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Diversity and enzyme activity of *Penicillium* species associated with macroalgae in Jeju Island

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A total of 28 strains of 19 *Penicillium* species were isolated in a survey of extracellular enzyme-producing fungi from macroalgae along the coast of Jeju Island of Korea. *Penicillium* species were identified based on morphological and β-tubulin sequence analyses. In addition, the halo-tolerance and enzyme activity of all strains were evaluated. The diversity of *Penicillium* strains isolated from brown algae was higher than the diversity of strains isolated from green and red algae. The commonly isolated species were *Penicillium antarcticum*, *P. bialowiezense*, *P. brevicompactum*, *P. crustosum*, *P. oxalicum*, *P. rubens*, *P. sumatrense*, and *P. terrigenum*. While many strains showed endoglucanase, β-glucosidase, and protease activity, no alginase activity was detected. There was a positive correlation between halo-tolerance and endoglucanase activity within *Penicillium* species. Among 19 *Penicillium* species, three species–*P. kongii*, *P. olsonii*, and *P. viticola*–have not been previously recorded in Korea.

Keywords: *BenA*, endoglucanase, β-glucosidase, protease

Introduction

Macroalgae are primary producers in the marine ecosystem. They play important ecological roles by providing a food source and habitat for marine organisms and they produce compounds that can be used for medicinal, industrial, and biofuel applications (Graham *et al*., 2009). They are categorized into three groups, i.e., brown, green, and red algae, based on their components, color pigments, and nutrients (Chanda, 2010). Their cell walls are composed of polysaccharides, including alginate, cellulose, and xylans (Domozych, 2011). Jeju Island in Korea is known as one of the best places for macroalgal growth. Over 400 species have been reported to date in Jeju Island (Lee, 2008). However, several environmental issues have recently been reported, such as macroalgal bloom and the occurrence of subtropical species as a consequence of climate change and human activity along the coast of Jeju Island (Kang *et al*., 2011, 2014). These environmental problems are likely to lead second environmental pollution when the species reach the shore and rot.

Marine fungi have been found in a wide range of environments, such as macroalgae, coral, plant, sediments, and wood (Bugni and Ireland, 2004). Marine fungi are frequently isolated from macroalgae and commonly belong to the genera *Acremonium*, *Cladosporium*, and *Penicillium* (Kohlmeyer and Kohlmeyer, 1979; Zuccaro *et al*., 2004; Janso *et al*., 2005). Algicolous fungi are the second largest source of new metabolites (Bugni and Ireland, 2004). In particular, marine-derived *Penicillium* species are producers of secondary metabolites, such as those with anti-tumor, anti-fungal, and anti-bacterial activity (Numata *et al*., 1996; Iwamoto *et al*., 1999; Bugni and Ireland, 2004; Park *et al*., 2014), and extracellular enzymes, such as extracellular alginase, endoglucanase, β-glucosidase (Park *et al*., 2014). Useful materials of marine-derived *Penicillium* from macroalgal biomass can be used as a potential solution for the environmental problems to degrade macroalgae biomass

The genus *Penicillium* is commonly isolated from various environments, including soil, air, indoor, and marine environments (Pitt, 1979; Raghukumar, 2008; Li and Wang, 2009; Paz *et al*., 2010; Samson *et al*., 2010). This genus is well-known as decomposers, plant pathogens, and producers of bioactive compounds (Frisvad and Samson, 2004; Samson *et al*., 2010). *Penicillium* morphology is difficult to define owing to the plasticity of its distinguishing features according to growth conditions, such as nutrients and temperature (Visagie *et al*., 2014). Recently, Visagie *et al*. (2014) offered standardized methods for the identification of *Penicillium* as well as species descriptions. Based on standardized methods, including a multigene phylogenetic analysis of nuc rDNA internal transcribed spacer (ITS), β-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*), there are currently 354 accepted species in *Penicillium*, and these are divided into two subgenera (*Aspergilloides* and *Penicillium*) and 25 sections (Visagie *et al*., 2014). To date, over 100 *Penicillium* species have been recorded from terrestrial (soil and crop) and marine environments in Korea (Lee *et al*., 2003; Yu, 2006; Kim *et al*., 2009; Park *et al*., 2014, 2015).

We examined *Penicillium* species associated with macroalgae in Jeju Island in Korea with respect to the biomass degradation of macroalgae to discover potential industrial applications. Our main objective was to investigate the diversity of *Penicillium* associated with macroalgae along Jeju Island and to evaluate the halo-tolerance and the enzyme activity

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of *Penicillium* isolates, including extracellular alginase, endoglucanase, β-glucosidase, and protease activity. In our examination of *Penicillium* diversity, three unrecorded species in Korea–*P. kongii*, *P. olsonii*, and *P. viticola*–were found. We describe the macro- and micromorphological characteristics of these newly recorded species.

Materials and Methods

Materials

Fifty-one macroalgae were collected from nine sites along Jeju Island of Korea in July 2014. Each sample was gently washed with sterilized artificial sea water (ASW) (Huang *et al*., 2011) to remove surface debris and soil. Discs of 5 mm in diameter were cut from each sample and 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) supplemented with ASW. All plates were incubated at 25°C until the morphological features of the cultured fungi could be distinguished (7–15 days), then each *Penicillium* strain was transferred to a new PDA plate. The isolated strains were stored 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 of Rogers and Bendich (1994). Portions of the *BenA* gene were amplified and sequenced for 28 strains. PCR reactions were performed using the primers Bt2a and Bt2b (Glass and Donaldson, 1995). Each PCR reaction was performed in a C1000 Thermal Cycler (Bio-Rad) following Park *et al*. (2015). The PCR products were purified using the Expin^{TM} PCR Purification Kit (GeneAll Biotechnology) according to the manufacturer's instructions. Sequencing was performed in both forward and reverse directions using the corresponding PCR primers at Macrogen, using an ABI Prism 3700 Genetic Analyzer (Life Technologies).

Phylogenetic analysis

Sequences were assembled, proofread, edited, and aligned using MEGA5 (Tamura *et al*., 2011). The resulting consensus sequences were deposited in GenBank (accession nos. are shown in Table 1). Molecular identification was performed by a section-by-section approach. First, *BenA* sequences were analyzed with 114 type strains to determine the section to which each strain belongs. Next, to identify strains to the spe-

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

Species	Strain No.	Collection No.	Substrate	Sampling site / Location	Accession No.
P. antarcticum	JM0840	SFC20150317-M06	*Dictyota* (B)[b]	S8 / Hamdeok-ri, Jocheon-eup, Jeju-si	KU600412
	JM0852	SFC20150812-M04	*Colpomenia* (B)	S8 / Hamdeok-ri, Jocheon-eup, Jeju-si	KU600413
P. bialowiezense	JM1502	SFC20150812-M12	*Sargassum thunbergii* (B)	S9 / Yongdam-dong, Jeju-si	KU600414
	JM1458	SFC20150402-M21	*Ulva* (G)	S9 / Yongdam-dong, Jeju-si	KU600415
	JM1491	SFC20150812-M11	*Colpomenia* (B)	S9 / Yongdam-dong, Jeju-si	KU600416
P. brevicompactum	JM1454	SFC20150812-M09	*Ulva* (G)	S9 / Yongdam-dong, Jeju-si	KU600417
	JM1463	SFC20150812-M10	*Colpomenia* (B)	S9 / Yongdam-dong, Jeju-si	KU600418
P. copticola	JM0860	SFC20150812-M05	*Colpomenia* (B)	S8 / Hamdeok-ri, Jocheon-eup, Jeju-si	KU600419
P. corylophilum	JM0171	SFC20141123-M44	*Undaria pinnatifida* (B)	S1 / Gwakji-ri, Aewol-eup, Jeju-si	KU600420
P. crustosum	JM0843	SFC20150812-M03	*Dictyota* (B)	S8 / Hamdeok-ri, Jocheon-eup, Jeju-si	KU600421
	JM0895	SFC20150402-M26	*Sargassum* (B)	S8 / Hamdeok-ri, Jocheon-eup, Jeju-si	KU600422
P. hetheringtonii	JM1466	SFC20141120-M06	*Colpomenia* (B)	S9 / Yongdam-dong, Jeju-si	KP235302
P. kongii[a]	JM0233	SFC20150812-M01	*Codium* (G)	S1 / Gwakji-ri, Aewol-eup, Jeju-si	KU600423
P. olsonii[a]	JM0768	SFC20150812-M02	*Ulva* (G)	S5 / Hahyo-dong, Seogwipo-si	KU600424
P. oxalicum	JM1147	SFC20150812-M07	*Lomentaria catenata* (R)	S6 / Hado-ri, Gujwa-eup, Jeju-si	KU600425
	JM1408	SFC20150812-M08	*Chondrus* (R)	S7 / Gimnyeong-ri, Gujwa-eup, Jeju-si	KU600426
P. paxilli	JM0601	SFC20141120-M03	*Sargassum* (B)	S4 / Saekdal-dong, Seogwipo-si	KP235303
P. radicicola	JM1536	SFC20150402-M25	*Undaria pinnatifida* (B)	S9 / Yongdam-dong, Jeju-si	KU600427
P. rubens	JM0894	SFC20150402-M12	*Sargassum* (B)	S8 / Hamdeok-ri, Jocheon-eup, Jeju-si	KU600428
	JM1109	SFC20150812-M06	*Galaxaura* (R)	S6 / Hado-ri, Gujwa-eup, Jeju-si	KU600429
P. sumatrense	JM0173-1	SFC20141120-M04	*Undaria pinnatifida* (B)	S1 / Gwakji-ri, Aewol-eup, Jeju-si	KP235306
	JM0854-1	SFC20141120-M01	*Colpomenia* (B)	S8 / Hamdeok-ri, Jocheon-eup, Jeju-si	KP235305
P. swiecickii	JM1552	SFC20150812-M13	*Undaria pinnatifida* (B)	S9 / Yongdam-dong, Jeju-si	KU600430
P. terrigenum	JM0439	SFC20141120-M05	*Sargassum* (B)	S2 / Ongpo-ri, Hallim-eup, Jeju-si	KP235309
	JM1453	SFC20141120-M02	*Ulva* (G)	S9 / Yongdam-dong, Jeju-si	KP235308
P. trzebinskii	JM0128	SFC20150812-M13	*Hydroclathrus clathratus* (B)	S1 / Gwakji-ri, Aewol-eup, Jeju-si	KU600431
P. viticola[a]	JM1436	SFC20150402-M20	*Ulva* (G)	S9 / Yongdam-dong, Jeju-si	KU600432
Penicillium sp. 1	JM1494	SFC20150402-M23	*Colpomenia* (B)	S9 / Yongdam-dong, Jeju-si	KU600433

[a] Newly recorded species in Korea
[b] Alga classification: brown algae (B), green algae (G), red algae (R).

cies level, *BenA* data were analyzed with type strain sequences and confirmative sequences of the corresponding *Penicillium* section. Multiple sequence alignments were performed following the methods of Park *et al.* (2015). Maximum likelihood phylogenetic analyses were performed using RAxML (Stamatakis, 2006) with the GTR+gamma model of evolution and 1,000 bootstrap replicates.

Morphological analysis

To observe macroscopic culture characteristics, 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. In addition, CYA plates were inoculated and incubated for 7 days at 4°C, 30°C, and 37°C. All media were prepared as described in Visagie *et al.* (2014). After incubation, the culture characteristics were recorded using the recommended methods by Visagie *et al.* (2014). The color name and code of each culture were based on the "Methuen Handbook of Colour" (Kornerup and Wanscher, 1963). Microscopic characteristics were evaluated following the methods of Park *et al.* (2015).

Halo-tolerance assay and enzyme assay

Halo-tolerance was determined by measuring colony diameter of the strains on MEA supplemented with ASW and without ASW. Colony diameter was measured after the cultures were incubated for 5 days at 25°C. Differences in growth between MEA supplemented with ASW and without ASW were compared using Student's *t*-tests and *P*-values were corrected by controlling the false discovery rate using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995).

Extracellular alginase, endoglucanase, β-glucosidase, and extracellular protease activity were assessed for each strain using modified plate screening methods. We screened for extracellular alginase activity using the plate methods of Park *et al.* (2014). Endoglucanase and β-glucosidase activity were assayed by growing the fungi on Mandels' medium (Lee *et al.*, 2015) with 1% carboxymethylcellulose (Sigma-Aldrich) and 0.5% D-cellobiose (Sigma-Aldrich) as the primary carbon sources, respectively. Extracellular protease activity was assayed by growing the fungi for 5 days on yeast extract agar (Oxoid) supplemented with 1.5% skim milk (Difco-Becton) as the primary carbon source (Teng *et al.*, 2001).

Halo-tolerance assay and enzyme assay were conducted in triplicate. Halo-tolerance and enzyme activity were compared among the sections of the *Penicillium* using analysis of variance. Tukey's post-hoc tests were used to compare pairs of sections. The correlation between halo-tolerance and enzyme activity was analyzed using Pearson's correlation test.

Results

On the basis of morphological characteristics, 51 macroalgae were identified as 24 distinct taxa at the genus or species level, including brown algae (10 species, 26 samples), green algae (3 species, 10 samples), and red algae (11 species, 15 samples). More various macroalgae were collected at site 1 (9 macroalgae species) and site 6 (10 macroalgae species) than other sites. *Colpomenia* (brown algae), *Sargassum* (brown algae), *Ulva* (green algae), and *Undaria pinnatifida* (brown algae) were the most common algae (Fig. 1).

Various *Penicillium* strains were isolated from 11 species of 16 macroalgae samples, including brown algae (6 species, 10 samples), green algae (2 species, 3 samples), and red algae (3 species, 3 samples) (Table 1). The largest number of *Penicillium* strains were isolated at site 9 (11 strains), followed by site 8 (7), and site 1 (4) (Fig. 1). No *Penicillium* was isolated at site 3.

Identification

In total, 19 *Penicillium* species in ten sections were recovered from 28 strains using a section-by-section phylogenetic analysis based on *BenA* sequence. These strains formed monophyletic groups with type strains (Fig. 2). Although *Penicillium* sp. (JM1494) formed a group with the type strain of *P. guanacastense*, the strain could not be confidently identified because the morphological characteristics differed. The strain was designated *Penicillium* sp. 1. The most abundant species was *P. bialowiezense* (3 strains). More *Penicillium* species were

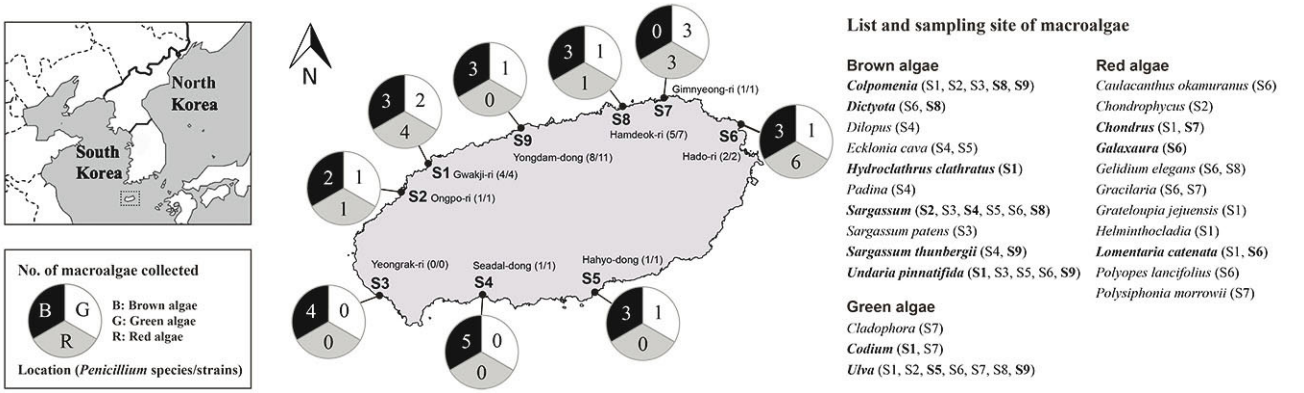

Fig. 1. Map showing the species and locations of the sampling sites for macroalgae collected along the coastline of Jeju Island. Numbers in the circles for each site indicate the number of macroalgae collected. Bold font represents algae species where *Penicillium* were isolated.

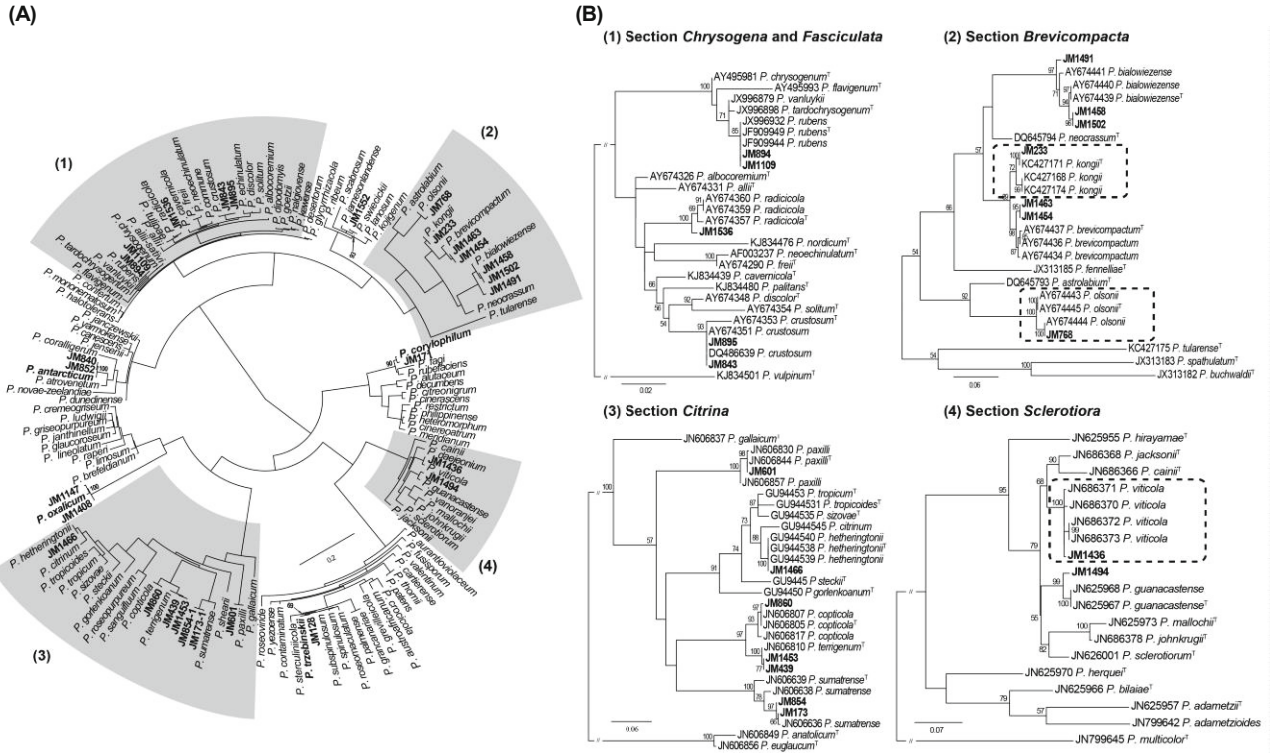

Fig. 2. The maximum likelihood phylogenetic tree based on the partial β-tubulin (*BenA*) gene sequences. (A) Relationships among ex-type strains of *Penicillium* and strains from macroalgae. (B) Identification of strains to the species level for each *Penicillium* section. "T" indicates ex-type strains. Bootstrap scores of > 50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site.

detected from brown algae (15 *Penicillium* species) than green algae (6 species) or red algae (2 species) (Table 1). Of the locations surveyed, site 9 showed the highest *Penicillium* diversity (8 species), followed by site 8 (5 species) and site 1 (4 species) (Fig. 1).

Halo-tolerance and enzyme assays

Although *P. corylophilum* (JM0171), *P. terrigenum* (JM1453),

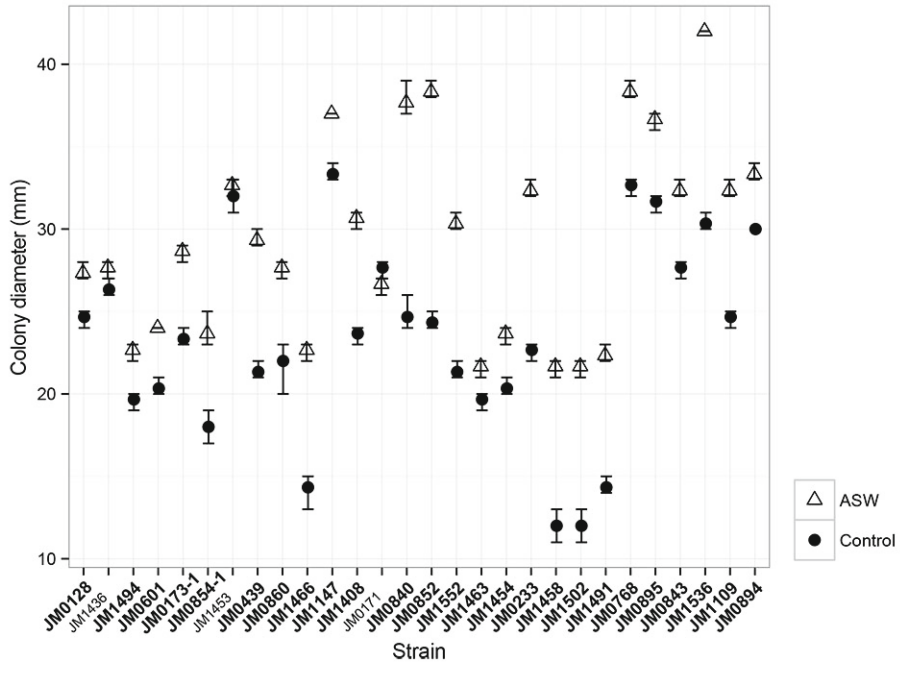

Fig. 3. Halo-tolerance of *Penicillium* strains on MEA supplemented with artificial sea water (ASW) and not supplemented with ASW. Strains that were significantly different between MEA supplemented with ASW and without ASW (Corrected $P < 0.05$) are represented in bold font.

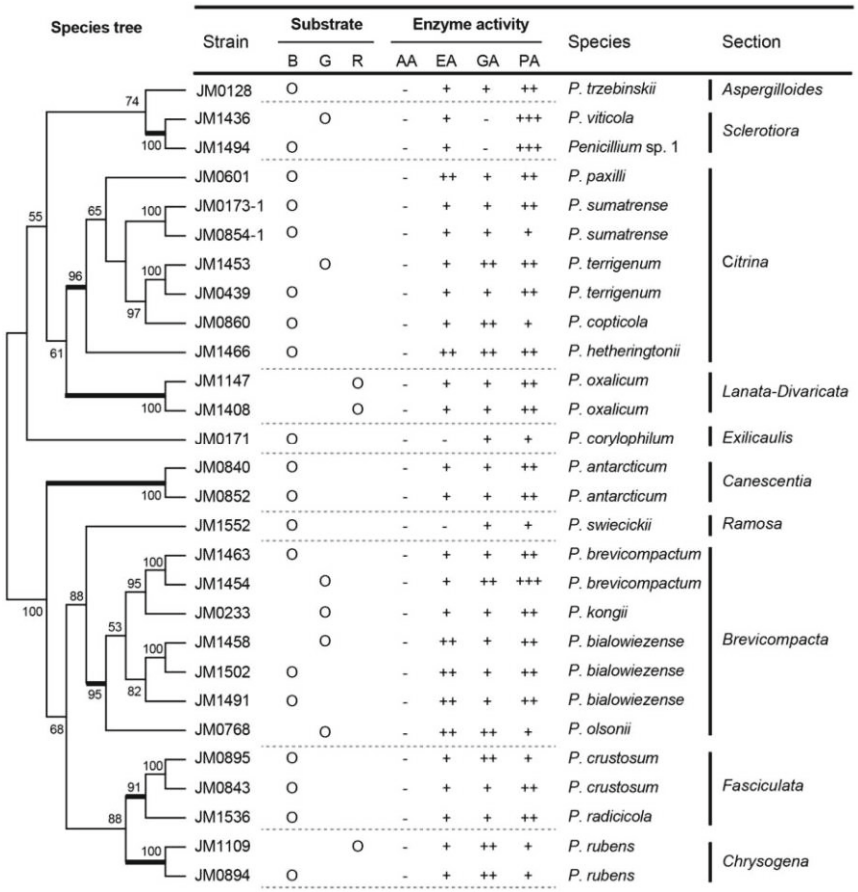

Fig. 4. Phylogeny, substrate, and enzyme activity of the *Penicillium* strain. The maximum likelihood phylogenetic tree was based on partial β-tubulin (*BenA*) gene sequences. Bootstrap scores of > 50 are presented at the nodes. Thicker branches in the phylogeny represent section of *Penicillium*. Substrates are classified as brown algae (B), green algae (G), and red algae (R). Alginase (AA), endoglucanase (EA), β-glucosidase (GA), and protease (PA) activity of *Penicillium* strains exhibit no activity (-), 0.5–5 mm (+), 5–10 mm (++), and over 10 mm (+++).

and *P. viticola* (JM1436) showed similar growth rates on the two different media (i.e., with and without ASW), the other strains exhibited significantly different growth under saline conditions (Fig. 3). In particular, *P. antarcticum* (JM0840 and JM0852) showed the best growth under saline conditions compared to MEA without ASW.

Most strains exhibited endoglucanase, β-glucosidase, and protease activity, but none of the strains exhibited alginase activity (Fig. 4). Twenty-six strains of 17 species showed endoglucanase activity. In particular, *P. bialowiezense* (JM1491, JM1458, and JM1502), *P. hetheringtonii* (JM1466), *P. olsonii* (JM0768), and *P. paxilli* (JM0601) showed relatively high enzyme activity. Twenty-six strains of 17 species showed β-glucosidase activity. *P. brevicompactum* (JM1454), *P. crustosum* (JM0895), *P. copticola* (JM0860), *P. hetheringtonii* (JM1466), *P. olsonii* (JM0768), *P. rubens* (JM0894 and JM1109), and *P. terrigenum* (JM1453) showed relatively high enzyme activity. All strains used in this study exhibited extracellular protease activity. *P. brevicompactum* (JM1454), *P. viticola* (JM1436), and *Penicillium* sp. 1 (JM1494) showed the highest extracellular protease activity.

We analyzed differences in both halo-tolerance and enzyme activity depending on the section of *Penicillium*. Halo-tolerance did not differ between sections of *Penicillium*. Enzyme activity was statistically significantly different between sections of *Penicillium*; however, pairwise tests did not indicate significant differences for most pairs of sections (Fig. 5). We analyzed the correlation between halo-tolerance and enzyme activity. Halo-tolerance was positively correlated with endo-

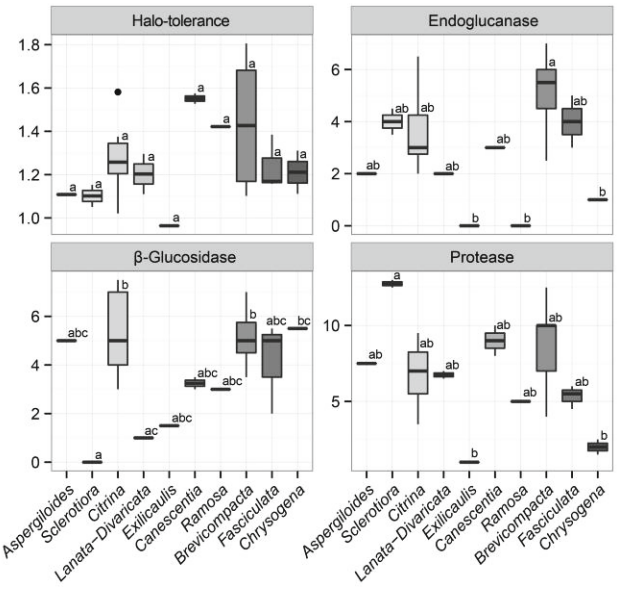

Fig. 5. Differences in halo-tolerance and enzyme activity among sections of *Penicillium*. Post-hoc multiple pairwise comparisons for each section were conducted using Tukey's tests. Statistically significant differences are denoted by different lowercase letters above each boxplot.

glucanase activity ($R = 0.45$, $P = 0.02$), but not with β-glucosidase ($P = 0.45$) or protease activity ($P = 0.53$).

Taxonomy

***Penicillium kongii* L. Wang 2013 (Fig. 6)**

Description: Colony diameters, 7 d, 25°C (unless otherwise stated), in mm: CYA, 22–24; CYA 4°C, 3.0–3.2; CYA 30°C, no growth; CYA 37°C, no growth; MEA, 17–23; YES, 30–36.
Colonies on CYA: colonies radially sulcate, sporulation moderate, non-sporulating margins 1–2 mm; colony texture velvety; conidia dull green (29E3); exudate droplets clear; soluble pigments absent; reverse color dull yellow (3B4), with yellow (3A6) at center.
Colonies on MEA: colonies radially sulcate, sporulation moderate to strong, non-sporulating margins 1–2 mm; colony texture velvety, floccose near center; conidia dull green (27D3); exudate droplets absent; soluble pigments absent; reverse color pale orange (5A3).
Colonies on YES: colonies slightly wrinkled, sporulation moderate to strong, non-sporulating margins 1–2 mm; colony texture velvety, floccose at center; conidia dull green (26D3); exudate droplets absent; soluble pigments reddish brown (8E7); reverse color pale red (7D7), with reddish yellow (4B7) at margins.
Sclerotia absent. Asci and ascospores not observed. Conidiophores terverticillate, smooth, 3.3–4.4 μm wide; 4–6 metulae per ramus, 10–14 × 3.1–3.9 μm; phialides ampulliform, 7–10 × 2.6–3.8 (–4.0) μm (Fig. 6D–F). Conidia globose to ellipsoidal, 2.8–3.5 × 2.4–2.3 μm, with smooth or finely roughed walls (Fig. 6G).
Strain examined: JM0233 (SFC20150812-M01)
Remarks: *P. kongii* is morphologically similar to *P. bialowiezense* and *P. brevicompactum*. *P. kongii* can be distinguished from *P. bialowiezense* by the production of soluble reddish brown pigments on YES and from *P. brevicompactum* by fast growth on CYA and the production of soluble reddish brown pigments on YES. *P. kongii* (JM0233) was isolated from green algae (*Codium* sp.) in Jeju Island.

***Penicillium olsonii* Bain & Sartory 1912 (Fig. 6)**

Description: Colony diameters, 7 d, 25°C (unless otherwise stated), in mm: CYA, 30–33; CYA 4°C, 2.5–3.0; CYA 30°C, 13–15; CYA 37°C, no growth; MEA, 29–33; YES, 46–51.
Colonies on CYA: colonies plane or slightly radially sulcate, sporulation moderate, non-sporulating margins 1 mm; colony texture velvety; conidia grayish green (26D3), bluish gray (22B3) at center; exudate droplets absent; soluble pigments absent; reverse color yellowish white (3A2).
Colonies on MEA: colonies plane, sporulation moderate, non-sporulating margins 1 mm; colony texture velvety; conidia dull green (28D3); exudate droplets clear; soluble pigments absent; reverse color light yellow (4A4).
Colonies on YES: colonies radially sulcate, sporulation moderate, non-sporulating margins 1–2 mm; colony texture velvety; conidia dull green (28D3); exudate droplets absent; soluble pigments absent; reverse color pale yellow (3A3).
Sclerotia absent. Asci and ascospores not observed. Conidiophores terverticillate, smooth or finely roughened walls, 3.6–4.3 μm wide, 3–5 metulae per ramus, 11–13 × 3.6–4.2 μm; phialides ampulliform, 10–13 × 2.3–3.2 (–3.5) μm (Fig. 6D–F). Conidia ellipsoidal, 3.8–4.9 × 2.8–3.8 μm, with smooth or finely roughened walls (Fig. 6G).
Strain examined: JM0768 (SFC20150812-M02)
Remarks: *P. olsonii* is phylogenetically related to *P. astrolabium*. The former can be distinguished from *P. astrolabium* by slow growth and yellowish white reverse on CYA. Although the conidia and phialide size of *P. olsonii* (JM0768) are slightly larger than those of the previous description of *P. olsonii* (Pitt 1979), it formed a monophyletic group with other *P. olsonii* based on a phylogenetic analysis of *BenA* (sequence similarity = 98.7–100%; bootstrap support = 100%). *P. olsonii* (JM0768) was isolated from green algae (*Ulva* sp.) in Jeju Island.

***Penicillium viticola* Nonaka & Masuma 2011 (Fig. 6)**

Description: Colony diameters, 7 d, 25°C (unless otherwise stated), in mm: CYA, 37–39; CYA 4°C, No growth; CYA 30°C, 20–22; CYA 37°C, no growth; MEA, 35–36; YES, 38–39.
Colonies on CYA: colonies radially sulcate, 2–3 rings at center, sporulation moderate, non-sporulating margins 1–2 mm; colony texture velvety; conidia greenish gray (26D2); exudate droplets absent; soluble pigments absent; reverse color reddish orange (6B7), with light yellow (4A4).
Colonies on MEA: colonies radially sulcate, 3–4 rings at cen-

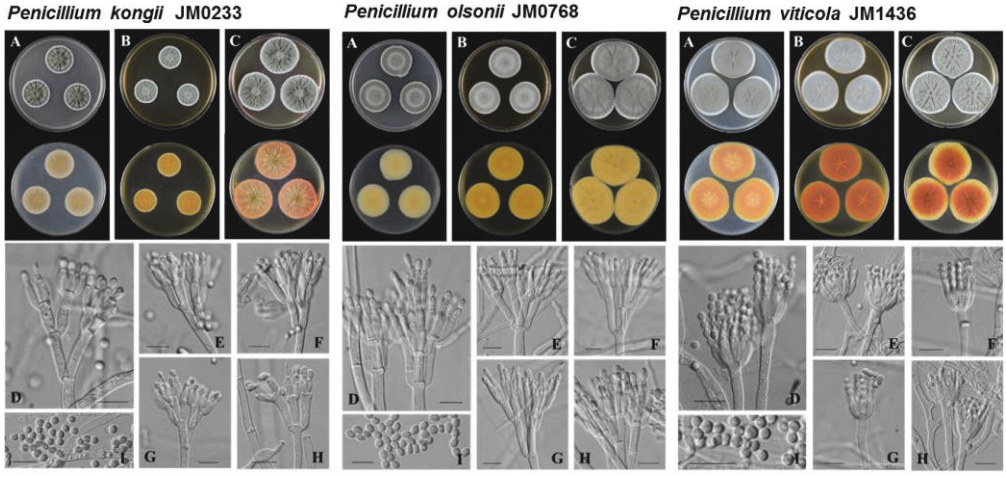

Fig. 6. *Penicillium kongii* JM0233, *P. olsonii* JM0768, and *P. viticola* JM1436 for 7-day-old cultures at 25°C. (A-C) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar, and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse). (D–H) Conidiophores. (I) Conidia (scale bars: D–H = 10 μm)

ter, sporulation moderate, non-sporulating margins 2–3 mm; colony texture velvety; conidia grayish green (27D3); exudate droplets absent; soluble pigments absent; reverse color reddish brown (8D7).
Colonies on YES: colonies radially sulcate, sporulation moderate, non-sporulating margins 1–2 mm; colony texture velvety; conidia dull green (28E3); exudate droplets absent; soluble pigments absent; reverse color orange (6B8), with dull yellow (3B3) at margin.
Sclerotia absent. Asci and ascospores not observed. Conidiophores monoverticillate, slight walls, 2.5–3.9 μm wide, phialides ampulliform, 8–11 (–12) × 2.6–3.4 μm (Fig. 6D–F). Conidia globose to broadly ellipsoidal, 3.8–4.9 × 2.8–3.8 μm, with smooth walls (Fig. 6G).
Strain examined: JM1436 (SFC20150402-M20)
Remarks: *P. viticola* (JM1436) is morphologically similar to *P. decumbens* and *P. adametzii*. It can be distinguished from *P. adametzii* by a velvety texture on CYA and globose to broadly ellipsoidal conidia, and from *P. decumbens* by no growth on CYA at 37°C. *P. viticola* (JM1436) was isolated from green algae (*Ulva* sp.) in Jeju Island.

Discussion

Marine fungi play an important ecological role in decomposing marine macroalgae and wood (Hyde *et al*., 1998; Kohlmeyer and Kohlmeyer, 2013). *Acremonium*, *Aspergillus*, *Cladosporium*, and *Penicillium* are commonly isolated from marine environments (Khlmeyer and Kohlmyer, 1979; Khudykova *et al*., 2000; Zuccaro *et al*., 2004; Janso *et al*., 2005). Studies of marine-derived *Penicillium* have usually focused on discovering new bioactive compounds and their enzyme activity. *Penicillium* species are known for producing bioactive compounds (Frisvad and Samson, 2004) and enzymes for the degradation of various compounds, such as alginase, β-glucosidase, endoglucanase, pectinase, and xylanase (Krogh *et al*., 2004; Yoon *et al*., 2007; Park *et al*., 2014). However, the diversity of *Penicillium* from macroalgae has not been examined. In this study, 28 strains of 19 species were identified. *Penicillium* species in sections *Citrina* and *Brevicompacta* were commonly isolated from macroalgae. *P. hetheringtonii*, *P. paxilli*, *P. sumatrense*, and *P. terrigenum* in section *Citrina* have been reported in a previous study (Park *et al*., 2015). *P. copticola* was first isolated from macroalgae.

More *Penicillium* species were isolated from brown algae than from other types of algae. These results may reflect the higher number of brown algae samples used in the study. In the case of red algae, however, a lower diversity of *Penicillium* was detected compared with green algae, even though the number of green algae samples was the smallest. In addition, we examined the similar number of brown and red algae species. In red algae, however, only half as many *Penicillium* species were isolated compared to brown algae, which may suggest that *Penicillium* species have different preferences for macroalgae substrates. The cell wall components differ between types of macroalgae (Popper *et al*., 2011). Therefore, differences in *Penicillium* diversity between macroalgae may be associated with differences in substrate preferences. Additional studies on *Penicillium*-degrading macroalgae may reveal the preferences of *Penicillium* with respect to the degradation of macroalgae.

Marine fungi can be divided into two distinct groups based on a broad ecological definition; obligate marine fungi exclusively grow and sporulate in marine habitats; facultative marine fungi can grow in terrestrial and marine habitats (Kohlmeyer and Kohlmeyer, 1979). Facultative marine fungi commonly belong to *Penicillium*, *Aspergillus*, *Trichoderma*, and *Cladosporium* (Khudyakova *et al*., 2000; Cantrell *et al*., 2006). The growth of *Aspergillus* and *Penicillium* from marine environments is optimal in media with salinity similar to that of seawater and a temperature of 25–37°C (Dunn and Baker, 1983). Most *Penicillium* species isolated from macroalgae in Jeju Island have also been found in terrestrial environments, e.g., in soil, crop, and indoor environments (Frisvad and Samson, 2004; Houbraken and Samson, 2011). In this study, the strains grew faster on media with ASW than without ASW. Salinity adaption of *Penicillium* in marine environments varied depending on species and strain. A previous study reported variation in salinity adaption within strains depending on salinity conditions, such as fresh, brackish, and sea water (Ristanović and Miller, 1969).

Marine fungi produce various enzymes to decompose organic material, such as alginate and cellulose (Hyde *et al*., 1998). Although some *Penicillium* species produce alginase to degrade alginate (Burtseva *et al*., 2010; Dubrovskaya *et al*., 2012; Park *et al*., 2014), no alginase activity was found for any strain in this study. Endoglucanase and β-glucosidase are important enzymes that assist in the degradation of cellulose (Pointing, 1999). Many *Penicillium* species from marine environments produce endoglucanase and β-glucosidase (Adsul *et al*., 2004; Dutta *et al*., 2008; Park *et al*., 2014). Most *Penicillium* strains in this study exhibited endoglucanase, β-glucosidase, and protease activity. We previously reported enzyme activity for species in *Penicillium* section *Citrina* isolated from marine environments (Park *et al*., 2015). *P. hetheringtonii* (JM1466), *P. paxilli* (JM601), *P. sumatrense* (JM173 and JM854), and *P. terrigenum* (JM439 and JM1453) showed unclear zones for endoglucanase on cellulolysis basal medium agar supplemented with 2% carboxymethylcellulose. However, in this study, using Mandels' medium (Lee *et al*., 2015) supplemented with 1% carboxymethylcellulose, the strains showed endoglucanase activity. The endoglucanase activity assay using Mandels' medium can more clearly detect strains that exhibit activity than assays using cellulolysis basal medium agar. Protease is important for the degradation of proteins; the compound is commonly found in sea waste, and the activity of this enzyme has the potential ability to convert waste to useful biomass (Wang and Chio, 1998; Jo *et al*., 2008). Proteases are produced by a variety of sources, including marine fungi (Pisano *et al*., 1964) and bacteria (Wang and Chio, 1998; Jo *et al*., 2008). In particular, *Aspergillus* and *Penicillium* isolated from the deep sea were found to produce protease (Germano *et al*., 2003; Agarwal *et al*., 2004; Damare *et al*., 2006). In this study, all strains showed protease activity. The strains with strong activity were *P. brevicompactum* (JM1454), *P. viticola* (JM1436), and *Penicillium* sp. 1 (JM1494). Of the strains tested in our study, *P. hetheringtonii* (JM1466) exhibited relatively high activity for all enzymes tested. *P. hetheringtonii* is commonly isolated

from soil (Houbraken *et al*., 2010) and produces the mycotoxins citrinin, quinolactacin, and unknown extrolites (Houbraken *et al*., 2010) as well as β-glucosidase (Park *et al*., 2015). Marine-derived *Penicillium* from algae is the second largest source of new metabolites (Bugni and Ireland, 2004). It is possible for marine-derived *Penicillium* to evolve different bioactive compounds and enzymes than those of *Penicillium* from terrestrial environments. In a previous study, *P. expansum* and *P. chrysogenum* showed different metabolite patterns between terrestrial and marine-derived fungal strains (Vansteelandt *et al*., 2012). *P. chrysogenum* isolated from a marine environment showed higher endoglucanase activity in salinity conditions than *P. chrysogenum* isolated from a terrestrial environment (Lee *et al*., 2015). We also observed that halo-tolerance is positively correlated with endoglucanase activity, but not with other enzymes, such as β-glucosidase and protease. Neither halo-tolerance nor enzyme activity showed a distinct pattern among species or phylogenetic groups.

In conclusion, we isolated 28 marine-derived *Penicillium* strains associated with macroalgae in Jeju Island, including three newly recorded species in Korea. Although higher *Penicillium* diversity was observed for brown algae compared to green and red algae, substrate specificity was not detected in this study. *Penicillium* species associated with macroalgae showed endoglucanase, β-glucosidase, and protease activity; accordingly, they can be of valuable resources for the conversion of plant biomass to fuels and chemicals. Although a relationship among macroalgae, *Penicillium*, and enzyme activity was not detected, we believe that our data will provide a basis for further, more extensive studies to determine whether a correlation exists.

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