioides* with the bootstrap value of 100% (Fig. 17).

#### *Leptosphaerulina chartarum* (KNU14-16)

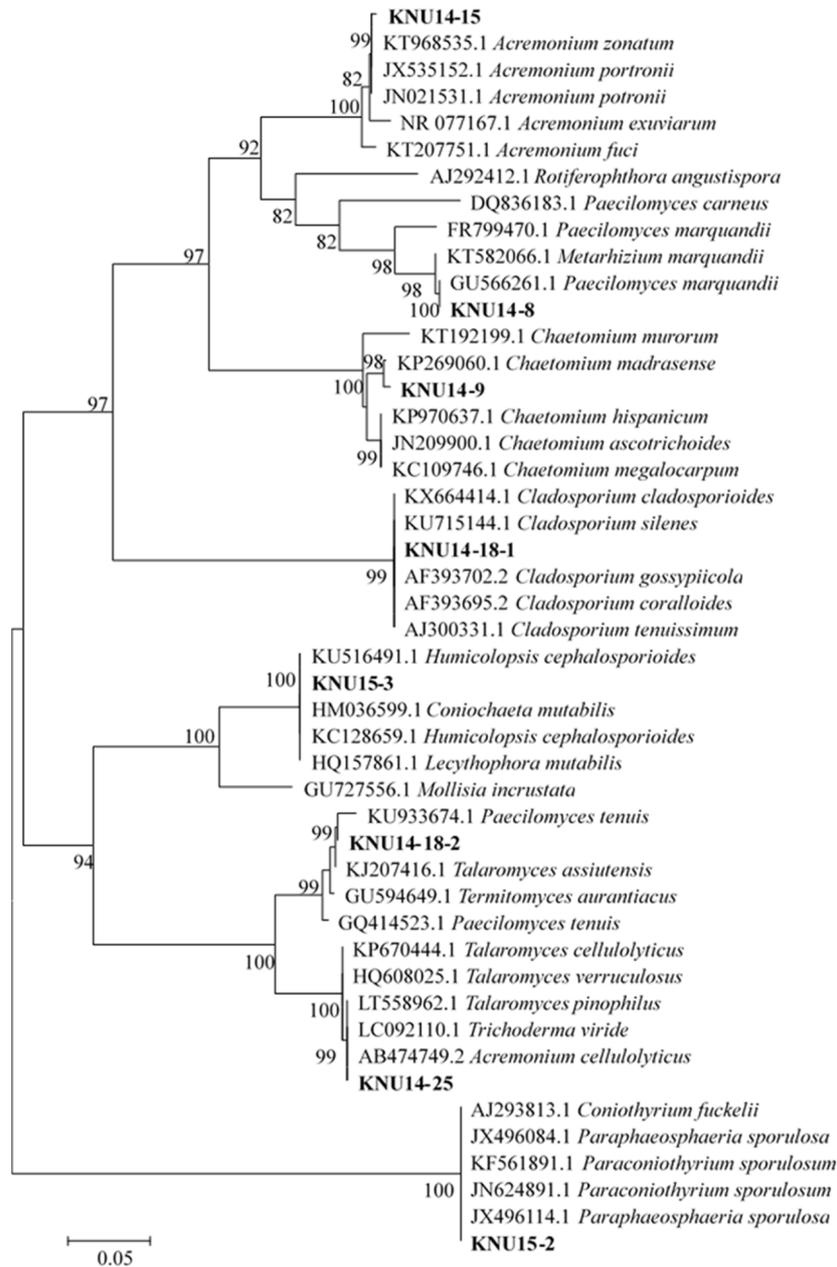
KNU14-16 was isolated from crop field soil in July 2014. ITS rDNA sequences were used to compare and determine the phylogenetic relationship between the isolate KNU 14-8 and previously described *Leptosphaerulina* species. All the retrieved sequences were aligned by using the Multalin program. Bootstrap analysis was carried out with 1,000 replications to determine the support for each clade. According to the results, ITS sequence of KNU14-16 matched with *L. chartarum* (HQ248491) with 99% similarity (Fig. 18).

#### *Paecilomyces marquandii* (*Metarahzium marquandii*) (KNU14-8)

KNU14-8 was isolated from crop field soil in July 2014. ITS rDNA sequences were used to compare and determine the phylogenetic relationship between the isolate KNU 14-8 and previously described *Paecilomyces* species. The phylogenetic relationship was constructed by using the neighbor joining search option with nearest neighbor interchange. Bootstrap analysis was performed with 1,000 replicates to determine the support for each clade. The ITS rDNA region were identical to that of *P. marquandii* (GU566261) with the bootstrap value of 100% (Fig. 17). These results suggested that our study isolate, KNU14-8 matched with *P. marquandii*.

#### *Paecilomyces tenuis* (KNU14-18-2)

KNU14-18-2 was isolated from crop field soil in July 2014. ITS rDNA sequences were compared to determine the phylogenetic relationship between the isolate KNU14-18-2 and previously described *Paecilomyces* species. The isolate was most closely related to *Paecilomyces tenuis* (KU 933674) and formed monophyletic group with bootstrap value of 99% (Fig. 17). The phylogenetic analysis showed



**Fig. 17.** Neighbor-joining phylogenetic analysis of the partial 18S-ITS1-5.8S-ITS2-28S rDNA region of (KNU14-25), (KNU14-15), (KNU14-8), (KNU14-9), (KNU14-18-1), (KNU14-18-2), (KNU15-2) and (KNU15-3) obtained from crop field soil in Korea. Numerical values (>50) on branches are the bootstrap with 1,000 replicates.

that the isolate is *Paecilomyces tenuis*. This is a common species found especially in soil but this is the first report of its occurrence in Korea.

***Paraphaeosphaeria sporulosa* (KNU15-2)**

KNU15-2 was isolated from crop field soil in July 2014. ITS rDNA sequences were compared to determine the phylogenetic relationship between the isolate KNU15-2 and previously described *Paraphaeosphaeria* species. The

isolate was assigned to *Paraphaeosphaeria* on the basis of ITS sequences. The isolate KNU15-2 formed a monophyletic clade with the sequences of *P. sporulosa* and had high sequence similarity with *P. sporulosa* (JX496114) with bootstrap value of 100% (Fig. 17). This is the first report of its existence in Korea.

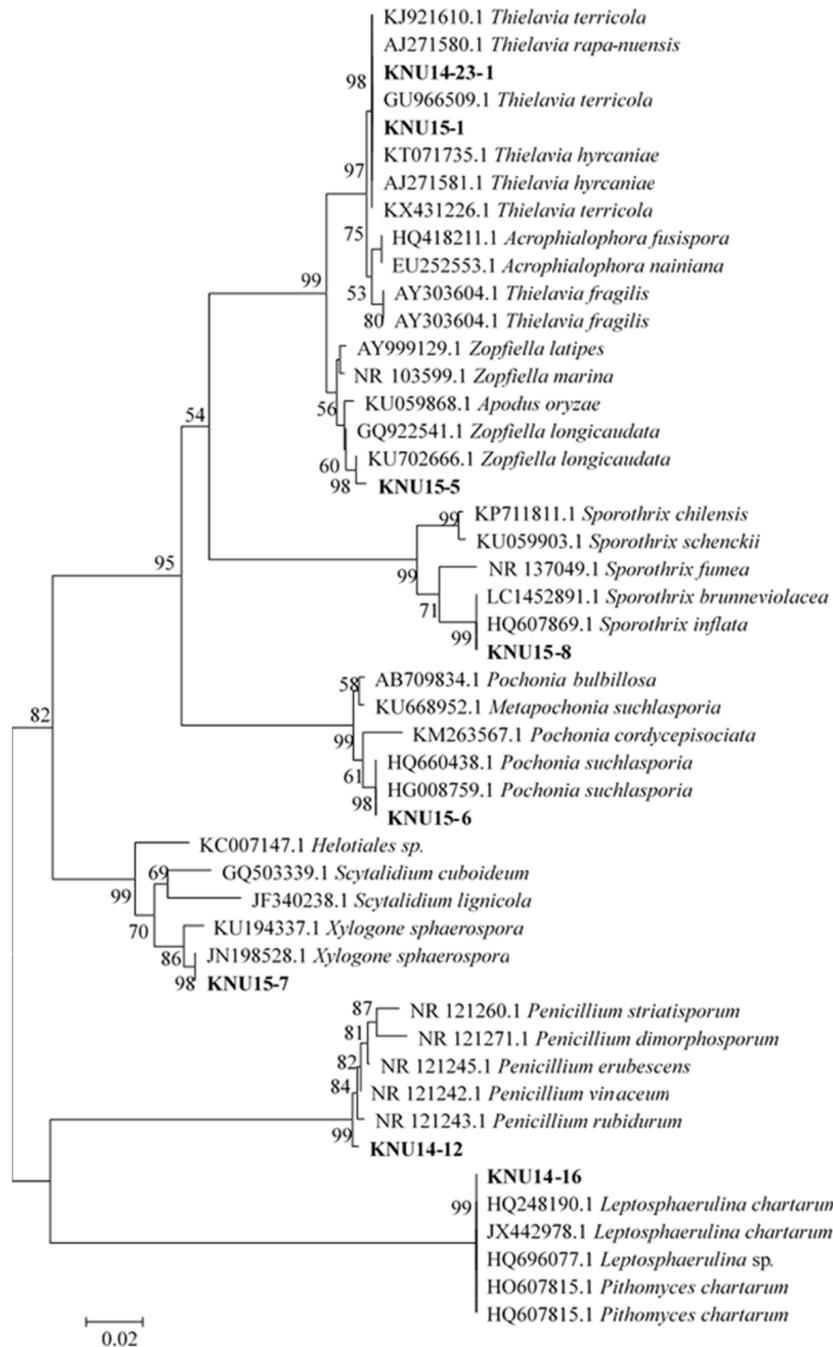
***Penicillium rubidurum* (KNU14-12)**

ITS rDNA sequences of the isolate KNU14-12 were

compared to determine the phylogenetic relationship between the isolate KNU14-12 and previously described *Penicillium* species. The isolate was most closely related to *Penicillium rubidurum* and formed a monophyletic group with bootstrap value of 99% (Fig. 18). The phylogenetic analysis showed that the isolate is *Penicillium rubidurum*.

#### *Pochonia suchlasporia* (KNU15-6)

ITS and rDNA sequences of the isolate KNU15-6 were compared to determine the phylogenetic relationship between the isolate KNU15-6 and previously described *Pochonia* species. The ITS region of the KNU15-6 were 98% identical to the culture collection of *P. suchlasporia* (HG 008759) (Fig. 18). The results strongly suggests that that



**Fig. 18.** Neighbor-joining phylogenetic analysis of the partial 18S-ITS1-5.8S-ITS2-28S rDNA region of (KNU14-23-1), (KNU15-1), (KNU15-5), (KNU15-6), (KNU15-7), (KNU15-8), (KNU14-16) and (KNU14-23-1) obtained from crop field soil in Korea. Numerical values (>50) on branches are the bootstrap with 1,000 replicates.

our study isolate KNU15-6 is *P. suchlasporia* which is not reported previously in Korea.

#### ***Sporothrix inflata* (KNU15-8)**

KNU15-8 was isolated from crop field soil in July 2014. ITS rDNA sequences were employed to determine the phylogenetic relationship between the isolate KNU15-8 and previously defined *Sporothrix* species. The ITS sequence of KNU15-8 was aligned with the available sequences of *S. inflata* strains and its nearest species through the BLAST sequence using the MultAlin program, and a neighbor joining tree was constructed from the aligned sequences. The phylogenetic analysis suggests that the isolate KNU15-8 was 99% similar with the *S. inflata* (HQ607879) (Fig. 18). The phylogenetic analysis showed that the isolate is *S. inflata*.

#### ***Thielavia hyrcaniae* (KNU15-1)**

KNU15-1 was isolated from crop field soil in July 2014. ITS rDNA sequences were employed to determine the phylogenetic relationship between the isolate KNU15-1 and previously defined *Thielavia* species. The isolate showed strong sequence similarity to *T. hyrcaniae* and formed monophyletic group with bootstrap value of 98% (Fig. 18). The phylogenetic analysis showed that the isolate is *Thielavia hyrcaniae*.

#### ***Thielavia terricola* (KNU14-23-1)**

KNU14-23-1 was isolated from crop field soil in July 2014. ITS rDNA sequences were employed to determine the phylogenetic relationship between the isolate KNU14-23-1 and previously defined *Thielavia* species. The isolate was most closely related to *T. terricola* and formed monophyletic group with bootstrap value of 98% (Fig. 18). The phylogenetic analysis showed that the isolate is *Thielavia terricola*.

#### ***Xylogone sphaerospora* (KNU15-7)**

KNU15-7 was isolated from crop field soil in July 2014. ITS rDNA sequences were employed to determine the phylogenetic relationship between the isolate KNU15-7 and previously defined *Xylogone* species. The isolate was most closely related to *Xylogone sphaerospora* and formed monophyletic group with bootstrap value of 98% (Fig. 18). The phylogenetic analysis showed that the isolate is *Xylogone sphaerospora* which is not reported previously in Korea.

#### ***Zopfiella longicaudata* (KNU15-5)**

KNU15-5 was isolated from crop field soil in July 2014. ITS rDNA sequences were employed to determine the phylogenetic relationship between the isolate KNU15-5 and previously defined *Zopfiella* species. The isolate was most closely related to *Zopfiella longicaudata* and formed monophyletic group with bootstrap value of 98% (Fig. 18). The phylogenetic analysis showed that the isolate is *Zopfiella longicaudata*.

## Discussion

It has been reported that the *Paecilomyces* is mainly distinguished by the shape of the phialides that tapering into a long distinct neck and the divergent aggregation of whorls [25]. Morphologically these two isolates showed variable colony color in all tested growth media. Isolate KNU14-8 grew slowly as compared to the isolate KNU14-18-2 in all the tested growth media. On the basis of above mentioned taxonomical properties of the isolate KNU14-8 was considered to be *P. marquandii*. Taxonomical properties of our study isolate KNU14-8 reasonably fits with the description made by Hughes [26]. In addition, *P. marquandii* can be distinguish from all the other species in the genus by producing phialides that often rise from undifferentiated hyphae in whorls, white colonies on cza-pak agar and subglobose conidia [27]. Moreover, Phylogenetic analysis revealed that the isolate is *P. marquandii*. Thus based on these morphological and molecular characteristics it can be concluded that the isolate KNU14-8 is *P. marquandii*. The other isolate KNU14-18-2 was presumed to be a *Paecilomyces* on the basis on its phialides. The study isolate reasonably fits with the description of *Paecilomyces tenuis* [10]. Our study isolate KNU14-18-2 also consists of similar morphological characteristics as described by Han *et al.* [10]. Moreover, Phylogenetic analysis revealed that the isolate is *P. tenuis*. Thus based on these morphological and molecular characteristics it can be concluded that the isolate KNU14-18-2 is *P. tenuis*. In addition, *P. tenuis* has an importance in producing Huperzine (Hup A) which has a potential application in Alzheimer disease therapy [28]. However further studies in this aspect would be worthwhile.

*Penicillium* is a genus of ascomycetous fungi that are saprobic, filamentous and typically monomorphic [29]. *Penicillium* species have septate hyphae (2~5 um in diameter) that give rise to branched or unbranched conidiophores with secondary branches that give *Penicillium* a

brush-like appearance [29]. We also found the similar brush-like appearance of conidiophore in our isolate (Fig. 10L~10N). Moreover, conidiophore and cleistothecium characters of *Penicillium* are of great taxonomic importance [30]. The isolate KNU14-12 was presumed to be a *Penicillium* on the basis of its conidiophores, conidia and its colony appearance. The isolate reasonably fits with the description of *Penicillium rubidurum* which was described in Udagawa and Horie [31]. The study isolate KNU14-12 from crop field soil only differs slightly from the original description by size of conidiophores, and conidia. We also found the similar morphological characteristics in the present isolate. Moreover, several studies showed that *Penicillium* has a large impact on human life and main function in nature is the decomposition of organic materials, where species cause devastating rots as pre and post-harvest pathogens on food [32]. Further studies on these aspects by the present isolate are needed. On the basis of above mentioned taxonomical properties of the isolate KNU14-12 was considered to be *P. rubidurum*. This is a common species found especially in soil but this is the first report of the isolation from crop field soil in Korea.

Morphology features and molecular characteristics of KNU15-8 isolate was similar to the morphology *Sporothrix inflata* that has been previously reported [15, 17, 18, 33]. In addition, the results were also in agreement with the previous study [10] that dark, thick-walled conidia referred to as the 'Humicola-type' were observed rarely. Colony morphology of the present isolate was similar to the colony image of *Cladostidium silenes* [18]. Moreover, the morphological feature of KNU 14-18-1 isolate was very similar to *C. silenes* [18] that *C. silenes* differs from *C. cladosporioides* in having shorter ramo- and intercalary conidia. ITS rDNA sequences were also employed to determine the phylogenetic relationship between the isolate KNU14-18-1 and previously defined *Cladostidium* species. Hence, the isolate was found to be more closely related to *C. silenes* with bootstrap value of 98%. In addition, the morphological features of the isolates KNU15-2, KNU15-6, KNU15-7, KNU15-5, KNU15-1, KNU14-23-1, KNU14-16 and KNU15-3, was found similar to that of previously reported *Paraphaeosphaeria sporulosa*, *Pochonia suchlasporia*, *Xylogone sphaerospora*, *Zopfiella longicaudata*, *Thielavia hyrcaniae*, *Thielavia terricola*, *Leptosphaerulina chartarum*, and *Humicolopsis cephalosporioides* isolates respectively. Further studies regarding their biotechnological importance are worthwhile in the future.

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