Mycobiology

Research Article

Diversity of Marine-Derived *Aspergillus* from Tidal Mudflats and Sea Sand in Korea

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Abstract *Aspergillus* (Trichocomaceae, Eurotiales, and Ascomycota) is a genus of well-defined asexual spore-forming fungi that produce valuable compounds such as secondary metabolites and enzymes; however, some species are also responsible for diseases in plants and animals, including humans. To date, 26 *Aspergillus* species have been reported in Korea, with most species located in terrestrial environments. In our study, *Aspergillus* species were isolated from mudflats and sea sand along the western and southern coasts of Korea. A total of 84 strains were isolated and identified as 17 *Aspergillus* species in 11 sections on the basis of both morphological characteristics and sequence analysis of the calmodulin gene (*CaM*) locus. Commonly isolated species were *A. fumigatus* (26 strains), *A. sydowii* (14 strains), and *A. terreus* (10 strains). The diversity of *Aspergillus* species isolated from mudflats (13 species) was higher than the diversity of those from sea sand (five species). Four identified species—*A. caesiellus*, *A. montenegroi*, *A. rhizopodus*, and *A. tabacinus*—are in the first records in Korea. Here, we provide detailed descriptions of the morphological characteristics of these four species.

Keywords *Aspergillus*, *CaM*, Marine environment, Morphology, Phylogeny

Genus *Aspergillus* consists of well-defined asexual spore-forming fungi and is classified as Trichocomaceae, Eurotiales, Eurotiomycetes, and Ascomycota. Approximately 64% of the 250 described species of aspergilli have not been studied regarding their sexual state [1]. Historically, species of *Aspergillus* were called as several names: *Chaetosartorya*, *Cristaspora*, *Dichotomomyces*, *Emericella*, *Eurotium*, *Fennellia*, *Neocarpenteles*, *Neopetromyces*, *Neosartorya*, and *Petromyces* [2]. However, genus *Aspergillus* has been recently unified by mycologists in an effort to simplify the taxonomy by using a single genus name following ICN guidelines (International Code of Nomenclature for algae, fungi, and plants) [3].

Since *Aspergillus* was first described in the 18th century [4], 339 species have been reported worldwide and have been categorized into four subgenera (*Aspergillus*, *Circumdati*, *Fumigati*, and *Nidulantes*) and 20 sections [2]. Morphological features of *Aspergillus* are crucial for initial identification [2]; however, morphology alone has proven unreliable because several morphological features are influenced by environmental conditions such as composition of the medium, pH, additives, or temperature [5]. To overcome these limitations, mycologists recently proposed standardized methods involving polyphasic analysis including morphological and molecular analyses and extrolite profiling [2]. Molecular identification methods were first introduced into fungal taxonomy as sequencing of the internal transcribed spacer (ITS) region, and this locus has been proposed as the universal DNA barcode marker for all fungi. More recently, additional DNA markers for *Aspergillus* such as calmodulin (*CaM*), β-tubulin (*BenA*), and the RNA polymerase II second largest subunit (*RPB2*) have been used in *Aspergillus* taxonomy [2]. In particular, *CaM* has proven a useful marker for *Aspergillus* identification because the *CaM* database is well established, the locus is relatively easy to amplify, and adequate polymorphism allows for accurate identification of *Aspergillus* species [2].

Aspergillus is known for plant pathogen and cause of respiratory diseases in humans, and produces various mycotoxins such as aflatoxin and ochratoxin A [6], however, *Aspergillus* plays important ecological roles by degrading starches, hemicelluloses, celluloses, and other polysaccharides [7]. Therefore, *Aspergillus* species have been industrially exploited for potential applications of their enzymes [6, 8]. *Aspergillus* species are isolated from various environments [2]. In addition, many *Aspergillus* species have been reported

Mycobiology 2016 December, **44**(4): 237-247
https://doi.org/10.5941/MYCO.2016.44.4.237
pISSN 1229-8093 • eISSN 2092-9323

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Received September 22, 2016
Revised October 9, 2016
Accepted October 29, 2016

from marine environments including sponges and corals, but most *Aspergillus* studies have been focused on their bioactive compounds rather than their diversity [9, 10].

There are various types of the intertidal zone depending on various geographical factors [11, 12]. The intertidal zone in Korea usually appears as mudflats or sea sand. Intertidal zones are ecologically valuable and provide habitats and feeding areas for diverse flora and fauna populations [13]. In addition, many microbes live in intertidal zones and perform critical ecological roles such as degradation of organic materials and synthesis of various bioactive compounds [14, 15]. On the other hand, most researches about intertidal zones have been focused on bacterial communities [16, 17] rather than fungal communities.

To date, 26 species of *Aspergillus* have been reported in Korea [18]. Although some *Aspergillus* species were reported from soil and inside buildings, most of them were isolated from fermented foods and studied as a potential fermentation initiator for production of foods and drinks such as meju, soy sauce, and traditional alcohol [19]. On the other hand, diversity of marine-derived *Aspergillus* has been poorly studied. Since the Ministry of Ocean and Fisheries established the Marine Fungal Resource Bank, marine-derived fungi have been studied to promote exploration of marine biodiversity and biological resources. We studied *Aspergillus* from intertidal mudflats and sea sand on both the western and southern coasts of Korea from 2014 to 2015. Based on sequence analysis of the *CaM* locus, several *Aspergillus* strains were identified at the species level. During the study, we found four previously unrecorded species in Korea (*A. caesiellus*, *A. montenegroi*, *A. rhizopodus*, and *A. tabacinus*). In this study, we describe macromorphological and micromorphological characteristics of these four species.

Table 1. Information of isolated *Aspergillus* strains from mudflat and sea sand in different seasons

Section	Species	Strain No.	Collection No.[a]	Substrate (No. of strains)		Season (No. of strains)		GenBank accession No. (*CaM*)
				Mudflat	Sea sand	Summer	Winter	
Aspergillus	*A. ruber*	8M62	SFC20160317-M25	2	–	1	1	KX845511
		P239	SFC20160805-M29	–	–	–	–	KX845512
	A. pseudoglacus	8M859	SFC20160317-M27	–	2	2	–	KX845513
Circumdati	*A. westerdijikiae*	8M709	SFC20160112-M05	1	–	–	1	KX845514
Clavati	*A. clvatus*	P6	SFC20150303-M01	6	–	5	1	KX845515
	A. rhizopodus	SP53	SFC20160407-M08	2	–	1	1	KX845516
Flavi	*Aspergillus* sp. 1	P102	SFC20160805-M30	3	2	–	5	KX845517
		SEP13	SFC20160805-M31	–	–	–	–	KX845518
Flavipedes	*Aspergillus* sp. 2	SP112	SFC20160805-M32	1	–	–	1	KX845519
Fumigati	*A. fumigatus*	8M56	SFC20160805-M33	23	3	11	15	KX845520
		8M59	SFC20160805-M34	–	–	–	–	KX845521
		8M87	SFC20160805-M35	–	–	–	–	KX845522
		8M111	SFC20160805-M36	–	–	–	–	KX845523
		MT195	SFC20160805-M37	–	–	–	–	KX845524
		P230	SFC20160805-M38	–	–	–	–	KX845525
	Aspergillus sp. 3	SP66	SFC20160805-M39	1	–	–	1	KX845526
Nidulantes	*A. nidulans*	8M315	SFC20150812-M13	1	–	1	–	KX845527
	A. montenegroi	P40	SFC20160805-M50	1	–	–	1	KX845528
Nigri	*A. welwischiae*	SA8	SFC20160805-M40	5	1	2	4	KX845529
		SA13	SFC20160805-M41	–	–	–	–	KX845530
		SA14	SFC20160805-M42	–	–	–	–	KX845531
		SP100	SFC20160317-M20	–	–	–	–	KX845532
Restriciti	*A. caesiellus*	MT127	SFC20160112-M06	1	–	1	–	KX845533
Terrei	*A. terreus*	P212	SFC20160805-M45	9	1	3	7	KX845517
		P213	SFC20160805-M43	–	–	–	–	KX845535
Versicolores	*A. tabacinus*	P39	SFC20160407-M11	2	–	–	2	KX845536
	A. venenatus	SP57	SFC20160407-M10	2	1	–	3	KX845537
	A. sydowii	MT147	SFC20160407-M09	14	–	4	10	KX845538
		MT172	SFC20160805-M44	–	–	–	–	KX845539
		MT349	SFC20160805-M46	–	–	–	–	KX845540
		P17	SFC20160805-M47	–	–	–	–	KX845541
		P49	SFC20160805-M48	–	–	–	–	KX845542
		P206	SFC20160805-M49	–	–	–	–	KX845543
		P207	SFC20160805-M51	–	–	–	–	KX845544

[a]SFC stands for abbreviation of 'Seoul National University Fungus Collection'.

MATERIALS AND METHODS

Collection and isolation. Samples were collected from both the western and southern coasts of Korea. Collection was carried out during summer and winter from 2014 to 2015. Each collection was repeated five times after removal of the top 0.5 to 1.0 cm of soil, and each collection site was randomly chosen within a 10 m distance. After collection, the samples were stored at 4°C prior to isolation. Each sample was diluted 10-fold with artificial sea water [20]. Next, 100 µL of each dilution was spread on three different media plates, which contained artificial sea water: potato dextrose agar (PDA; Difco, Becton Dickinson, Sparks, MD, USA), 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 (Difco, Becton Dickinson). All plates were incubated at 25°C for 1 wk to confirm distinguishable morphological features. Each *Aspergillus* strain was transferred to a new PDA plate. Each strain was then stored in 20% glycerol at −80°C at the Seoul National University Fungus Collection (SFC).

Molecular procedures and phylogenetic analysis. DNA was extracted using a modified cetyltrimethylammonium bromide protocol [21]. The PCR amplification of the *CaM* locus was conducted as described by Park *et al.* [22]. Gel electrophoresis was carried out for each amplicon on a 1% agarose gel, and these PCR products were purified using the Expin PCR Purification Kit (Geneall Biotechnology, Seoul, Korea) following the manufacture's instruction. The purified amplicons were sequenced using corresponding PCR primers by Macrogen (Seoul, Korea) in both forward and reverse directions using an ABI Prism 3700 genetic analyzer (Life Technologies, Gaithersburg, MD, USA).

Sequences were assembled, proofread, edited, and aligned using MEGA v.5 [23] and were deposited in GenBank (Table 1). For multiple sequence alignments, MAFFT v.7 [24] was used, and each sequence was checked and adjusted by eye. After alignment, maximum likelihood phylogenetic trees were constructed. The phylogenetic trees were generated using RAxML 8.0.2 [25] and the GTR-GAMMA model of evolution with 1,000 bootstrap replicates.

Morphological analysis. To examine both macro- and micromorphological characteristics of the unrecorded species, each strain was inoculated as a spore suspension at three points on plates with one of three different media: Czapek yeast autolysate agar (CYA, yeast extract; Difco), malt extract agar (MEA, Oxoid), and yeast extract sucrose agar (YES, yeast extract; Difco). All CYA, MEA, and YES plates were incubated at 25°C for 7 days, and CYA plates were incubated at 30°C, 37°C, and 50°C for additional 7 days. All plates with media were prepared as described by Samson *et al.* [2]. The color names and alphanumeric codes for macromorphological characteristics were assigned using the criteria of Kornerup and Wanscher [26]. Analysis of micromorphological characteristics was performed following the methods of Samson *et al.* [2].

RESULTS

Totally, 84 *Aspergillus* strains were isolated from two intertidal substrates (mudflats and sea sand) at 30 sites from the western and southern coasts of Korea. More *Aspergillus* strains were isolated (74 strains) from mudflats rather than from sea sand (10 strains). Furthermore, higher diversity of *Aspergillus* was observed in mudflats (16 species) compared to sea sand (six species). Five species (*A. fumigatus*, *A. terreus*, *A. welwitschiae*, *A. venenatus*, and *Aspergillus* sp. 1) were commonly found from both substrates, and *A. sydowii* was found only from mudflats. More *Aspergillus* strains were isolated during winter (52 strains) than summer (32 strains). Diversity of *Aspergillus* species was higher in winter (14 species) than in summer (10 species), and six species (*A. clavatus*, *A. fumigatus*, *A. rhizopodus*, *A. ruber*, *A. sydowii*, *A. terreus*, and *A. welwitschiae*) were commonly found during both seasons (Table 1). On the basis of *CaM* sequences, 84 isolates were identified as 17 species in 11 sections, and three strains were identified as *Aspergillus* sp. due to ambiguous phylogenetic relationship (Table 1). For convenience, sections were grouped into four groups regardless of phylogenetic relationship.

Three sections were included in group 1: *Versicolores*, *Nidulantes*, and *Flavi*. Three species (*A. sydowii*, *A. tabacinus*, and *A. venenatus*) were confirmed to belong to section *Versicolores*. Seven representative strains (SFC20160407-M09, SFC20160805-M44, SFC20160805-M46, SFC20160805-M47, SFC20160805-M48, SFC20160805-49, and SFC20160805-M51) were identified as *A. sydowii* (sequence similarity, 99%; bootstrap support, 99%) (Fig. 1). Although one isolated strain (SFC20160407-M11) formed a monophyletic group with low bootstrap support, its morphology and *CaM* sequence similarity were very similar to those of strains of *A. tabacinus* (NRRL 4791^{T}, NRRL5031, S823, and NRRL A-23173); thus, it was identified as *A. tabacinus* (sequence similarity, 99.8~100%; bootstrap support, 78%) (Fig. 1). One strain (SFC20160407-M10) was identified as *A. venenatus* (sequence similarity, 99.8%; bootstrap support, 99%) (Fig. 1). In section *Nidulantes*, two strains (SFC20150812-M13 and SFC20160805-M50) were isolated from mudflats (Table 1), and each strain was identified as either *A. nidulans* (sequence similarity, 99.8%; bootstrap support, 100%) or *A. montenegroi* (sequence similarity, 99.3%; bootstrap support, 100%), respectively. Two strains (SFC20160805-M30 and SFC20160805-M31) in section *Flavi* were identical to type strains of *A. flavus* (NRRL 1957^{T}) and *A. oryzae* (NRRL 447^{T}), with 100% sequence similarity (Fig. 1). However, we identified these specimens as *Aspergillus* sp. 1 because it was difficult to distinguish these two species by *CaM* sequences.

Group 2 included sections *Terrei* and *Flavipedes* and showed high bootstrap support. Two strains (SFC20160805-

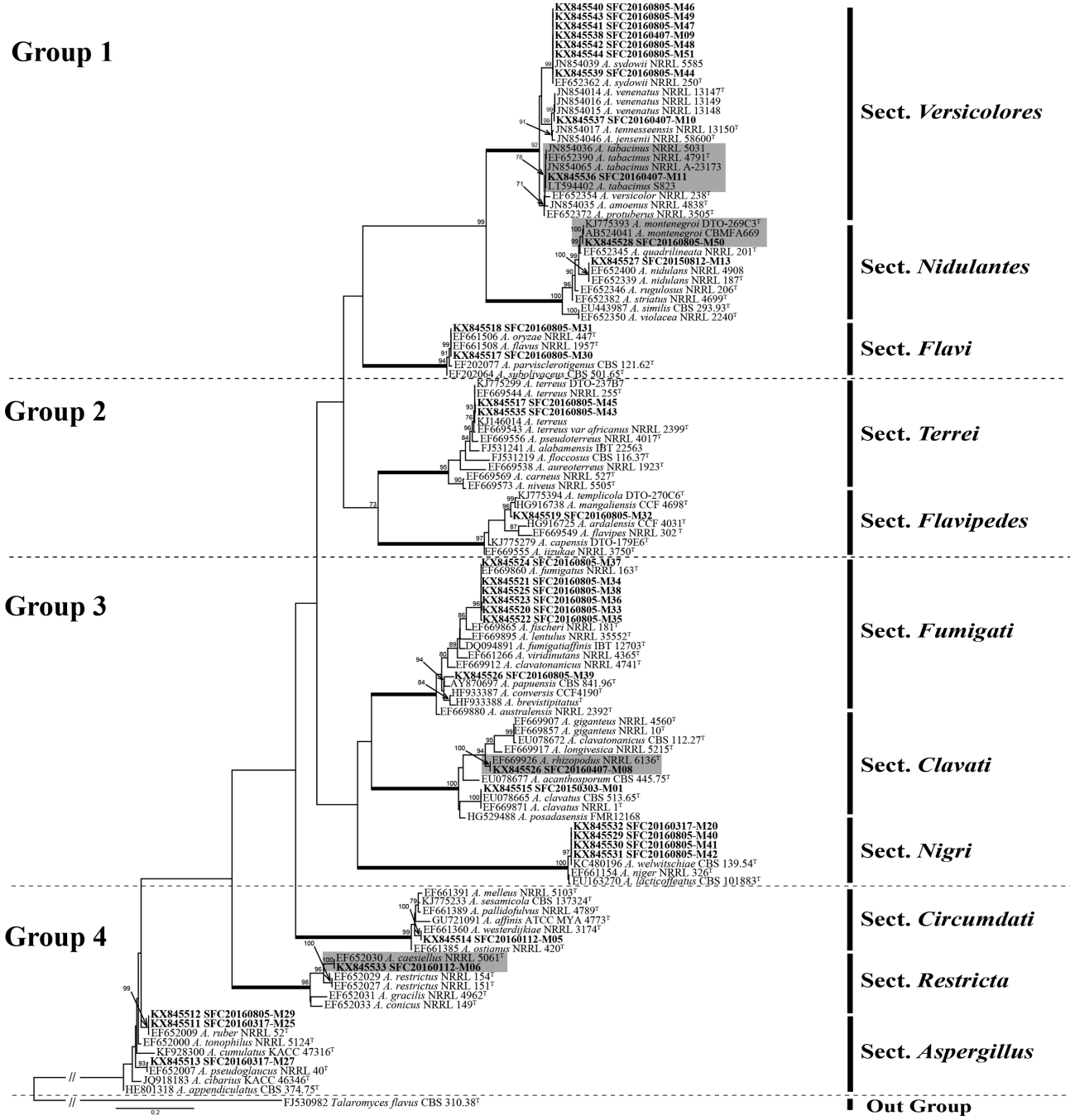

Fig. 1. A maximum likelihood phylogenetic tree of *Aspergillus* on the basis of sequences of the calmodulin (*CaM*) locus. Bootstrap scores > 70 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. "T" indicates ex-type strains. Each group was designed depending on the arrangement of sections in the phylogenetic tree.

M43 and SFC20160805-M45) were identified as *A. terreus* in section *Terrei* (sequence similarity, 99.6~100%; bootstrap support, 76%) (Fig. 1). In section *Flavipedes*, SFC20160805-M32 formed a group with type strains of *A. mangaliensis* (CCF4698^{T}: sequence similarity, 96.5%) and *A. templicola* (DTO270C6^{T}: sequence similarity, 97.1%), but we identified it as *Aspergillus* sp. 2 because of unclear phylogenetic relationship (Fig. 1).

Group 3 included three sections (*Clavati*, *Fumigati*, and *Nigri*). Six strains (SFC20160805-M33, SFC20160805-M34, SFC20160805-M35, SFC20160805-M36, SFC20160805-M37, and SFC20160805-M38) were confirmed to belong to section *Fumigati* and were identified as *A. fumigatus* (sequence similarity, 99.6~100%; bootstrap support, 96%) (Fig. 1). SFC20160805-M39 was found to be closely related to *A. papuensis* (CBS 841.96^{T}), *A. coversis* (CCF4190^{T}), and *A. brevistipitatus* (CCF419^{T}) (sequence similarity, 93.1~95.3%), but it was named *Aspergillus* sp. 3 due to unresolved

phylogeny. In section *Clavati*, the strains (SFC20150303-M01 and SFC20160407-M08) were identified as *A. clavatus* (sequence similarity, 99.8%; bootstrap support, 100%) and *A. rhizopodus* (sequence similarity, 99.9%; bootstrap support, 100%), respectively (Fig. 1). In section *Nigri*, four strains (SFC20160317-M20, SFC20160805-M40, SFC20160805-M41, and SFC20160805M-42) were identified as *A. welwitschiae* (sequence similarity, 100%; bootstrap support, 97%) (Fig. 1).

Group 4 included three distantly related sections (*Aspergillus*, *Restricta*, and *Circumdati*). In section *Aspergillus*, two strains (SFC20160317-M25 and SFC20160805-M29) were identified as *A. ruber* (sequence similarity, 99.8~100%; bootstrap support, 99%), and the other strain (SFC20160317-M27) was identified as *A. pseudoglaucus* (sequence similarity, 99.6%; bootstrap support, 94%) (Fig. 1). Strain SFC201601112-M06 was identified as *A. caesiellus* in section *Restricta* (sequence similarity, 100%; bootstrap support, 100%). One strain (SFC20160112-M05) was identified as *A. westerdijkiae* in section *Circumdati* (sequence similarity, 99.83%; bootstrap support, 100%).

Taxonomy.

Aspergillus caesiellus Saito, J. Coll. (1904) (Fig. 2A)

Description: Colony diameter, at 25°C for 7 days, CYA 8~13 mm; CYA 30°C: 14~15 mm; no growth at 37°C and 50°C; MEA 13~14 mm; YES 29~31 mm.

Colonies on CYA, lightly sulcate, heavy sporulation, velutinous, central part gray (26C1) to grayish green (25C3) with 3-mm gray margin (26C1), no exudates, no pigmentation, reverse central part light brown (6D4) to dull green (28E4).

Colonies on MEA, sporulation, floccus, central part dull green (25D3) to dark turquoise (24F5) with 1-mm white margin, no exudates, no pigmentation, reverse central part dull green (27D3) to dull green (30D4).

Colonies on YES, sporulation, central floccus, velutinous, central part greenish gray (25D2) to dark green (25F4) with 1 mm white margin, no exudates, no pigmentation, reverse grayish yellow (4C4).

Conidiophore smooth with slightly green wall (61~) 82~95 (~114) × 4.6~5.2 (~5.5) μm wide, *vesicles* spatulate (10.7~) 11.1 × 13.5 (~15.4), *conidial head* uniseriate, *metulae* covering half of upper surface, (4.4~) 4.5~5.6 (~5.8) × (2.6~) 2.8~3.4 (~3.9) μm, *phialide* (2.4~) 2.5~2.6 × 7.0~8.2 (~8.6) μm, conidia ellipse and pyriform with greenish gray (4.4~) 4.5~5.5 (~6) × (2.4~) 2.6~2.8 μm diam.

Strain examined: SFC20160112-M06.

Remarks: *A. caesiellus* is similar to *A. penicillioides* but is distinguished by more vigorous growth and formation of conidia on CYA and MEA media. *A. caesiellus* grows more slowly and its cylinder becomes more round.

Aspergillus montenegroi Y. Horie, Miyaji & Nishim (1996) (Fig. 2B)

Description: Colony diameter, at 25°C for 7 days, CYA 52~58 mm; CYA 30°C 64~70 mm; CYA 37°C 66~72 mm; no growth at 50°C; MEA 64~72 mm; YES 66~70 mm.

Colonies on CYA, not sulcate, sporulation, floccus, and velutinous, central part yellowish gray (3C2) to olive (3D3) and grayish green (28E5) with 3 mm white margin, no exudate, pigmentation grayish red (7B3), reverse central part reddish gray (7B2) to brownish orange (7C4) with reddish gray (8B2) margin.

Colonies on MEA, not sulcate, sporulation velutinous, dull green (27E3) with 1-mm white margin, no exudate, no pigmentation, reverse central part brown (6E4) to light brown (6D4).

Colonies on YES, sulcate, sporulation, velutinous, central part purplish gray (13B2) to greenish yellow (1B8) and grayish green (27E6) with 2 mm white margin, no exudate, no pigmentation, reverse yellowish brown (5D5).

Conidiophore smooth and thick-walled with orange-brown (63.9~) 65~88.8 (~110.8) × (3.5~) 4.3~5.3 (~5.5) μm, *vesicles* hemispherical with orange-brown to dark brown 9.7~9.9 × (10.8~) 11.8~13.5 (~13.8) μm, *conidial heads* biseriate, *metulae* covering over half of upper surface (4.4~) 4.5~5.6 (~5.8) × (2.6~) 2.8~3.4 (~3.9) μm, *phialide* (4.7~) 5.2~5.6 (~6.1) × (2~) 2.1~2.6 (~2.7) μm, *conidia* hyaline with grayish green to green (2.8~) 2.9~4.3 (~4.7) × (2.1~) 2.9~3.4 (~3.6) μm.

Strain examined: SFC20160805-M50.

Remarks: The spore of *Aspergillus montenegroi* has similar morphology to that of the *Aspergillus nidulans*. However, the spore of *A. montenegroi* has reticulate ornamentation on the convex wall, whereas the spore of *A. nidulans* has a smooth wall.

Aspergillus rhizopodus J. N. Rai, Wadhwani & S. C. Agarwal (1975) (Fig. 2C)

Description: Colony diameter, at 25°C for 7 days, CYA 58~62 mm; CYA 30°C 65~68 mm; CYA 37°C 19~22 mm; no growth at 50°C; MEA 52~53 mm; YES 74~76 mm.

Colonies on CYA, weakly sulcate, heavy sporulation, velutinous with a floccus central part, dull green (25D3) with 2 mm white margin, no exudate, no pigmentation, reverse central part light brown (5D4) to olive brown (4D4).

Colonies on MEA, weakly sulcate, heavy sporulation floccus, dull green (25D3) with 7-mm white margin, slight exudates, no pigmentation, reverse central part brownish orange (5C6) to grayish orange (5B4).

Colonies on YES, weakly sulcate, heavy sporulation, floccus, central part grey (24C1) to grayish turquoise (24D3) with 2 mm white margin, no exudates, no pigmentation, reverse grayish yellow (4C5).

Conidiophore smooth-walled with light brown (210~) 340~502 (~550) × (5.9~) 6.3~8.2 μm, *vesicles* clavate form 14.9~15.9 (~16.4) × 19~20.8 μm, and *conidial heads* biseriate, *metulae* covering entire vesicle surface, (4~) 4.9~5.5 (~5.8) × (2.4~) 2.5~3.1 (~3.2) μm, *phialide* (5.2~) 5.6~5.9 (~6) × (1.4~) 1.6~2 (~2.1) μm, *conidia* spherical or subspherical with a light green smooth wall (1.9~) 2.1~2.7 μm.

Strain examined: SFC20160407-M08.

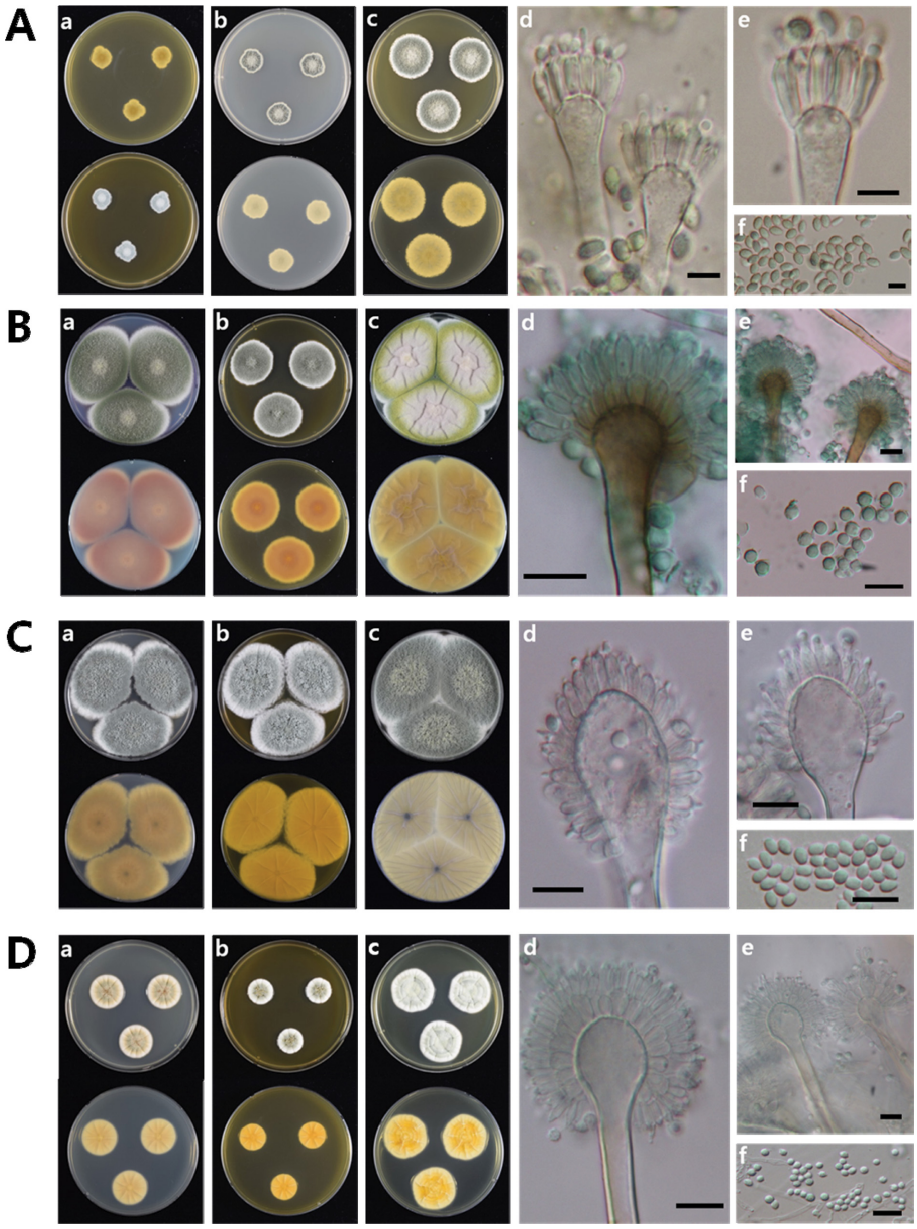

Fig. 2. Morphology of *Aspergillus caesiellus* (SFC201601112-M06) (A), *A. montenegroi* (SFC20160805-M50) (B), *A. rhizopodus* (SFC20160407-M08) (C), and *A. tabacinus* (SFC20160407-M11) (D). a~c, incubation for 7 days at 25°C. Upper: a, Czapek yeast autolysate agar (CYA); b, malt extract agar (MEA); c, yeast extract sucrose agar (YES). Lower: a, CYA reverse; b, MEA reverse; c, YES reverse. d, e, conidiophores; f, conidia (scale bars = 10 μm).

Remarks: *A. rhizopodus* is similar to *A. giganteus* and *A. longivesica* but is distinguished by variously shaped foot cells with fingerlike projections and large conidial heads in the presence of light.

Aspergillus tabacinus Nakaz., Y. Takeda, Simo & A. Watan (1934) (Fig. 2D)

Description: Colony diameter, at 25°C for 7 days, CYA 25~26 mm; CYA 30°C 22~24 mm; no growth at 37°C and 50°C; MEA 18~19 mm; YES 31~33 mm.

Colonies on CYA, sulcate, sporulation in central area, central part grayish beige (4C2) to greenish grey (27D2), brownish orange (5C4) with 2-mm white margin, no exudate, no pigmentation, reverse central part grayish orange (5B3) to yellowish white (4A2). No growth at 37°C and 50°C.

Colonies on MEA, sulcate, sporulation moderate, dull green (26E2) with 2-mm white margin, no exudates, no pigmentation, reverse brownish orange (5C5).

Colonies on YES, lightly sulcate, rings in central area, central part yellowish white (2A2) to grayish green (27C3) with 3-mm white margin, no exudates, no pigmentation, reverse central part deep yellow (4A8) to pale yellow (4A3).

Conidiophore smooth-walled, light brown (353~) 368~448 (~514) × (5.2~) 5.4~6.3 μm wide, *vesicles* spatulate form

14.9~15.9 (~16.4) × 19~20.8 μm, *conidial heads* biseriate, *metulae* covering entire vesicle surface (4~) 4.9~5.5 (~5.8) × (2.4~) 2.5~3.1 (~3.2) μm, *phialide* (5.2~) 5.6~5.9 (~6) × (1.4~) 1.6~2 (~2.1) μm, *conidia* spherical or subspherical with light green smooth wall (1.9~) 2.1~2.7 μm.

Strain examined: SFC20160407-M08.

Remarks: *A. tabacinus* has smooth-walled conidia similar to those of *A. amoenus* and *A. austroafricanus*, but *A. tabacinus* produces no soluble pigment, whereas *A. austroafricanus* produces a brown soluble pigment on the CYA medium.

DISCUSSION

In this study, we explored the diversity of *Aspergillus* derived from marine environments in Korea and identified each *Aspergillus* strain using *CaM* sequences, a locus that has previously been shown to be an effective DNA marker for *Aspergillus* identification [2]. A total of 17 species in 11 sections were identified, while three species were left unidentified (*Aspergillus* sp. 1~3) due to their ambiguity in morphological and phylogenetic analyses. Several *Aspergillus* species (*A. fumigatus*, *A. nidulans*, *A. sydowii*, *A. terreus*, *A. versicolor*, and *A. westerdijkiae*) have been reported from marine substrates such as deep sea sediments, marine sponges, corals, and mudflat [27-29], and particularly *A. fumigatus* and *A. sydowii* were dominant in mud substrates [30]. Similarly, in our study, higher diversity of *Aspergillus* was detected in mudflats, and both *A. fumigatus* and *A. sydowii* were the most abundant species, as another study has shown [30]. The number of isolated strains and diversity of *Aspergillus* were similar in winter and summer, and we could not find a significant difference between the seasons. *A. montenegroi*, *A. venenatus*, and *A. tabacinus* are reported for the first time in marine environments.

Group 1: sections *Versicolores*, *Nidulantes*, and *Flavi*. Section *Versicolores* was first introduced by Thom and Church [31], and 13 species are included in this section [32]. Pathogenic species in section *Versicolores* are generally isolated from various substrates including hypersaline water [33]. In our study, *A. sydowii*, *A. tabacinus*, and *A. venenatus* were isolated from a marine environment. *A. sydowii* is commonly isolated from marine environments as well and is known as a serious pathogen of marine organisms [34]. *A. venenatus* and *A. tabacinus* have previously been isolated from plant materials [32], but our study is the first report of these species in a marine environment. This study is the first report of *A. tabacinus* in Korea.

Species in section *Nidulantes* are usually found in soil, indoor environments, and foods, and are characterized by red or purple pseudoparenchymatous cleistothecium surrounded by hülle cells and purple ascospores [35]. In the present study, we identified two strains as *A. nidulans* and *A. montenegroi*. *A. nidulans* is characterized by a "nestlike" fruiting body [36] and is known for producing xylanase [37]. *A. montenegroi* was first isolated from soil by Horie *et al.* [38] (as *Emericella montenegroi*). *A. montenegroi* is characterized by ascospores with reticulate and convex walls [38]. This is the first report of this species in a marine environment and in Korea.

Species in section *Flavi* have a relatively thin and rough cell wall as compared to species in other sections [39]. Species in this section are found in soil, air, organic materials, and plants [40]. In our study, five isolated strains were confirmed within section *Flavi* and form a monophyletic group with type strains of *A. flavus* and *A. oryzae*. Both species are generally found in terrestrial environments such as soil and plant products, but are also found in marine environments [41, 42]. *A. flavus* is a representative species in that section and is known as an opportunistic pathogen of plants and animals due to aflatoxin production, whereas *A. oryzae* is used in food fermentation [42, 43]. However, it is difficult to distinguish *A. flavus* and *A. oryzae* because of their similar morphological characteristics and ambiguous phylogenetic relationship [44]. Therefore, we denoted five strains as *Aspergillus* sp. 1, which showed high sequence similarity to both *A. flavus* and *A. oryzae*; thus, further research is necessary to unambiguously identify these strains.

Group 2: sections *Terrei* and *Flavipedes*. Species in section *Terrei* were first described by Gams *et al.* [45] and commonly have brown columnar conidial heads. They are ubiquitous and considered economically important, especially in the fermentation industry [46]. In section *Terrei,* 10 strains were identified as *A. terreus*. This species is commonly isolated from terrestrial habitats such as deserts, grassland soils, and plant products but has also been isolated from a soft coral [28]. *A. terreus* is used for food fermentation because of its production of itaconic or itatartaric acid [47, 48]; however, *A. terreus* is also a known human pathogen that induces an inflammatory response [49]. *A. terreus* produces aspernolides A and B as well as butenolides [28]. In section *Flavipedes*, only one strain, *Aspergillus* sp. 2, is present, and it could not be clearly distinguished from either *A. mangaliensis* or *A. templicola* due to weak phylogenetic support and ambiguous morphological features.

Group 3: sections *Fumigati, Clavati,* and *Nigri*. Section *Fumigati* was first categorized as an *A. fumigatus* group by Raper and Fennel [40]. This identification was based on morphology and has remained a controversial issue due to the similarity of these morphological features among other taxa [50, 51]. Most species in this section are pathogenic or allergenic to humans owing to mycotoxins [52, 53]. In our study, two *Aspergillus* species (*A. fumigatus* and *Aspergillus* sp. 3) were found in this section. A total of 26 strains were identified as *A. fumigatus* in section *Fumigati*. *A. fumigatus* is the most abundant species isolated in this study. *A. fumigatus* is generally found in forest soils and air. *A. fumigatus* is often the dominant fungal species in mudflats [30, 54, 55]. Like other species in section *Fumigati*, *A. fumigatus* is generally characterized by green conidia

and phialides [56, 57]. *A. fumigatus* is known for causing a pulmonary disease and an allergic reaction in the respiratory tract via mycelia colonization [57]. In addition, one strain, *Aspergillus* sp. 3 (SFC20160805-M39), was confirmed to belong to section *Fumigati*; however, it could not be identified at the species level because it is phylogenetically and morphologically different from reported type species such as *A. papuensis* (CBS 841.96^T), *A. coversis* (CCF4190^T), and *A. brevistipitatus* (CCF419^T).

Section *Clavati* was initially classified as a part of the *Aspergillus clavatus* group along with *A. giganteus* by Thom and Church [31]. Section *Clavati* is considered a sibling group of section *Fumigati* and includes six *Aspergillus* species that show alkali tolerance and produce various mycotoxins such as patulin and tryptoquivalones [58-60]. In the present study, two species (*A. clavatus* and *A. rhizopodus*) were found in section *Clavati*. *A. clavatus* is generally found in soil, dung, and stored foods [61] and produces various compounds such as hydroxylamine [59]. *A. rhizopodus* was first reported by Rai *et al.* [62] and was considered a synonym of *A. giganteus* [63] because of their similar macromorphology. In contrast, the former species is characterized by distinct, long conidiophores and is an alkalophilic fungus [60, 62]. *A. rhizopodus* has never been recorded in Korea.

Section *Nigri*, which is generally called black molds, currently consists of 26 species [64]. Prior to the introduction of molecular markers, this section was difficult to identify to the species level because of very similar morphology within the section. In our study, six strains were isolated and identified as *A. welwitschiae*. The latter was first introduced by Wehmer [65] and is sometimes confused with *A. niger* owing to similar morphological characteristics, but it can be distinguished by the ability to produce ochratoxin A [66].

Group 4: sections *Circumdati*, *Aspergillus*, and *Restricta*. Section *Circumdati* was initially classified as a part of the *A. ochraceus* group by Raper and Fennell [40] but later categorized into section *Circumdati* [67]. Twenty-seven species are currently recognized in this section and are characterized by yellow conidia [68]. Species in section *Circumdati* are economically important due to their production of various bioactive compounds such as ochratoxin [68]. In the present study, one isolated strain of *A. westerdijkiae* was identified as belonging to section *Circumdati*. *A. westerdijkiae* is commonly isolated from fruits or grains but has been isolated from deep-sea environments as well [69]. *A. westerdijkiae* produces various alkaloid compounds [69].

Section *Aspergillus* was first reported by Raper and Fennell [40] and is considered one of the main sections in subgenus *Aspergillus* [2]. Most species in section *Aspergillus* were formerly classified within *Eurotium* [7]. Species in section *Aspergillus* are generally distinguished from other sections by their distinct morphological characteristics such as yellowish hyphae, greenish yellow conidial heads, and red hyphae [7]. *A. pseudoglaucus* and *A. ruber* were isolated from sea sand and mudflats, respectively. These species are generally isolated from fermented foods [70, 71]. *A. ruber* is recognized as a highly valuable species via its production of tannase and various secondary metabolites including aflatoxin B [70, 72]. *A. pseudoglaucus* was first named *Eurotium repens* by de Bary [73] but was recently renamed *A. pseudoglaucus* by Hubka *et al.* [7]. *A. pseudoglaucus* is known for cytotoxic activity and production of echinulin [74].

Section *Restricta* was first classified within the *A. glaucus* group by Thom and Raper [75], but later renamed as section *Restricta* by Gams *et al.* [45]. In our study, *A. caesiellus* was isolated from mudflats, and this is the first report of this species in Korea. *A. caesiellus* has been generally found in plants [76]; several enzymes, including keratinase, cellulases and xylanases, have been isolated from this species [76, 77].

In conclusion, in this study, we explored the diversity of *Aspergillus* in marine environments (mudflats and sea sand) during both summer and winter in Korea. We identified 17 species in 11 sections based on *CaM* sequences, whereas three specimens were identified to the genus level. This study shows four previously unrecorded *Aspergillus* species in Korea: *A. caesiellus*, *A. montenegroi*, *A. rhizopodus*, and *A. tabacinus*. Collectively, marine-derived *Aspergillus* species have been shown to produce over 120 secondary metabolites that have cytotoxic and antimicrobial properties and are therefore potentially important pharmacologically [78, 79]. Despite the high known diversity within the *Aspergillus* genus and the remarkable potential for production of useful industrial and medical compounds that have been found in this group, most of the recorded taxa within this genus remain unidentified to the species level. Therefore, we believe that this study will spur future research into the diversity of marine *Aspergillus* and will form the basis for the discovery of new bioresources through accurate identification of species in this genus.

ACKNOWLEDGEMENTS

This study was supported by the Marine BioResource Bank Program of the Ministry of Ocean and Fisheries, Korea.

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