

A New record of four *Penicillium* species isolated from *Agarum clathratum* in Korea

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***Agarum clathratum*, brown algae, play important ecological roles in marine ecosystem, but can cause secondary environment pollution when they pile up on the beach. In order to resolve the environment problem by *A. clathratum*, we focus to isolate and identify *Penicillium* because many species are well known to produce extracellular enzymes. A total of 32 *Penicillium* strains were isolated from *A. clathratum* samples that collected from 13 sites along the mid-east coast of Korea in summer. They were identified based on morphological characters and phylogenetic analysis using β -tubulin DNA sequences as well as a combined dataset of β -tubulin and calmodulin. A total of 32 strains were isolated and they were identified to 13 *Penicillium* species. The commonly isolated species were *Penicillium citrinum*, *P. roseomaculatum*, and *Penicillium* sp. Among 13 *Penicillium* species, four species – *P. bilaiae*, *P. cremeogriseum*, *P. madriti*, and *P. roseomaculatum* – have not been previously recorded in Korea. For these four new species records to Korea, we provide morphological characteristics of each strain.**

Keywords: β -tubulin, calmodulin, marine environments, morphology, phylogenetic analysis

Introduction

Macroalgae play important ecological roles by providing a food source and habitat for marine organisms in marine ecosystems (Graham *et al.*, 2009), and 908 macroalgae species have been recorded in Korea (Kim *et al.*, 2013). *Agarum clathratum* Dumortier, a brown alga, has been reported on the coast of Hokkaido in Japan, the Kuril Islands, and the Bering Sea. It is commonly found in the mid-east coast of Korea (Kang, 1966). *A. clathratum* is composed of carbohydrates, proteins, lipids and polyphenolic compounds (Park *et al.*, 2012). Some of these compounds have potential medicinal applications as antioxidants (Park *et al.*, 2012) and some compounds exhibit immunomodulatory activity (Cho

et al., 2014). While the whitening of the rocky shore caused a decrease of macroalgae biomass and species diversity on the east coast of Korea (Choi *et al.*, 2006), the *A. clathratum* biomass has significantly increased (Sohn *et al.*, 2007). Biomass increase in *A. clathratum* is likely to cause secondary environmental pollution as the species piles up on the beach and rots. Marine fungi, such as *Acremonium*, *Cladosporium*, and *Penicillium*, are frequently isolated from macroalgae (Kohlmeyer and Kohlmeyer, 1979; Zuccaro *et al.*, 2004; Jones and Pang, 2012) and play an important ecological role in decomposing macroalgae (Schaumann and Weide, 1990; Hyde *et al.*, 1998). In particular, marine-derived *Penicillium* species have demonstrated the ability to degrade alginate and/or cellulose, which are cell wall components of macroalgae (Dubrovskaya *et al.*, 2012; Park *et al.*, 2014a)

Marine-derived *Penicillium* have been isolated from various marine environments such as sand, seawater, and macroalgae (Park *et al.*, 2014a, 2015; Nicoletti and Trincone, 2016) and are known to produce secondary metabolites and extracellular enzymes (Edrada *et al.*, 2002; Dubrovskaya *et al.*, 2012; Park *et al.*, 2014a). Since the main purpose of studies of marine-derived *Penicillium* was to find natural bioactive compounds from macroalgae-derived fungi (Nicoletti and Trincone, 2016), identification of fungi was not conducted at the species level. Many of the isolates, such as *Penicillium* sp., remain to be identified (Nicoletti and Trincone, 2016).

The genus *Penicillium* is easily identified on the artificial medium at the genus level based on the morphological features; however, it is difficult to identify at the species level due to similar morphology and morphological variation due to different growth conditions (Visagie *et al.*, 2014b). Visagie *et al.* (2014b) introduced standardized methods for identification and characterization of *Penicillium* species. The taxonomy of *Penicillium* was studied using a polyphasic approach including morphology, multigene phylogenetic analysis of nuclear DNA internal transcribed spacer (ITS), β -tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*), and extrolite profiling. DNA-based methods have revolutionized fungal taxonomy, most significantly by circumventing problems inherent in morphological-based identification. Most fungal phylogenies to date have been carried out based on the ITS region, which is developed as fungal DNA barcode gene (Schoch *et al.*, 20012). However, molecular phylogenies based on other loci, such as *BenA*, *CaM*, and *RPB2* have provided higher resolution in *Penicillium* than those constructed from ITS sequences (Visagie *et al.*, 2014b). Among these markers, *BenA* has proven to be the most useful marker for species identification in *Penicillium*, however, some species require the use of combined datasets from multiple loci (*BenA*+*CaM*) for accurate spe-

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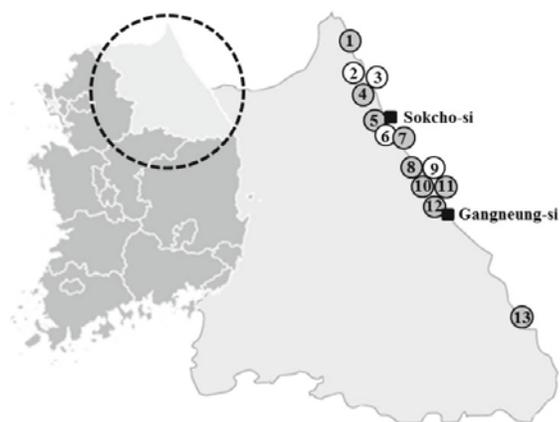


Fig. 1. Map of mid-east coast of Korea showing sampling sites of *Agarum clathratum*. Sampling sites (*Penicillium* strains/species): 1, Chodo-ri (0/0); 2, Gajin-ri (8/3); 3, Oho-ri (1/1); 4, Sampo-ri (1/1); 5, Joyang-dong (2/2); 6, Jeong-am-ri (4/2); 7, Jucheong-ri (2/1); 8, In-gu-ri (1/1); 9, Namae-ri (2/2); 10, Wonpo-ri (0/0); 11, Hyangho-ri (10/6); 12, Dongdeok-ri (0/0); 13, Hamaengbang-ri (1/1). White circle indicate locations of the sampling sites found in new records in Korea.

cies identification (Visagie *et al.*, 2014b). *Penicillium* contains 354 accepted species in two subgenera (*Aspergilloides* and *Penicillium*) and 25 sections. Over 100 *Penicillium* species have been recorded from various habitats, including marine environments, in Korea (Lee *et al.*, 2003; Yu, 2006; Kim *et al.*, 2009; Park *et al.*, 2014a).

Since the Ministry of Ocean and Fisheries established the Marine Fungal Resource Bank (MFRB) to promote the exploration of marine-derived fungi, we have collected many fungal isolates from various marine environments in Korea including seaweed such as *A. clathratum*. As a long-term goal, it is prerequisite to survey *Penicillium* species associated with *A. clathratum* in order to resolve environmental problems caused by *A. clathratum* as well as to identify *Penicillium* strains that potentially have medicinal or other human applications. Therefore, the main objective of this work was to isolate *Penicillium* species from *A. clathratum* along the mid-east coast of Korea and to identify these isolates at the species level using both morphology and sequence analysis of the *BenA* and *CaM* loci.

Table 1. Summary and GenBank accession numbers for *Penicillium* strains isolated from *Agarum clathratum*

Section	Species	Collection No.	Location	Accession No.		
				<i>BenA</i>	<i>CaM</i>	
<i>Aspergilloides</i>	<i>P. roseomaculatum</i> ^a	SFC20151118-M27	Jeong-am-ri, Ganghyeon-myeon, Yangyang-gun, Gangwon-do	KX712462	KX712494	
		SFC20160805-M01	Jeong-am-ri, Ganghyeon-myeon, Yangyang-gun, Gangwon-do	KX712463	KX712495	
		SFC20160805-M02	Oho-ri, Jugwang-myeon, Goseong-gun, Gangwon-do	KX712464	KX712496	
	<i>P. spinulosum</i>	SFC20160805-M03	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712465	KX712497	
		SFC20160317-M23	Joyang-dong, Sokcho-si, Gangwon-do	KX712466	KX712498	
		SFC20160805-M04	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712467		
	<i>Brevicompecta</i>	<i>P. bialowiezense</i>	SFC20160805-M05	Sampo-ri, Jugwang-myeon, Goseong-gun, Gangwon-do	KX712468	
	<i>Canescentia</i>	<i>P. antarcticum</i>	SFC20160805-M06	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712469	
	<i>Citrina</i>	<i>P. citrinum</i>	SFC20160805-M07	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712470	
SFC20160805-M08			In-gu-ri, Hyeonnam-myeon, Yangyang-gun, Gangwon-do	KX712471		
SFC20160805-M09			Joyang-dong, Sokcho-si, Gangwon-do	KX712472		
SFC20160805-M10			Gajin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do	KX712473		
SFC20160805-M11			Gajin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do	KX712474		
SFC20160805-M12			Gajin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do	KX712475		
SFC20160805-M13			Gajin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do	KX712476		
<i>Fasciculata</i>	<i>P. aurantiogriseum</i>	SFC20160805-M14	Gajin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do	KX712477		
		SFC20160805-M15	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712478		
<i>Lanata-Divaricata</i>	<i>P. cremeogriseum</i> ^a	SFC20160805-M16	Hamaengbang-ri, Geundeok-myeon, Samcheok-si, Gangwon-do	KX712479		
		SFC20160805-M17	Namae-ri, Hyeonnam-myeon, Yangyang-gun, Gangwon-do	KX712480		
<i>Ramosa</i>	<i>P. oxalicum</i>	SFC20160805-M18	Gajin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do	KX712481		
		SFC20160805-M19	Jeong-am-ri, Ganghyeon-myeon, Yangyang-gun, Gangwon-do	KX712482		
		SFC20160805-M20	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712483		
<i>Sclerotiora</i>	<i>P. bilaiae</i> ^a	SFC20160805-M21	Jeong-am-ri, Ganghyeon-myeon, Yangyang-gun, Gangwon-do	KX712484		
		SFC20151118-M26	Namae-ri, Hyeonnam-myeon, Yangyang-gun, Gangwon-do	KX712485	KX712499	
		SFC20160805-M22	Jucheong-ri, Ganghyeon-myeon, Yangyang-gun, Gangwon-do	KX712486	KX712500	
	<i>P. daejeonium</i>	SFC20160805-M23	Jucheong-ri, Ganghyeon-myeon, Yangyang-gun, Gangwon-do	KX712487	KX712501	
		SFC20160805-M24	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712488	KX712502	
	<i>Penicillium</i> sp.	SFC20160805-M25	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712489	KX712503	
		SFC20160805-M26	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712490	KX712504	
<i>Turbata</i>	<i>P. madriti</i> ^a	SFC20160805-M27	Hyangho-ri, Jumunjin-eup, Gangneung-si, Gangwon-do	KX712491	KX712505	
		SFC20160317-M24	Gajin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea	KX712492		
		SFC20160805-M28	Gajin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea	KX712493		

^a New to record in Korea.

Materials and Methods

Samples

Agarum clathratum samples were collected from 13 sites along the mid-east coast of Korea in August, 2015 (Fig. 1). Five samples were randomly chosen from each site. Each sample was washed with artificial sea water (ASW) to remove surface debris and soil. For each sample, 5 mm disks were placed on three different culture media plates: potato dextrose agar (PDA, Difco, Becton Dickinson), glucose yeast extract agar (1 g/L glucose, 0.1 g/L yeast extract, 0.5 g/L peptone, and 15 g/L agar), and dichloran rose bengal chloramphenicol agar (DRBC; Difco, Becton Dickinson) and supplemented with artificial sea water (ASW). The plates were incubated at 25°C for 7–15 days, and then each strain was transferred to a PDA plate with ASW. The isolated strains were maintained in 20% glycerol at –80°C at the Seoul National University Fungus Collection (SFC) (Table 1).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) extraction protocol described by Rogers and Bendich (1994). PCR amplifications of *BenA* and *CaM* genes were performed using the primers Bt2a and Bt2b (Glass and Donaldson, 1995) and CF1 and CF4 (Peterson *et al.*, 2005), respectively. Each PCR was performed in a C1000 Thermal Cycler (Bio-Rad) following the methods of Park *et al.* (2015). The PCR products were purified with the ExpinTM PCR Purification Kit (GeneAll Biotechnology) according to the manufacturer's instructions. DNA sequencing was performed in both forward and reverse directions with the same PCR primers at Macrogen, using an ABI Prism 3700 Genetic Analyzer (Life Technologies).

Phylogenetic analysis

Sequences were assembled, proofread, and edited using MEGA v. 5 (Tamura *et al.*, 2011) and were deposited in GenBank (accession numbers are shown in Table 1). Phylogenetic analysis was performed using a two-step approach. First, we conducted a BLAST search of *BenA* sequences from each isolate and the sequences with the highest similarity were downloaded and used for further phylogenetic analysis. A second analysis was employed when identification based on *BenA* sequences was unclear. A combined dataset of two loci (*BenA* + *CaM*) was used in second analysis. Multiple sequences were aligned using the default settings of MAFFT v7 (Kato and Standley, 2013), and ambiguously aligned positions were adjusted manually. Maximum likelihood phylogenetic analyses were performed using RAxML (Stamatakis, 2006), with the GTR+G model of evolution and 1,000 bootstrap replicates.

Morphological analysis

All strains were inoculated at three points on Czapek yeast autolysate agar (CYA), yeast extract sucrose agar (YES), and malt extract agar (MEA; Oxoid) and incubated at 25°C for 7 days (Visagie *et al.*, 2014b). In addition, CYA plates were incubated at 30°C and 37°C. After incubation, the culture

characteristics and microscopic characteristics were evaluated following the methods of Park *et al.* (2015).

Results and Discussion

A total of 32 *Penicillium* strains were isolated from ten of sixteen sites along the mid-east coast of Korea. Of all regions assessed, Hyangho-ri had the largest number of strains (10), followed by Gajin-ri (8) (Table 1). Sequences from the *BenA* locus identified 32 *Penicillium* strains in 13 species of nine sections (Fig. 2). Eight species were clearly identified at the species level by *BenA* sequence analysis (Fig. 2), however, four species in sections *Aspergilloides* and *Sclerotiora* could not be accurately identified based on *BenA* sequences alone. The combined dataset (*BenA* + *CaM*) improved resolution of closely related species and supported the classification of these strains as different species (Fig. 3). These results show that the *BenA* locus usually provides adequate resolution for *Penicillium* species identification; however, we also show that the combined dataset from two loci (*BenA* + *CaM*) has higher resolution than *BenA* sequences alone. One species (four strains) could not be confidently identified to species level due to ambiguity in the molecular and morphological results; it was designated as *Penicillium* sp. in this study.

The most common species associated with *A. clathratum* was *P. citrinum* (9 isolates), followed by *Penicillium* sp. (4), *P. roseomaculatum* (3), and *P. virgatum* (3). Some species were reported from marine environments for the first time and were also new records to Korea. A detailed description of the morphology and taxonomy of these four new recorded species in Korea is given below: *P. bilaiiae*, *P. cremeogriseum*, *P. madriti*, and *P. roseomaculatum*.

Penicillium section *Aspergilloides*

Five strains were determined to section *Aspergilloides* using the *BenA* sequences. Based on the combined dataset of both the *BenA* and *CaM* loci, three strains (SFC20151118-M27, SFC20160805-M01, and SFC20160805-M02) formed a monophyletic group with *P. roseomaculatum* DTO 225-E3 (type strain), DTO 035-A1, DTO 084-F8, and DTO 100-A7 (97% bootstrap support) (Fig. 3A). These strains had 99.5–99.7% sequence similarity to *P. roseomaculatum* at the *BenA* locus and 99.5–99.7% at the *CaM* locus. The other strains (SFC-20160805-M03 and SFC20160317-M23) were identified as *P. spinulosum*, with 100% sequence similarity at both the *BenA* and *CaM* loci.

The species in *Penicillium* section *Aspergilloides* were commonly isolated from soil, food, and indoor environments. The phylogeny for this section was constructed using the combined dataset from the *BenA*, *CaM*, and *RPB2* loci and was divided into 12 clades and 51 species (Houbraken *et al.*, 2014). *P. roseomaculatum* and *P. spinulosum* belonged to the *P. spinulosum*-clade in section *Aspergilloides*. There are currently seven accepted species in the *P. spinulosum*-clade: *P. gran Canariae*, *P. palmense*, *P. roseomaculatum*, *P. spinulosum*, *P. sterculiicola*, *P. subspinulosum*, and *P. trzebinskii*. Although species belonging to the *P. spinulosum*-clade appeared closely related based on morphology, the species was clearly separated based on the combined dataset using the

BenA and *CaM* loci (Houbraken et al., 2014). Especially, *P. roseomaculatum*, *P. spinulosum*, *P. subspinulosum*, and *P. trzebinskii* have a close phylogenetic relationship in this section, therefore it is required to analyze with combined analysis of *BenA* and *CaM*. *P. roseomaculatum* is a new distribution record for both marine environments and Korea. *P.*

spinulosum was reported from soil, forest litter, decaying vegetation, food, and marine environments (Pitt, 1979; Lee et al., 2003; Panno et al., 2013; Houbraken et al., 2014).

Penicillium section *Brevicompecta* and *Ramosa*

One strain was confirmed within section *Brevicompecta*

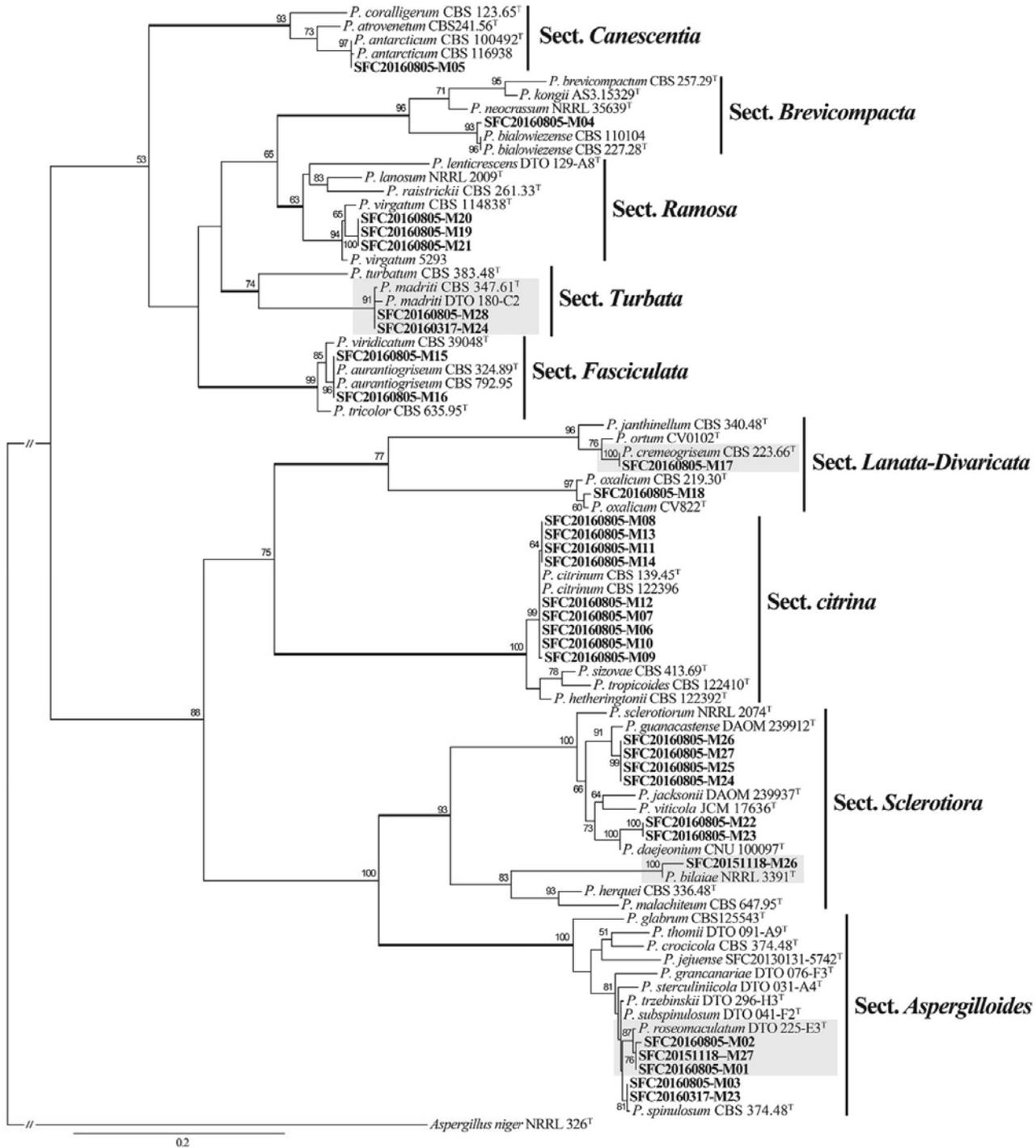


Fig. 2. Phylogenetic tree for *Penicillium* strains isolated from *Agarum clathratum* and related species based on maximum likelihood (ML) of β -tubulin (*BenA*). Bootstrap scores of > 50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site, and “^T” indicates ex-type strains.

(SFC20160805-M04) and three strains were confirmed in section *Ramosa* (SFC20160805-M19, SFC20160805-M20, and SFC20160805-M21). The section *Brevicompecta* strain SFC20160805-M04 formed a monophyletic group with *P. bialowiezense* CBS 227.28 (type strain) and CBS 110104 when the phylogeny was constructed using the *BenA* sequences (sequence similarity = 99.6%; bootstrap support = 100%). The section *Ramosa* strain SFC20160805-M19, SFC20160805-M20, and SFC20160805-M21 were identified as *P. virgatum* for *BenA* sequence (sequence similarity = 97.7–100%; bootstrap support = 94%).

P. bialowiezense is commonly isolated from soil, seaweed, and indoor environments (Samson *et al.*, 2004; Scott *et al.*, 2008). This species was previously isolated from grapes (Kim *et al.*, 2007) and macroorganisms in marine environments in Korea (Park *et al.*, 2014a). Strains of this species collected from marine environments showed β -glucosidase and antifungal activity (Park *et al.*, 2014a). *Penicillium virgatum* was previously isolated from the soil of a soybean field (Kwaśna and Nirenberg, 2005) and marine sponge from the Atlantic Ocean (Passarini *et al.*, 2013). In our previous study, we isolated this species from marine macro-organisms and showed its antifungal activity against the plant pathogens *C. acutatum* and *F. oxysporum* (Park *et al.*, 2014a).

Penicillium section *Canescentia*, *Fasciculata*, and *Turbata*

Five strains were identified to be within sections *Canescentia* (SFC20160805-M05), *Fasciculata* (SFC20160805-M15 and SFC20160805-M16), and *Turbata* (SFC20160317-M24 and SFC20160805-M28). The section *Canescentia* strain SFC-

20160805-M05 was identified as *P. antarcticum* at the *BenA* locus (sequence similarity = 99.8–100%; bootstrap support = 96%). The section *Fasciculata* strain SFC20160805-M15 and SFC20160805-M16 formed a monophyletic group with *P. aurantiogriseum* CBS 324.89 (type strain) and CBS 792.95 using the *BenA* sequences (sequence similarity = 100%; bootstrap support = 96%). Strains SFC20160317-M24 and SFC-20160805-M28 were grouped into section *Turbata* and formed a monophyletic group with the *P. madriti* CBS 347.61 (type strain) and DTO 180-C2 using the *BenA* sequences (99.5–99.8% sequence similarity), with 96% bootstrap support.

P. antarcticum was firstly isolated from the Antarctic (McRae *et al.*, 1999). In Korea, *P. antarcticum* was isolated from sea water, sponges, and macroorganisms in marine environments and showed β -glucosidase and antifungal activity (Park *et al.*, 2014a, 2014b). *Penicillium aurantiogriseum* is commonly isolated from cereals (Pitt, 1979) and known to cause postharvest disease in apples and pears (Jones and Aldwinke, 1990), and strains isolated from marine environments showed cytotoxicity (Ma *et al.*, 2016). In Korea, this species causes blue mold in pears (Shim *et al.*, 2002). *P. madriti* was previously isolated from soil and house dust (Smith, 1961; Visagie *et al.*, 2014a). *P. madriti* is known to produce orsellinic acid (Birkinshaw and Gowlland, 1962). This species is a new distribution record in marine environments and in Korea.

Penicillium section *Citrina* and *Lanata-Divaricata*

Large number of isolates (9 strains; SFC20160805-M06, SFC-20160805-M07, SFC20160805-M08, SFC20160805-M09, SFC-20160805-M10, SFC20160805-M11, SFC20160805-M12, SFC-

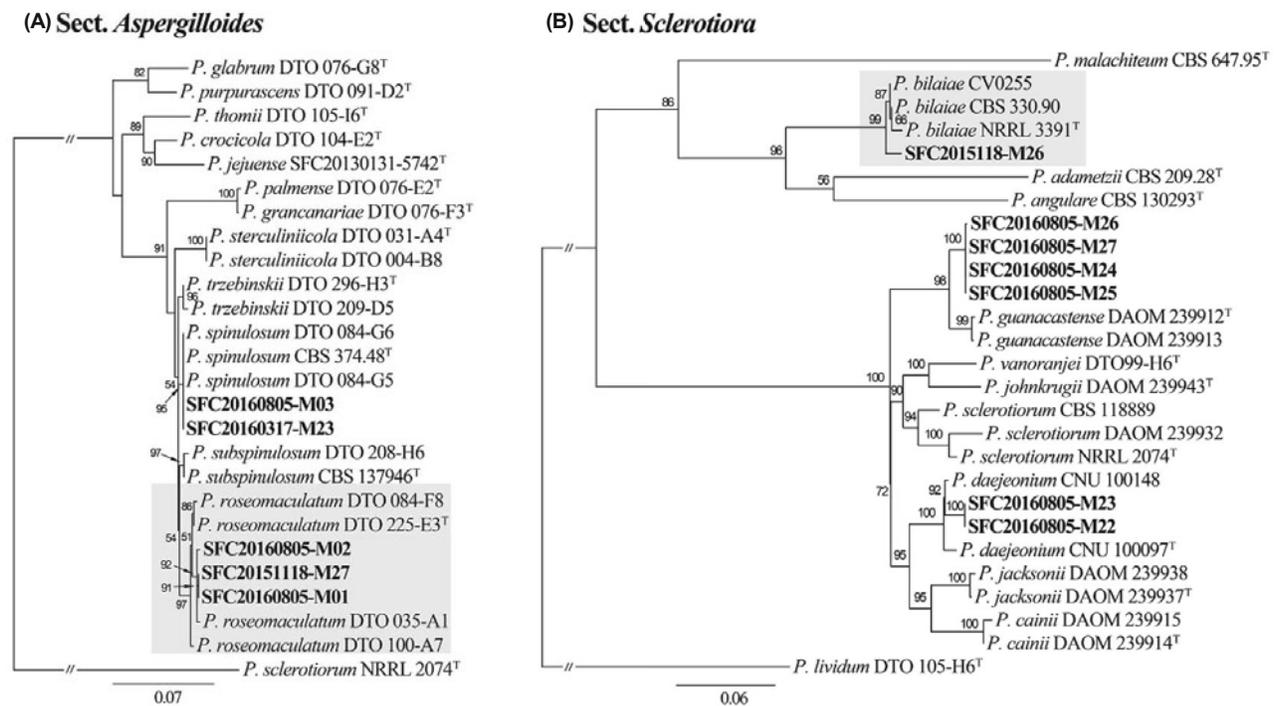


Fig. 3. Phylogenetic tree based on maximum likelihood (ML) of the combined data set of β -tubulin (*BenA*) and calmodulin (*CaM*) used to identify strains to species in section *Aspergilloides* (A) and *Sclerotiora* (B). Bootstrap scores of > 50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site, and ^T indicates ex-type strains.

20160805-M13, and SFC20160805-M14) were identified as *P. citrinum* (section *Citrina*). These isolates formed a monophyletic group with *P. citrinum* CBS 139.45 (type strain) and CBS 122396 strains at the *BenA* locus (99.8–100% sequence similarity) with 98% bootstrap support. Two strains SFC-20160805-M17 and SFC20160805-M18 were grouped into in section *Lanata-Divariata* at the *BenA* locus and were identified as *P. cremeogriseum* (sequence similarity = 100%; bootstrap support = 100%) and *P. oxalicum* (sequence similarity = 99.3–99.6%; bootstrap support = 100%), respectively.

P. citrinum was previously isolated from various environments including marine, soil, indoor, air, and food (Houb-raken et al., 2011). In Korea, this species was isolated from grapes (Kim et al., 2007), and was commonly found in marine environments such as seaweed, macroorganisms, and macroalgae (Park et al., 2014a, 2015). This species is known to produce a variety of active compounds and enzymes (Dutta et al., 2007; Houb-raken et al., 2011; Park et al., 2014a). *P. cremeogriseum* was previously isolated from agricultural and forest soil (Sonjak et al., 2007) and has been shown to exhibit antitumor activity (Frisvad and Filtenborg, 1990). *P. cremeogriseum* is new distribution records in marine environments and in Korea. *P. oxalicum* was previously isolated from soil, air, indoor environments, and marine environments (Visagie et al., 2014a, 2015; Ma et al., 2016). In Korea, this species was isolated from barely and maize (Kim et al., 2005), as well as macroorganisms in marine environments (Park et al., 2014a). *P. oxalicum* produces a variety of active compounds and enzymes (Li et al., 2007; Park et al., 2014a; Ma et al., 2016) and is known as a biological agent against plant pathogens (Sabuquillo et al., 2006).

Penicillium section *Sclerotiora*

BenA sequence analysis confirmed that seven strains belonged to section *Sclerotiora*. The combined dataset (*BenA* + *CaM*) improved their resolution and supported the classification of these strains as different species (Fig. 3B). Strain SFC20151118-M26 formed a monophyletic group with the *P. bilaiae* NRRL 3391 (type strain), CBS 330.90, and CV0255 strains with 100% bootstrap support (98.0–98.3% sequence similarity for *BenA* and 97.9–99.0% for *CaM*). Two strains, SFC20160805-M22 and SFC20160805-M23, were identified as *P. daejeonium* with 100% bootstrap support (97.9–98.1% sequence similarity at *BenA* and 98.0–99.0% at *CaM*). Four strains (SFC20160805-M24, SFC20160805-M25, SFC20160805-M26, and SFC20160805-M27) formed a monophyletic group with the *P. guanacastense* DAOM 239912 (type strain) and DAOM 239913 strains with 98% bootstrap support (98.2% sequence similarity for *BenA* and 97.3% for *CaM*).

The species in *Penicillium* section *Sclerotiora* are commonly isolated from soil and have monoverticillate, orange to red colonies, and sclerotia production (Rivera and Seifert, 2011; Visagie et al., 2013). There are currently 24 accepted species in section *Sclerotiora* based on morphological characteristics and the combined dataset from the *BenA* and *CaM* loci (Visagie et al., 2014b). *P. bilaiae* and *P. daejeonium* in section *Sclerotiora* were reported from soil and known as plant growth promoting fungi (Wakein et al., 2007) and *Penicillium* rot of grape and schisandra fruits (Sang et al., 2013), respectively. This is the first report of these species in marine environments.

Four strains (SFC20160805-M24, SFC20160805-M25, SFC-20160805-M26, and SFC20160805-M27) were most genetically similar to, but differed in terms of growth morphology and growth rate to *P. guanacastense*. In addition, this species was reported from gut of caterpillars, while our strains were isolated from marine environment. Due to morphological and genetical ambiguity, we designated these strains as an unidentified species (*Penicillium* sp.) within section *Sclerotiora* to highlight the need for further study.

Taxonomy

Penicillium bilaiae Chalab 1950 (Fig. 4)

Description: Colony diameters, 7 d, 25°C (unless otherwise stated), in mm: CYA, 30–31; CYA 30°C, 34–36; CYA 37°C, no growth; MEA, 28–29; YES, 31–33.

Colonies on CYA: colonies radially sulcate; sporulation moderate; 3–4 mm of white to yellowish white (4A2) mycelium at the margin; colony texture velutinous; conidia dark green (26F3); exudate droplets absent; soluble pigments absent; reverse color brown (7E4), with light yellow (4A4) at margin. Colonies on MEA: colonies radially sulcate; sporulation moderate; 2–3 mm of white to yellowish white (4A2);

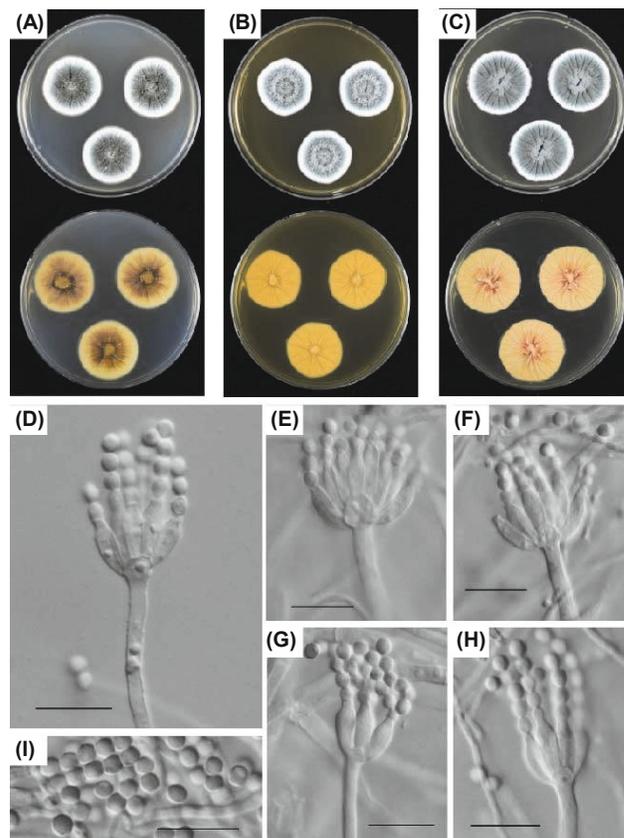


Fig. 4. *Penicillium bilaiae* SFC20151118-M26 for 7-day-old cultures at 25°C. (A–C) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse). (D–H) Conidiophores. (I) Conidia (scale bars: D–I = 10 µm)

colony texture velutinous; floccose near center; conidia dark green (27F4); exudate droplets absent; soluble pigments absent; reverse color grayish orange (5B5). Colonies on YES: colonies radially sulcate, slightly raised centrally; sporulation moderate; 2–3 mm of white at the margin; colony texture velutinous; conidia dark green (27F3); exudate droplets clear near margin; soluble pigments absent; reverse color light yellow (4A4).

Sclerotia absent; asci and ascospores not observed; conidiophores monoverticillate, smooth, 2.0–2.5 μm wide; phialides ampulliform, 6–8 \times 2.0–2.5 μm ; conidia globose to subglobose, 2.2–2.6 \times 2.0–2.6 μm , with roughed walls.

Strain examined: SFC20151118-M26

Remarks: *P. bilaiae* is morphologically similar to *P. alexiae* and *P. adametzioides*. This species can be distinguished from *P. alexiae* and *P. adametzioides* by rapid growth at 25, 30, and 37°C on CYA. Compared to type strain of *P. bilaiae*, Korean *P. bilaiae* has absence of orange brown pigment on CYA consistently produced in *P. bilaiae* and no growth at 37°C (Visagie *et al.*, 2013). Although strain SFC20151118-M26 collected from a marine environment in Korea showed slightly different culture characteristics from the type strain (IMI 113677) of *P. bilaiae*, phylogenetically, strain SFC2015-1118-M26 is closely related to *P. bilaiae*. Strain SFC2015-

1118-M26 was confidently identified as *P. bilaiae* because of phylogenetic relationships and other morphological characteristics.

Penicillium cremeogriseum Chalab 1950 (Fig. 5)

Description: Colony diameters, 7 d, 25°C (unless otherwise stated), in mm: CYA, 45–50; CYA 30°C, 34–36; CYA 37°C, 27–29; MEA, 45–52; YES, 44–46.

Colonies on CYA: colonies radially sulcate; sporulation weak; 5–6 mm of white mycelium at the margin; colony texture velutinous; conidia gray (27C1); exudate droplets clear to light yellow (4A4); soluble pigments absent; reverse color pale yellow (4A3). Colonies on MEA: colonies plate; sporulation weak at center; yellowish white (1A2) mycelium at margin, white elsewhere; colony texture velutinous; conidia greenish yellow (4C3) near center; exudate droplets clear to light yellow (4A4); soluble pigments absent; reverse color reddish yellow (4A6). Colonies on YES: colonies radially sulcate; sporulation weak; white to yellowish white (3A2) of mycelium at the margin; colony texture velutinous; conidia gray (4C1); exudate droplets absent; soluble pigments absent; reverse color olive brown (4F4) with orange yellow (4A6) at margin.

Sclerotia absent; asci and ascospores not observed; conidio-

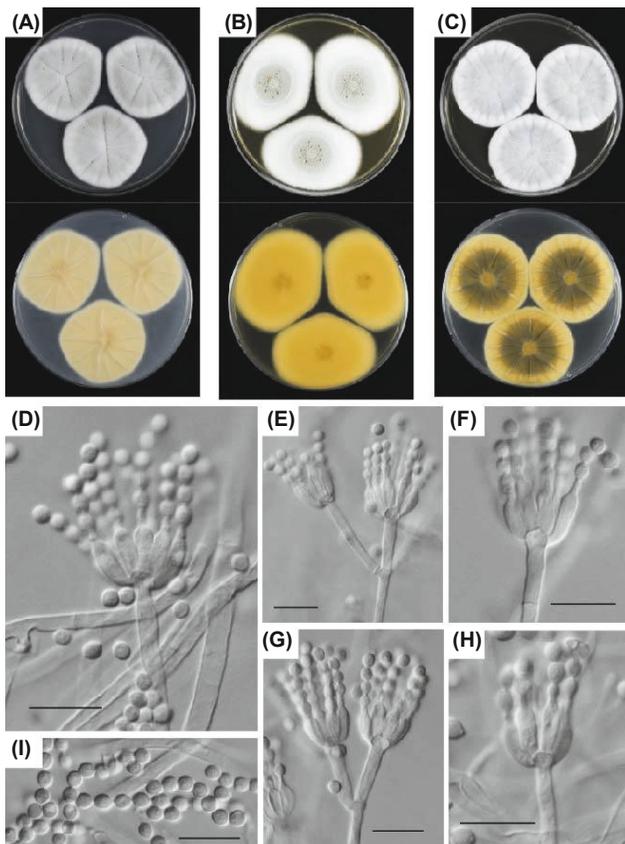


Fig. 5. *Penicillium cremeogriseum* SFC20160805-M17 for 7-day-old cultures at 25°C. (A–C) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse). (D–H) Conidiophores. (I) Conidia (scale bars: D–I = 10 μm)

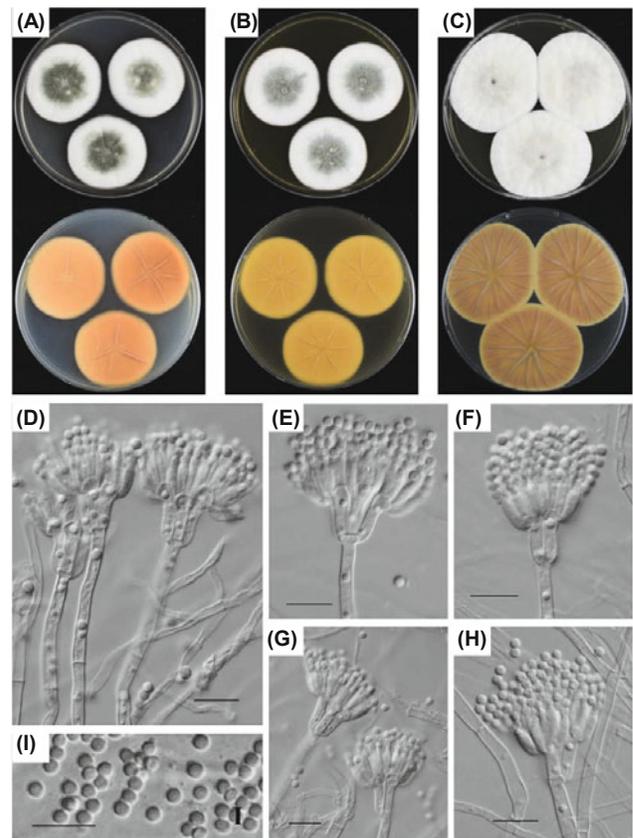


Fig. 6. *Penicillium madriti* SFC20160317-M24 for 7-day-old cultures at 25°C. (A–C) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse). (D–H) Conidiophores. (I) Conidia (scale bars: D–I = 10 μm)

phores monoverticillate and biverticillate, smooth, 2.0–2.5 μm wide; phialides ampulliform, 8–10 \times 2.2–2.8 μm ; conidia globose, 2.2 \times 2.8 μm , with smooth or finely roughed walls.

Strain examined: SFC20160805-M17

Remarks: *P. cremeogriseum* is morphologically similar to *P. ortum* and share identical ITS sequences with *P. ortum*. *P. cremeogriseum* can be distinguished from *P. ortum* by fast growth at 37°C on CYA. Compared to type strain of *P. cremeogriseum* (DTO225-E3), Korean *P. cremeogriseum* tends to grow faster at 25°C on CYA and YES (Visagie et al., 2014b). Although strain SFC20160805-M17 collected from marine environment in Korea showed slightly different culture characteristics with previously reported *P. cremeogriseum*, phylogenetically, strain SFC20160805-M17 share identical *BenA* sequences with *P. cremeogriseum*. Strain SFC20160805-M17 could be confidently identified as *P. cremeogriseum* because of phylogenetic relationship and other morphological characteristics.

Penicillium madriti G. Smith 1961 (Fig. 6)

Description: Colony diameters, 7 d, 25°C (unless otherwise stated), in mm: CYA, 39–41; CYA 30°C, 35–37; CYA 37°C, 5–7; MEA, 39–40; YES, 48–50.

Colonies on CYA: colonies radially sulcate, sporulation weak; 8–10 mm of white mycelium at the margin; colony texture velutinous; conidia dark green (29F7) at center; exudate droplets yellowish white (4A2); soluble pigments grayish orange (6B3); reverse color brownish orange (7C5). Colonies on MEA: colonies radially low sulcate; sporulation weak, 8–10 mm of white mycelium at the margin; colony texture velutinous; conidia greenish ray (27E2) at center; exudate droplets yellowish white (4A2); soluble pigments absent; reverse color light yellow (4A5). Colonies on YES: colonies radially low sulcate; sporulation weak; 11–14 mm of white mycelium at the margin; colony texture velutinous; conidia green gray (28B2) to gray (28B1); exudate droplets light yellow (4A4); soluble pigments absent; reverse color brownish orange (5C5).

Sclerotia absent; asci and ascospores not observed; conidiophores biverticillate, smooth, 2.5–2.8 μm wide; 3–5 metulae per ramus, 10–13 \times 2.5–3.0 μm ; phialides ampulliform, 6.5–9 \times 2.0–2.2 μm , conidia globose, 2.1 \times 2.5 μm , with smooth walls.

Strain examined: SFC20160317-M24

Remarks: *P. madriti* is morphologically similar to *P. bovisomum* in section *Turbata*. This species can be distinguished from *P. bovisomum* by fast growth on CYA and MEA and grayish green conidia.

Penicillium roseomaculatum Biourge 1923 (Fig. 7)

Description: Colony diameters, 7 d, 25°C (unless otherwise stated), in mm: CYA, 49–51; CYA 30°C, 26–29; CYA 37°C, no growth; MEA, 47–48; YES, 56–58.

Colonies on CYA: colonies sulcate at center, sporulation moderate; 4–5 mm of white mycelium at the margin; colony texture velutinous to floccose; conidia dull green (27E3); exudate droplets clear; soluble pigments absent; reverse color yellowish white (3A2). Colonies on MEA: colonies low sulcate at center; sporulation moderate, 3–4 mm of white mycelium

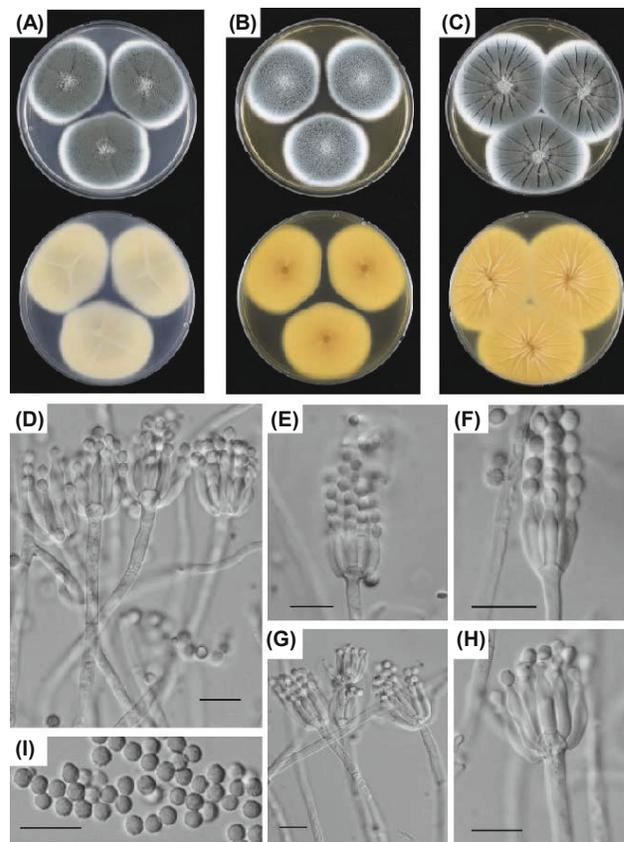


Fig. 7. *Penicillium roseomaculatum* SFC20160805-M01 for 7-day-old cultures at 25°C. (A–C) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse). (D–H) Conidiophores. (I) Conidia (scale bars: D–I = 10 μm)

at the margin; colony texture velutinous, floccose at center; conidia dull green (27E3); exudate droplets clear; soluble pigments absent; reverse color light yellow (4A4). Colonies on YES: colonies radially sulcate, slightly raised centrally; sporulation moderate; 5–6 mm of white mycelium at the margin; colony texture velutinous, floccose at center; conidia dull green (27E3), greenish gray (27D2) near center; exudate droplets absent; soluble pigments absent; reverse color light yellow (4A4).

Sclerotia absent; asci and ascospores not observed; conidiophores monoverticillate, smooth, 2.5–3.0 μm wide; phialides ampulliform, 8.0–11.0 \times 2.5–3.0 μm ; conidia globose, 2.7 \times 3.3 μm , with finely roughed walls.

Strain examined: SFC20151118-M27 and SFC20160805-M01

Remarks: *P. roseomaculatum* is morphologically and phylogenetically similar to species in the *P. spinulosum*-clade. *P. roseomaculatum* showed a large variation of growth characters among strains on CYA (Houbraken et al., 2014). Compared to type strain of *P. roseomaculatum* (DTO225-E3) Korean *P. roseomaculatum* tends to grow faster on CYA and MEA (Rivera et al., 2012). On the basis of *BenA* and *CaM* sequence analysis, *P. roseomaculatum* is clearly differs from species in *P. spinulosum*-clade.

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