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RESEARCH ARTICLE

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Fungal Diversity and Enzyme Activity Associated with the Macroalgae, *Agarum clathratum*

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ABSTRACT

Agarum clathratum, a brown macroalgae species, has recently become a serious environmental problem on the coasts of Korea. In an effort to solve this problem, fungal diversity associated with decaying *A. clathratum* was investigated and related β-glucosidase and endoglucanase activities were described. A total of 233 fungal strains were isolated from *A. clathratum* at 15 sites and identified 89 species based on morphology and a multigene analysis using the internal transcribed spacer region (ITS) and protein-coding genes including actin (*act*), β-tubulin (*benA*), calmodulin (*CaM*), and translation elongation factor (*tef1*). *Acremonium*, *Corollospora,* and *Penicillium* were the dominant genera, and *Acremonium fuci* and *Corollospora gracilis* were the dominant species. Fifty-one species exhibited cellulase activity, with *A. fuci*, *Alfaria terrestris*, *Hypoxylon perforatum*, *P. madriti*, and Pleosporales sp. Five showing the highest enzyme activities. Further enzyme quantification confirmed that these species had higher cellulase activity than *P. crysogenum*, a fungal species described in previous studies. This study lays the groundwork for bioremediation using fungi to remove decaying seaweed from populated areas and provides important background for potential industrial applications of environmentally friendly processes.

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KEYWORDS
β-glucosidase; cellulase activity; endoglucanase; fungal enzyme; seaweed

1. Introduction

Agarum clathratum, a brown macroalgal species, is generally found on rocks in the low intertidal to sub-tidal zones. It is widely distributed in Alaska and East Asia, including Hokkaido in Japan, the Kuril Islands, and northern area of Korea [1]. Brown algae, including *A. clathratum*, are composed of 30–50% carbohydrates as cellulose which is the primary component of the cell wall [2,3]. Interest in *A. clathratum* and other seaweed species has risen recently due to its immunomodulatory and antioxidant activities [4–6]. However, increasing masses of seaweed waste have been reported worldwide due to climate change and eutrophication by fertilizer runoff [7,8]. A large amount of seaweed wastes are deposited on the shores of Korea peninsula [9], and *A. clathratum* mass in particular has accumulated on the northeast coast of Korea causing serious environmental problems. Despite the severity of this problem, no effective method has been developed despite the severity of this problem.

Cellulose has previously been degraded using costly and energy demanding industrial methods such as chemical hydrolysis under high pressure and temperatures [10]. Hydrolysis of cellulose using microbial enzymes requires less energy and is less expensive and more environment-friendly process than industrial processes [11]. Microbial enzymes have been studied and applied in various industries such as bio-energy production from seaweed [12], mostly focused on bacterial cellulases. Numerous fungi have been reported from a wide range of brown seaweed, many with abundant cellulase, protease, and xylanase activities [13–15]. However, there is no study about fungal diversity and enzymes activity associated with *A. clathratum*.

Marine fungi interact with marine organisms as parasites, symbionts, or decomposers and are crucial for organic and inorganic nutrient cycling in marine ecosystems [13,16]. Approximately 1500 species of marine fungi have been reported worldwide [13], and are found in various substrates including seaweed, plants, sediments, and wood [16–18]. Marine fungi are categorized into two groups depending on their origin and salinity tolerance, obligate marine fungi from the marine origins and facultative fungi

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Supplemental data for this article can be accessed here.

from the terrestrial environments [19]. The number of fungi associated with seaweed accounts for one-third of reported marine fungi [13].

Historically, morphological characters have been used to identify fungi; however, they often lead to misidentification because many intraspecific fungal characters are variable depending on environmental conditions [20]. Thus, current identification methods employ both morphology and molecular markers. Among these molecular markers, the internal transcribed spacer (ITS) region has been widely used as a fungal barcode marker [21], and increased resolution can be attained using a multi-locus approach. Specifically, the actin locus (*act*) has been used for *Cladosporium,* β-tubulin (*benA*) for *Penicillium,* calmodulin (*CaM*) for *Aspergillus*, and translation elongation factor (*tef1*) for *Fusarium* and *Trichoderma* [20,22].

In this study, *A. clathratum* samples were collected from fifteen different sites along the northeast coast of Korea and fungal diversity associated with *A. clathratum* degradation was investigated. In addition, extracellular enzymes associated with fungi, including β-glucosidase and endoglucanase, were evaluated in order to discover potential candidates for the treatment of seaweed waste.

2. Materials and methods

2.1. Sampling and fungal isolation

Sampling was conducted from accumulated *A. clathratum* from fifteen different sites along the eastern coast of Korea in August 2015 (Figure 1). Before fungal isolation, each sample was washed with sterilized Artificial Sea Water (ASW) [23] to remove any debris on the surface. Sections of each sample (5 mm in diameter) were placed on three different media plates containing artificial seawater: potato dextrose agar (PDA; BD-Difco, Sparks, MD) and glucose yeast extract agar (GYA; 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; BD-Difco). All plates were incubated at 25°C and isolates with distinguishable morphology were transferred to a new PDA plate. Each pure culture was stored in 20% glycerol at −80°C at the Seoul National University Fungus Collection (SFC) or Marine Bio-Resource Information System (MRS).

2.2. Molecular identification procedures

Fungal isolates were grouped based on their morphological characteristics and several representative strains were chosen from each group for molecular identification. DNA was extracted using a cetyltrimethylammonium bromide (CTAB) protocol described by Rogers and Bendich [24].

PCR was conducted in two steps. First, the ITS region was amplified from all representative strains. Then, different protein-coding genes (*act, benA, CaM,* and *tef1*) were used to identify strains to the species level for the genera *Cladosporium* (*act*), *Penicillium* (*benA*), *Aspergillus* (*CaM*), *Fusarium* (*tef*1), and *Trichoderma* (*tef*1). Each PCR was performed using AccuPower® PCR PreMix (Bioneer, Daejeon, Korea) in a final volume of 20 μl, containing 10 pmol of each primer and 10 ng of gDNA. The PCR amplification of ITS, *act, benA, CaM,* and *tef*1 were performed using the primers ITS1F/ITS4 [25], ACT-512F/ACT-783R [26], Bt2a/Bt2b [27],

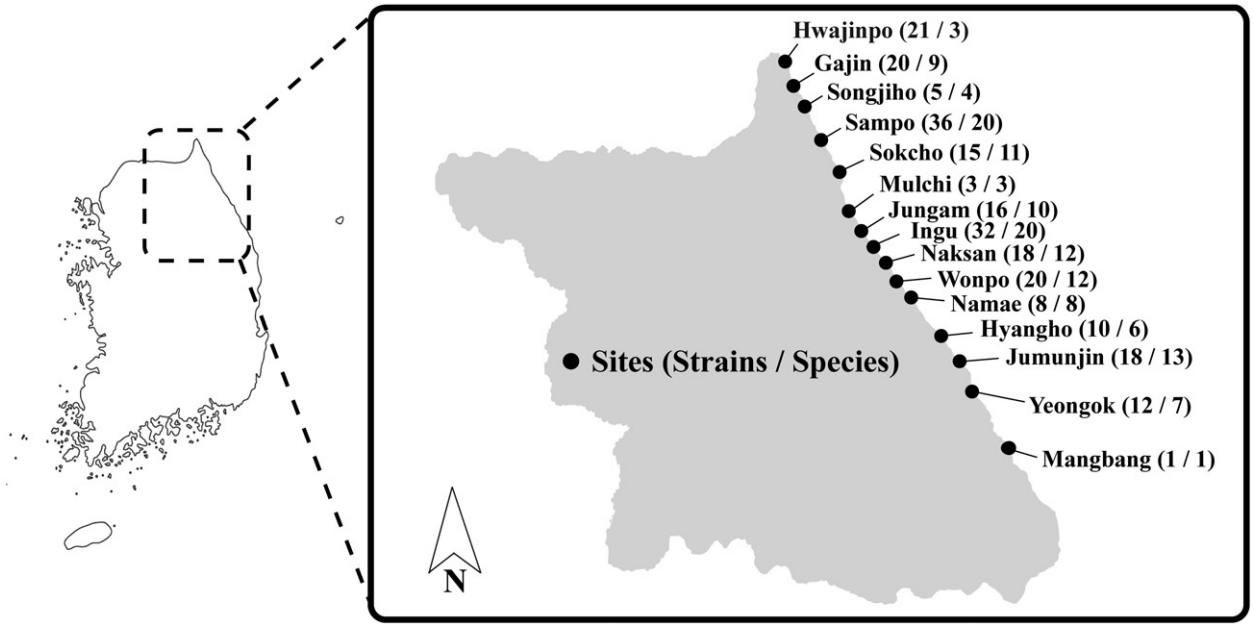

Figure 1. Map showing the location of sampling sites for *A. clathratum* collected along eastern coastline of Korea. Numbers in the parentheses for each site indicate strains and species isolated from decaying *A. clathratum*.

Table 1. GenBank accession numbers and clear zones of isolated strains from *Agarum clathratum*.

Representative strain	No. of strain	GenBank accession number					Top Scoring BLAST Mach in GenBank	Clear zone (mm)[a]	
		ITS	*ACT*	*BenA*	*CaM*	*tef1*		GA	EA
SFC102468	34	MH374541					*Acremonium fuci*	2.9	9
SFC102380	3	MH374571					*Alfaria terrestris*	14	10
SFC102269	1	MH374617					*Alternaria broccoli-italicae*	–	–
SFC102318	1	MH374593					*Arthrinium malaysianum*	–	–
SFC102355	1	MH374579			MH367021		*Aspergillus chevalieri*	–	–
SFC102407	4	MH374559			MH367023		*Aspergillus costaricaensis*	1	6
SFC102289	3	MH374606			MH367020		*Aspergillus fumigatus*	–	–
SFC102359	1	MH374578			MH367022		*Aspergillus insulicola*	5	5
SFC102419	1	MH374555			MH367024		*Aspergillus terreus*	1.5	4.5
SFC102281	1	MH374611			MH367019		*Aspergillus welwitschiae*	–	–
SFC102362	3	MH374575					*Asteromyces cruciatus*	–	–
SFC102361	1	MH374576					*Chaetomium globosum*	–	–
SFC102453	1	MH374547					*Chloridium* sp.	4.5	3.5
SFC102433	5	MH374553	MH367029				*Cladosporium cladosporioides*	1.5	–
SFC102263	1	MH374620	MH367026				*Cladosporium grevilleae*	–	4
SFC102255	1	MH374622	MH367025				*Cladosporium perangustum*	–	–
SFC102395	3	MH374563	MH367028				*Cladosporium rectoides*	3	1
SFC102352	1	MH374581	MH367027				*Cladosporium sphaerospermum*	–	–
SFC102394	11	MH374564					*Clonostachys miodochialis*	–	–
SFC102311	1	MH374597					*Clonostachys rosea*	5	5
SFC102291	2	MH374605					*Coniella quercicola*	–	–
SFC102400	26	MH374560					*Corollospora gracilis*	4.5	5
SFC102442	2	MH374552					*Corollospora maritima*	1	–
SFC102459	1	MH374544					*Corollospora* sp.	–	4
SFC102272	1	MH374616					*Diaporthe* sp.	–	–
SFC102458	1	MH374545					*Didymella bellidis*	1	1
SFC102377	1	MH374572					*Didymella pomorum*	–	–
SFC102337	2	MH374586					*Didymella* sp.	–	–
SFC102399	1	MH374561					*Discosia artocreas*	5	4
SFC102353	1	MH374580					*Epicoccum sorghinum*	1	–
SFC102301	1	MH374600					*Epicoccum* sp.	1	–
SFC102238	1	MH374628					*Eutypella scoparia*	7	3
SFC102386	4	MH374567				MH367033	*Fusairum* cf. *equiseti*	–	–
SFC102286	1	MH374609				MH367031	*Fusairum acuminatum*	1.5	1.5
SFC102314	2	MH374596				MH367032	*Fusairum graminearum*	–	–
SFC102248	1	MH374626				MH367030	*Fusarium* sp.	–	–
SFC102330	1	MH374590					*Galactomyces* sp.	–	–
SFC102393	1	MH374565					Hypocrealessp.	2	–
SFC102449	4	MH374551					*Hypoxylon perfoatum*	9	7.5
SFC102275	1	MH374614					*Lophiostoma* sp.	–	–
SFC102294	1	MH374602					*Myrmecridium schulzeri*	–	–
SFC102316	3	MH374594					*Neopestalotiopsis clavispora*	–	–
SFC102383	1	MH374569					*Nigrospora oryzae*	–	–
SFC102348	1	MH374582					*Paraconiothyrium fuckelii*	–	2
SFC102409	3	MH374558					*Paradendryphiella arenariae*	–	1.5
SFC102461	5	MH374543					*Paraphaeosphaeria* sp.	–	–
SFC102388	3	MH374566					*Paraphaeosphaeria sporulosa*	–	–
SFC102385	1	MH374568		MH367039			*Penicillium antarcticum*	–	2
SFC102451	1	MH374549		MH367035			*Penicillium aurantiogriseum*	–	5
SFC102254	1	MH374623		MH367045			*Penicillium bialowiezense*	8	5.5
SFC102288	1	MH374607		MH367043			*Penicillium bilaiae*	–	–
SFC102305	8	MH374599		MH367042			*Penicillium citrinum*	2	2.5
SFC102287	1	MH374608		MH367044			*Penicillium cremeogriseum*	–	–
SFC102320	2	MH374592		MH367041			*Penicillium daejeonium*	1	1
SFC102452	5	MH374548		MH367034			*Penicillium guanacastense*	2	2
SFC102420	2	MH374554		MH367037			*Penicillium madriti*	13.5	12.5
SFC102415	1	MH374557		MH367038			*Penicillium oxalicum*	3	2.5
SFC102343	3	MH374583		MH367040			*Penicillium roseomaculatum*	–	–
SFC102243	2	MH374627		MH367046			*Penicillium spinolusum*	–	–
SFC102450	3	MH374550		MH367036			*Penicillium virgatum*	2.5	3.5
SFC102471	1	MH374540					*Pestalotiopsis lespedezae*	3	1
SFC102282	4	MH374610					*Pestalotiopsis* sp.	–	2
SFC102465	3	MH374542					*Phaeosphaeria oryzae*	–	2
SFC102360	1	MH374577					Pleosporales sp. 1	8	6
SFC102369	5	MH374573					Pleosporales sp. 2	10	5
SFC102333	2	MH374589					Pleosporales sp. 3	7	1
SFC102334	1	MH374588					Pleosporales sp. 4	1	3
SFC102342	1	MH374584					Pleosporales sp. 5	11.5	6.5
SFC102260	1	MH374621					Pleosporales sp. 6	–	–
SFC102306	1	MH374598					Pleosporales sp. 7	1	3
SFC102397	4	MH374562					Pleosporales sp. 8	–	–
SFC102457	1	MH374546					Pleosporales sp. 9	2	2
SFC102382	1	MH374570					Pleosporales sp. 10	–	–
SFC102267	1	MH374619					*Porostereum spadiceum*	1	–
SFC102329	2	MH374591					*Roussoella* sp.	5	5

(*continued*)

Table 1. Continued.

Representative strain	No. of strain	GenBank accession number					Top Scoring BLAST Mach in GenBank	Clear zone (mm)[a]	
		ITS	*ACT*	*BenA*	*CaM*	*tef1*		GA	EA
SFC102268	1	MH374618					*Schizophylum commune*	1.5	–
SFC102315	1	MH374595					*Septoriellahubertusii*	4	3
SFC102335	1	MH374587					*Sesquicillium microsporum*	8.5	5.5
SFC102276	1	MH374613					*Stagonosporopsis cucurbitacearum*	–	–
SFC102417	1	MH374556					*Stemphylium solani*	7	5.5
SFC102277	3	MH374612					*Stereum* sp.	–	–
SFC102341	1	MH374585					*Teichospora* sp.	–	–
SFC102274	2	MH374615					*Trametes hirsuta*	–	–
SFC102367	1	MH374574				MH367051	*Trichoderma atroviride*	–	–
SFC102249	2	MH374625				MH367047	*Trichoderma guizhouense*	–	–
SFC102293	1	MH374603				MH367050	*Trichoderma* sp. 1	–	–
SFC102292	1	MH374604				MH367049	*Trichoderma* sp. 2	–	–
SFC102252	6	MH374624				MH367048	*Trichoderma* sp. 3	–	–
SFC102299	1	MH374601					*Zymoseptoria verkleyi*	1	2

The number in the last column indicates the diameter of the clear zone for each media plate.
[a]GA: β-glucosidase; EA: Endoglucanase.

CF1/CF4 [22], and EF1/EF2 [28], respectively, with C1000 Thermal Cycler (Bio-Rad, Hercules, CA) as described by Park et al. [29]. PCR products were purified using ExpinTM PCR Purification Kit (Geneall Biotechnol., Seoul, Korea) following the manufacturer's instruction. The purified amplicons were sequenced using corresponding PCR primers by Macrogen (Seoul, Korea) in both forward and reverse directions using ABI Prism 3730 genetic analyzer (Life Technol., Gaithersburg, MD).

Sequences were assembled, proofread, edited, and aligned using MEGA v.5 [30] and were deposited in GenBank (Table 1). For multiple sequence alignments, MAFFT v.7 [31] was used, and each sequence was checked and adjusted manually. After alignment, maximum likelihood (ML) phylogenetic trees were constructed. The phylogenetic trees were generated using RAxML 8.0.2 [32] and the GTR + GAMMA model of evolution with 1000 bootstrap replicates.

2.3. Enzyme assays

The plate screening assays for cellulase activity were conducted for representative strains from each species (Table 1). Mandel's medium was used supplemented with 0.5% D-cellobiose (CB; Sigma-Aldrich, St. Louis, MO) for β-glucosidase and 1% carboxymethylcellulose (CMC; Sigma-Aldrich) for endoglucanase [33]. The five species that showed the highest activity from each enzyme assay were chosen from the plate screening assays and then all strains of these five species were further screened for enzyme activity to select the strain with highest enzyme activity among them.

Fungal strains with the highest level of enzyme activity were incubated in a shaking bath at 25°C with minimal liquid media (0.3 g/L urea, 1.4 g/L KH_2PO_4, 2.0/L g of $(NH_4)2SO_4$, 0.3 g/L $CaCl_2$, 0.3 g/L $MgSO_4$, 0.25 g/L yeast extract, 0.75 g/L peptone, 5 mg/L $FeSO_4{\cdot}7H_2O$, 36 mg/L $COCl_2{\cdot}6H_2O$, 1.8 mg/L $MnSO_4{\cdot}H_2O$, and 2.5 mg/L $ZnSO_4{\cdot}7H_2O$) and ground *A. clathratum* as a carbon source. After a week of incubation, culture broths were collected by filtration and their cellulase activity was measured by micro-assay based on the dinitrosalicylic acid (DNS) method [34]. The cellulase activity was compared to that of *P. crysogenum* (FU42), which has shown high cellulase activity [33].

3. Results

3.1. Identification and diversity

A total of 233 fungal strains were isolated from decaying *A. clathratum* at 15 sites, and 89 fungal taxa were determined by morphological characters and ITS analysis. Next, the strains were identified using additional genetic markers: *act, CaM, benA,* or *tef1* (Table 1). The use of an additional locus (in conjunction with the ITS), significantly improved species identification. Sixty-two species were identified to the species level, while 27 remained unidentified species due to ambiguous phylogenetic relationships (Figure 2). The species were grouped into two phyla, five classes, 14 orders, 42 genera, and 89 species (Figure 2, Table 1). Ninety-seven percent of strains (226 strains of 85 species) were identified as Ascomycota and 3% (7 strains of 4 species) as Basidiomycota. At the order level, 30% of species (n = 70) belonged to the Hypocreales, 21% to the Pleosporales (n = 49), 18% to the Eurotiales (n = 42), 12% to the Microscales (n = 29), and 7% to the Xylariales (n = 14). At the genus level, over one-third of strains were represented by the *Acremonium* (15%), *Penicillium* (13%), and *Corollospora* (12%) (Figure 2). The genus *Penicillium* was represented by 13 species, while *Aspergillus* and *Cladosporium* were represented by 5 species. *Acremonium fuci* was the most dominant species (34 strains) and was found in most sites, followed by *Corollospora gracilis* (26 strains), *Clonostachys miodochialis* (11 strains), and *P. citrinum*

(8 strains) (Figure 2). Fungal abundance and diversity varied depending on the sampling site. The highest number of strains was recovered at Sampo, while only one strain was found at Mangbang (Figure 1).

3.2. Enzyme activity

Of 89 species, 49 exhibited cellulase activity: 41 species had β-glucosidase activity and 42 species had endoglucanase activity. The highest β-glucosidase activity was observed in five species: *A. terrestris, P. madriti*, Pleosporales sp. 5, *H. perforatum,* and *Sesquicillium microsporum*. The highest endoglucanase activity was observed in *A. fuci*, *A. terrestris*, *H. perforatum, P. madriti*, and Pleosporales sp. 5 (Table 1). Considering the isolation frequency and enzyme activity, five species were selected for further experiments; *A. fuci, A. terrestris, H. perforatum*, *P. madriti*, and Pleosporales sp. 5.

All strains were screened in order to choose strains with the highest enzyme activity. These strains were *A. fuci* (SFC102273; SFC20190110-M01; MRS002000115463), *A. terrestris* (SFC102380; SFC20190110-M02; MRS002000115464), *H. perforatum* (SFC102443; SFC20161014-M23; MRS002000066796), *P. madriti* (SFC102420; SFC20160317-M24; MRS002000066660) and Pleosporales sp. 5 (SFC102342; SCF20190110-M03; MRS002000115465) (Table S1). These selected fungal strains were then measured for cellulose enzyme activity with *A. clathratum* as a substrate. Endoglucanase activity from selected strains was higher than *P. crysogenum* (FU42). *Penicillium madriti* had the highest endoglucanase activity, which was approximately three times that of *P. crysogenum.* Most strains, however, had similar, or slightly less, β-glucosidase activity as *P. crysogenum,* with the exception of *P. madriti* (SFC102420), which had approximately twice as much higher β-glucosidase activity as *P. crysogenum* (Figure 3).

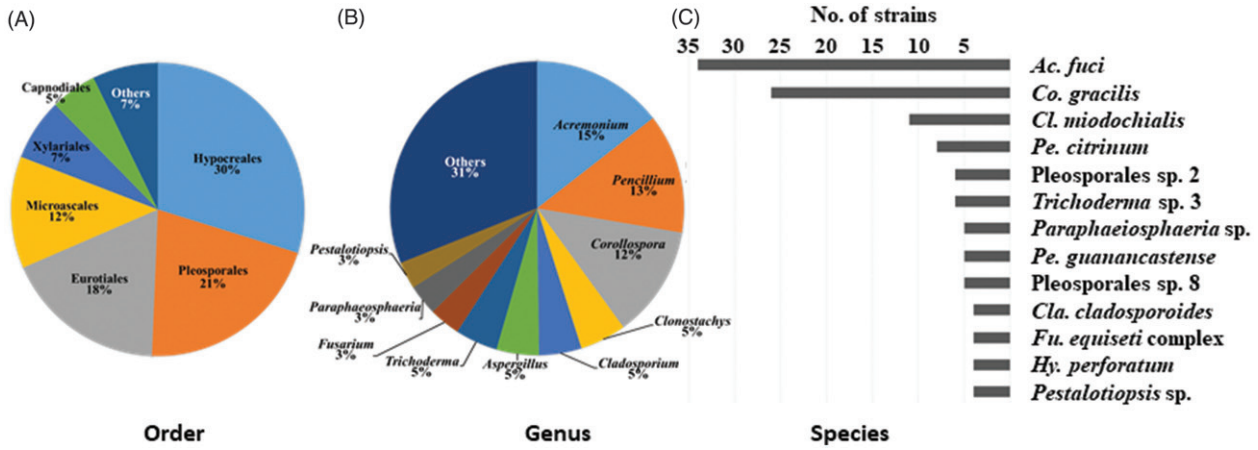

Figure 2. Composition of the dominant fungi isolated from *A. clathratum* at the order level (A); at genus level (B); and species level (C).

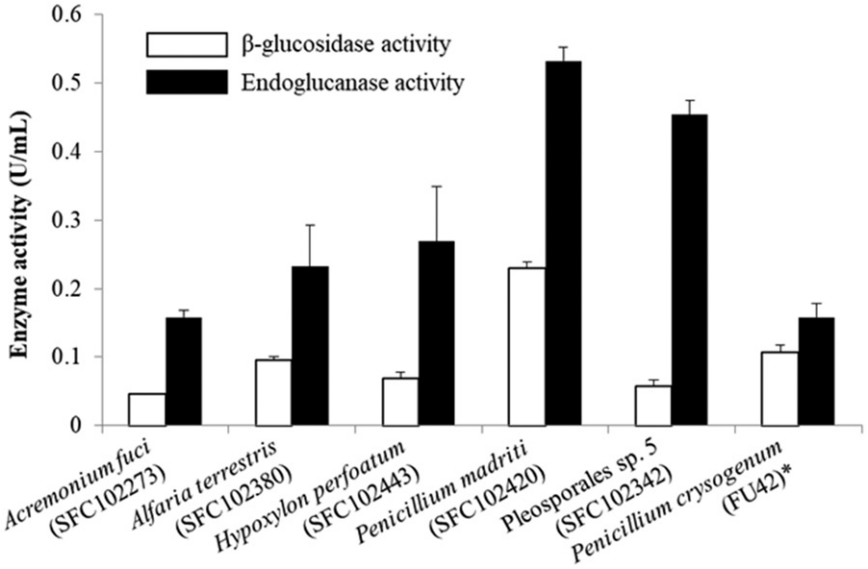

Figure 3. The enzyme quantification comparison of selected species when *A. clathratum* was given. '*' indicates positive control which showed good fungal enzyme activity in the previous study [33].

4. Discussion

Macroalgae is composed of large amounts of polysaccharides, primarily cellulose [35]. Marine fungi acquire nutrients from various organisms such as algae, sponges, and mangroves [13,16], and play a crucial role in ecosystem nutrient cycling by converting carbohydrate polymers (e.g., cellulose) to monosaccharides, which are easily metabolized by myriad organisms [13,16,36]. While macroalgae are a primary source of substrate for marine fungi [13,16], most studies have focused on their ecological role as symbionts within living algae, rather than as decomposers [37,38].

4.1. Diversity of fungi associated with *A. clathratum*

High fungal diversity (89 species) was detected from a single substrate, decaying *A. clathratum*, on the east coast of Korea. The recovery of such diversity is likely the result of using three different media used for isolation as well as a multi-locus molecular approach (ITS, ITS, *act, benA, CaM,* and *tef*1). The majority of species were classified into the phylum Ascomycota, which is consistent with a previous study [39]. However, four Basidiomycota wood decay fungi were also isolated. These fungi are commonly found in terrestrial environments, especially forests, thus, these species were likely opportunistic fungi of terrestrial origin. Similar results have been reported from intertidal zones and marine sediments [40].

The order Pleosporales is one of the most dominant fungal groups in marine environments, while species in the Hypocreales are rare in marine habitats, with the exception of the family Bionectriaceae, in which the *Acremonium* belong [13]. In our study, Hypocreales species, including *A. fuci*, were abundant. Within the order Pleosporales, several unidentified species were detected. In general, species in this order have similar morphological features and molecular markers for this order have not been developed, thus, many Pleosporales species remain unidentified [41,42].

A diverse array of species of *Penicillium* and *Aspergillus* were isolated from decaying *A. clathratum*. Although these species are also found in terrestrial environments, many species in these genera have been reported from marine environments such as macroalgae, coral, and sea sands [13,39]. In general, species diversity in these genera is much higher in brown algae than either red or green algae due to different cellulose components [13,36,38,43,44]. Many *Penicillium* species, in particular, have been reported in macroalgae including *A. clathratum* [15,44].

Acremonium fuci and *C. gracilis,* which are obligate marine fungi, accounted for most isolates of *Acremonium* and *Corollospora* (Figure 2). *Acremonium* separated into two main clades based on the ITS data: one clade likely had a terrestrial origin, while the other clade likely had a marine origin [45]. Specifically, *A. fuci* belongs to a marine-derived clade and is commonly isolated from brown seaweed [45]. *Corollospora* species are commonly found in sand, shell fragments, and algal thalli as obligate marine fungi [19,46,47], and species in this genus are produce antibacterial metabolites called corollosporine [48]. For example, *C. gracilis* has modified ascomycetous pores that can control the flow of seawater, a likely adaptation to aquatic habitats [13,47].

4.2. Fungal enzyme activity

Identifying fungi with high cellulolytic activity is the first step toward the application of these fungi to the removal of decaying seaweed in marine environments, in particular, populated shore areas and beaches. Some fungal spores of terrestrial origin that were isolated in this study may exhibit salt-resistance. Among these strains, five species were selected based on their high cellulase activity relative to other species. Species in the Pleosporales, Eurotiales, and Hypocreales degrade cellulose, and species in the genera *Aspergillus* and *Penicillium* are especially efficient at cellulose degradation [13].

When *A. clathratum* was chosen as a substrate, endoglucanase was higher than β-glucosidase activity and all species exhibited higher cellulase activity compared to *P. crysogenum* (Figure 3). Secretion of cellulolytic enzymes is likely influenced by the proportion of cellulose components but is also induced or repressed by other enzymes. For example, β-glucosidase is known to induce endoglucanase synthesis [49,50]. Thus, increased endoglucanase secretion of fungi associated with *A. clathratum* is expected compared to fungi provided with only a single carbon source. These cellulolytic enzymes are commonly found in other fungi from different substrates such as sediments and sponges, but they exhibited different enzyme activity depending on the substrate [51]. Both β-glucosidase and endoglucanase are used in the paper and detergent industries, however, both rely on massive amounts of water during cellulose hydrolysis and rely less on enzymatic catalysis of the natural hydrolytic process [52,53]. Thus, in addition to bioremediation using natural fungi, industrial applications of the fungi and their enzymes described here hold potential for more environmentally friendly manufacturing methods.

In this study, we found no significant relationship between fungal dominance and enzyme activity. This discordance in marine environments between microbial dominance and biological activity has previously been reported [54–56]. According to these previous studies, secondary metabolites and enzymes such as tannase were active in facultative fungi despite their lower abundance [54,55]. In nature, enzymatic activity of fungi is influenced by several factors including interactions with other microbial communities, the availability of organic matter, and other environmental factors [56]. Facultative fungi may produce increased metabolites and enzymes relative to obligate marine fungi in order to adapt to extreme environments [54,55].

Marine fungi have received increased attention in recent years, especially fungi associated with seaweed. These fungi play an important role in marine ecosystems as decomposers, and the use of fungal cellulases is potentially an important method with which combat environmental problems caused by seaweed waste. In this study, 89 fungal species were identified from 233 strains associated with the macroalgae, *A. clathratum*. Enzyme activity of ∼50% of the isolated strains exhibited β-glucosidase and endoglucanase activity. We expect that this study will provide critical, basic information regarding the fungi associated with *A. clathratum* decay in nature and that the enzymes produced by selected fungi have potential industrial applications.

Disclosure statement

No potential conflict of interest was reported by the authors.

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