이학석사 학위논문

Diversity and enzyme activity of fungal species associated with macroalgae, *Agarum clathratum*

. 구멍쇠 미역(*Agarum clathratum*) 에서 분리한 진균의 다양성과 효소활성

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이 서 빈

Diversity and enzyme activity of fungal species associated with macroalgae, *Agarum clathratum*

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Abstract

Agarum clathratum is one of brown macroalgae species. Recently, it has risen as a serious environmental issue being accumulated on coast in Korea. In order to discover fungal candidates to solve this problem, fungal diversity associated with *A. clathratum* in decay was investigated and their enzyme activities were confirmed; alginase, β-glucosidase and endoglucanase which participated in degrading alginate and cellulose of *A. clathratum*. A total 235 fungal strains were isolated from *A. clathratum* on 15 sites and identified to 89 species based on morphology and multigene analysis; using ITS and protein coding genes (*act, benA, CaM,* and *tef*). *Acremonium, Penicillium,* and *Corollospora* were shown up as dominant genera,

especially, *Acremonium fuci* and *Corollospora gracillis* were the most dominant species. 57 species exhibited alginase and/or cellulase activity by forming clear zone from plate assay. Among them, *Acremonium fuci, Alfaria terrestiris, Hypoxylon perforatum, Penicillium madriti,* and Pleosporales sp. 5 showed the highest enzyme activities. With these species, further enzyme quantification was conducted and most of species were confirmed that they had better enzyme activity than *Penicillium crysogenum* which was known for having good enzyme activity in previous studies, especially *Penicillium madriti*.

Keywords : Alginase, Cellulase, Fungal enzyme, Marine fungi, Seaweed

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Contents

List of Figures

List of Tables

1. Introduction

Macroalgae are called as seaweeds and they are considered as valuable organisms in marine environments because of their important roles such as providing good food sources to other herbivorous animals and controlling level of phosphates and nitrogen by fixation. Currently, over ten thousands of macroalga species have been reported in worldwide and they are categorized into three different types depending on their pigments; brown, green, or red (Jung et al., 2013). Among numerous macroalgae species, *Agarum clathratum* is categorized into brown macroalga species belonging to family Costariaceae.

A. clathratum is generally found on rock in the low intertidal to sub-tidal sites and is widely distributed in northern Pacific including Sakhalin, Kuriles and northern part of Japan and Korea (Kang, 1966; Yamada, 1972). Brown algae including *A. clathratum*, are composed of 30-50 % of carbohydrates content, and especially, alginate and cellulose are known as main components of their cell wall (Usov et al., 2001; Kim, 2013). Traditionally, it was used for foods in Asia countries due to good tastes, but also it is recently used for medicinal sources due to its immunomodulatory, antitumor, and antioxidant effect (Jeon et al., 2012; Park et al., 2012; Cho et al, 2013).

In recent years, however, huge seaweed waste have been reported in worldwide due to climate change and eutrophication (Lapointe and Bedford, 2006;

Hu et al., 2013) and it is no exception in Korea as well. According to Fishery Production Survey in Korea (2011), approximate over 800 thousand tons of seaweed waste occurred in coast, annually. Especially, it was observed that huge *A. clathratum* mass was accumulated on east-northen coast of Korea and serious environmental problems have been reported during summer in 2015 due to heavy biomass of seaweed. Despite severity of this problem, no proper method exists for disposal of seaweed waste up to now.

Chemical hydrolysis methods such as alkali swelling, acid hydrolysis, or supercritical fluids have been suggested to degrade cellulose and/or alginate for disposal of seaweed waste, but it required not only high costs and energies with high pressure, temperature, and pH sensitivity, but also physical treatment to remove sand, debris, and salt (McMillan, 1994). On the other hands, utilization method using microbial enzymes requires less cost and energy and is known for producing fewer side products with higher selectivity compared to chemical process (Kamaya, 1996; Bhat, 2000; Dincer and Telefoncu, 2007). Therefore, recent studies focused on their application. Various microbial enzymes have been studied and numerous fungal enzymes from seaweed have been reported such as cellulase, protease, and xylase (Lee et al., 2009; Jones and Pang, 2012; Hong et al., 2015; Park et al., 2016). Although marine fungi are known for producing a wide range of enzymes, studies for degrading seaweed waste have focused on bacterial enzymes rather than fungal enzyme. In addition, no study has been conducted showing whole fungal diversity and enzymes associated with *A. clathratum*, except a report about

diversity of *Penicillium* associated with *A. clathratum* (Park et al., 2016).

Marine fungi play important role by interacting with other marine organisms as parasites or symbionts and participating in nutrient cycle in marine ecosystem as decomposers by degrading organic substrates (Bugni and Ireland, 2004; Jones and Pang, 2012). Approximately 1,500 species of marine fungi have been reported in worldwide (Jones and Pang, 2012). These marine fungi are categorized into two groups depending on their origin and salinity tolerance; obligate fungi which is originated from marine environment, facultative fungi which is originated from terrestrial environment, but is able to grow in marine environment due to their salinity tolerance (Kohlmeyer, 1974). They are found in various substrates in marine such as seaweeds, plants, sediments, and wood (Bugni and Ireland, 2004; Schulz et al., 2008; Godinho et al. 2013). In particular, the number of fungi from seaweed accounts for one-third portion of reported marine fungi (Jones and Pang, 2012). Genus *Acremonium, Aspergillus, Cladosporium, Penicillium,* and *Trichoderma* are commonly found in marine habitats including seaweed and they account for most of marine fungi (Schulz et al., 2008; Zuccaro et al., 2008; Loque et al., 2010; Flewelling et al., 2013).

Historically, morphology was used to identify fungi as key characters, but it sometimes cause misidentification because it is vary under different conditions (Visagie et al., 2014). Since DNA sequencing was introduced in early 1990's, it started to be chosen for fungal identification. The ITS (Internal Transcribed Spacer) regions are used for fugal barcode marker due to sequence variations depending on

species (Schoch et al., 2012) and additional protein coding genes as secondary markers were used to improve resolution at species level such as actin (*act*) for *Cladosporium*, β -tubulin (*benA*) for *Penicillium*, calmodulin (*CaM*) for *Aspergillus*, and translation elongation factor (*tef1*) for *Fusarium* and *Trichoderma* (Glass and Donaldson, 1995; O'Donnell et al., 1998; Carbone & Kohn, 1999; Samson et al., 2014; Visagie et al., 2014).

In this study, *A. clathratum* samples were collected from fifteen different sites where accumulated seaweed waste occurred and fungal diversity associated with *A. clathratum* was investigated. Also, their extracellular enzyme activities including alginase, β-glucosidase, and endoglucanase were examined to discover potential candidates of useful fungal species to solve environmental problem by seaweed and for bio-industrial application using their enzymes.

2. Materials and Methods

2.1. Collection and isolation

A. clathratum samples were collected from fifteen different sites along eastern coast in Korea on August in 2015 (Figure 1-2).

Figure 1. *Agarum clathratum* (synonym *A. cribrosum*) from Japan. Retrieved from http://cfb.unh.edu/phycokey/Choices/Fucophyceae/AGARUM/Agarum_Image_page.htm

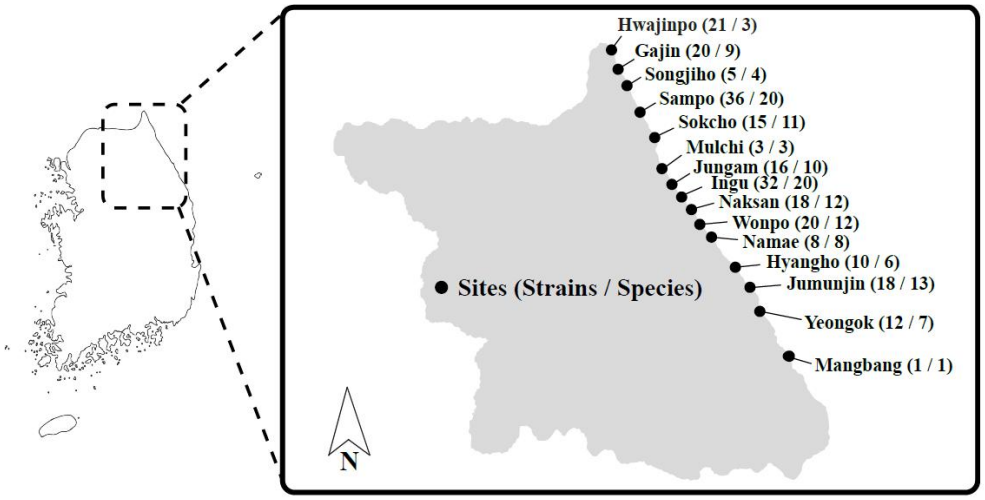


Figure 2. Map of eastern coastline of Korea showing sampling sites of *Agarum clatrhratum*.

Before isolation, each sample was washed with sterilized Artificial Sea Water (ASW) (Huang et al., 2011) to remove any debris on surface. Then, it was cut in 5 mm diameter and placed on three different media plates, which were supplemented with artificial sea water: potato dextrose agar (PDA; Difco, Becton Dickinson Sparks, MD, USA), glucose yeast extract agar (GYA; 1 g /L glucose, 0.1 g /L yeast extract, 0.5 g L^{-1} peptone, and 15 g /L agar), and dichloran rose bengal chloramphenicol agar (DRBC; Difco, Becton Dickinson, Sparks, MD, USA). All plates were incubated at 25°C and transferred to a PDA plate after distinguishable morphology was confirmed. All fungal strains were transferred to a new PDA plate and the strains were stored in 20% glycerol at – 80°C at the Seoul National University Fungus Collection (SFC).

2.2. Molecular Identification Procedures

For DNA extraction, cetyltrimethylammonium bromide (CTAB) protocol was used as Rogers and Bendich (1994) described. Each PCR reaction was performed AccuPower® PCR PreMix (Bioneer) in a final volume of 20 μl, containing 10 pmol of each primer and 10 ng of DNA. Each PCR were performed in a C1000 Thermal Cycler (Bio-Rad, Richmond, CA, USA) as following conditions: initial denature at 95°C for 5 mins, followed by 30 cycles at 95°C for 40 sec, at 55°C for 40 sec, and at 72°C for 1 min. Final step was run at 72°C for 10 mins. The PCR amplications of ITS, *act, benA, CaM,* and *tef1* were performed using ITS1F/ITS4 (White et al., 1990), ACT-512F/ACT-783R (Carbone & Kohn, 1999), Bt2a/Bt2b (Glass and Donaldson, 1995), CF1/CF4 (Samson et al., 2014), and EF1/EF2 (O'Donnell et al., 1998), respectively (Figure 3). Each PCR product was electrophoresed using 1 % agarose gel with LoadingSTAR (Dyne Bio, Seoul, Korea). PCR products were purified using the Expin^{TM} 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, Maryland, USA)

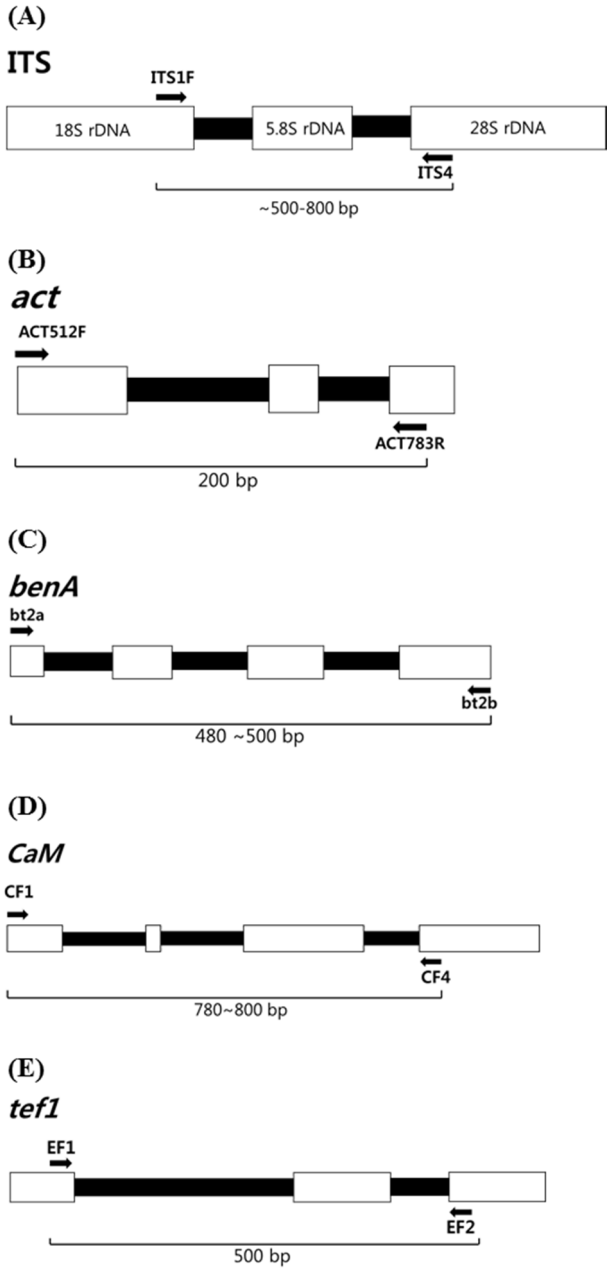


Figure 3. Information of DNA markers used in this study; Internal transcribed spacer (ITS) region primers (A), actin (B), β-tubulin (C), calmodulin (D), and translation elongation factor (E).

Sequences were assembled, proofread, edited, and aligned using MEGA v.5 (Tamura et al., 2011) and were deposited in GenBank (Table 1). For multiple sequence alignments, MAFFT v.7 was used (Katoh and Standley, 2013), and each sequence was checked and adjusted by eye. After alignment, the maximum likelihood (ML) phylogenetic trees were constructed. The phylogenetic trees were generated using RAxML 8.0.2 (Stamatakis, 2006) and the GTR+GAMMA model of evolution with 1,000 bootstrap replicates.

For identification of strains from *A. clathratum*, thr strains were grouped based on their morphological characteristics and a representative strain was chosen from each group to perform molecular identification. PCR was conducted in two steps. First, ITS was used for all representative strains. Then, different protein coding genes were used to identify at species level for genus *Cladosporium* (*act*), *Penicillium* (*benA*), *Aspergillus* (*CaM*), and *Fusarium* and *Trichoderma* (*tef1*), respectively.

2.3. Halo-tolerance and enzyme activity assays

To determine halo-tolerance of isolated fungal species, their growth rate was investigated by measuring diameter of each colony on PDA plates supplemented with ASW and without ASW. After inoculation, they were incubated at 25°C for 3-5 days depending on growth rate of each species

The plate screening assays were conducted to screen fungal enzyme activities. To screen enzyme activity of cellulase (β-glucosidase and endoglucancase), Mandel's medium was used supplemented with 0.5% D-cellobiose (CB; Sigma-Aldrich, St. Louis, MO, USA) for β-glucosidase and 1 % carboxymethylcellulose (CMC; Sigma-Aldrich, St. Louis, MO, USA) for endoglucanase (Pointing, 1999; Yoon et al., 2007, Lee et al., 2015). For alginase, modified 0.5% peptone agar was used supplemented with 0.8% sodium alginate (AL; Sigma-Aldrich, St. Louis, MO, USA) (Schaumann and Weide; 1990).

Each species was inoculated on each media plate and they were incubated for four days at 25°C. After incubation, the Gram's Iodine method was used to confirm clear zone and diameter of clear zone was measured (Kasana et al., 2008). Each plate assay was conducted in triplet. For species higher enzyme activity of either cellulose or alginase, every strain in that species was investigated to discover the best candidates. After screening, top 3 species from each enzymes were chosen which showed the highest enzyme activities for further enzymatic quantification (Table 1).

To confirm alginase, β-glucosidase, and endoglucanase activity of selected species which showed higher alginase and/or cellualse activity, modified DNS (3,5-Dinitrosalicylic acid) method was used (Chaplin and Kennedy, 1994). The species were inoculated liquid media which contained alginate, CB or CMC, respectively, as carbon source which are target substrate for each enzyme and incubated at 25°C in a shaking bath (180 rpm) for a week. After incubation, each media was filtered to

remove mycelium and centrifuged at 12,000 rpm for 10 mins. 300 μl of each supernatant was transferred to a conical tube and reagent was added to make 3 ml of total volume. The conical tubes were heated at 50°C for 1 hr and placed at room temperature to cool down. Each content was well mixed and absorbance was measured at 570 nm. Glucuronic acid and glucose were used as standard substrates for alginate and cellulose, respectively. The calibration curve was created by different concentration of glucuronic acid and glucose from 0.06 to 1.0 mg/ml (Figure 4). To compare amount of produced reducing sugar, both negative control and positive control were used. *Penicillium crysogenum* (FU42) was used as a positive control which was known for having high β-glucosidase and endoglucanase activity in previous study (Lee et al., 2015). Amount of produced reducing sugar was expressed as unit in mg in 1 ml of liquid media.

To investigate capability of fungal cellulase to degrade *A. clathratum* in practical when *A. clathratum* was given, *A. clathratum* was grinded and added as carbon sources into Mandel's medium instead of CB or CMC, respectively. Each inoculated sample was incubated at 25°C in a shaking bath (180 rpm) for a week and filtered to remove mycelium. The supernatant was collected and cellulase activity was measured as Lee et al. described (2011). The unit of enzyme activity was defined as required amount of enzyme to produce 1 μl of glucose.

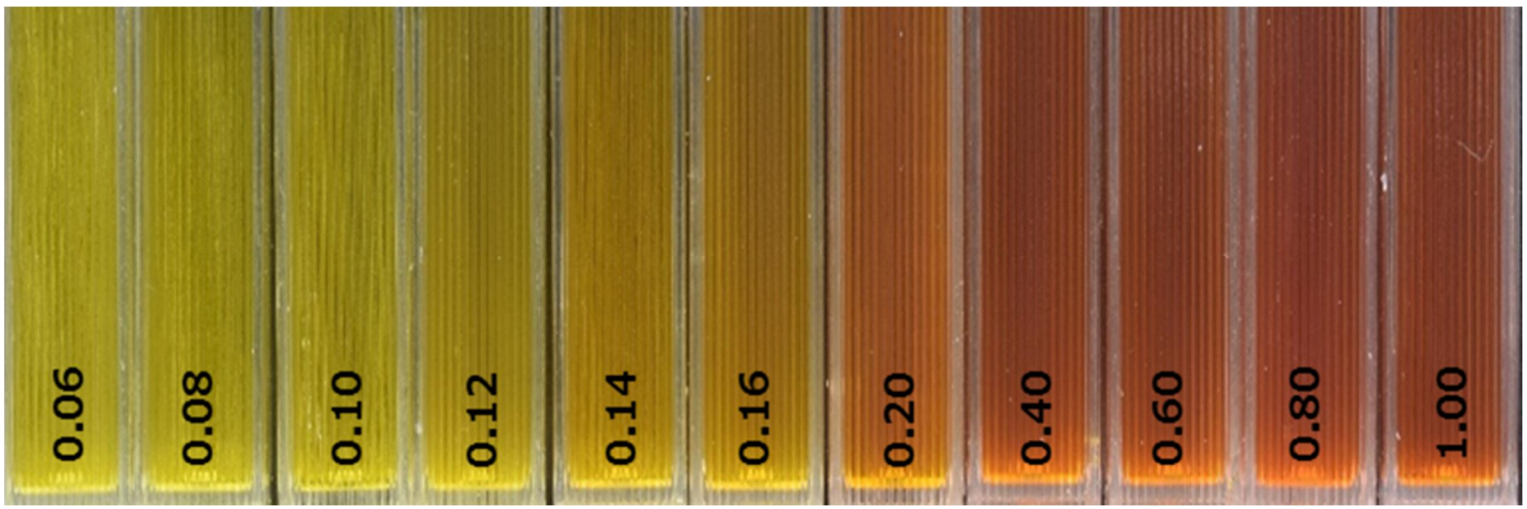


Figure 4. Color change of DNS depending on sugar concentration. From left to right, each number indicates the concentration of glucose from 0.06 to 1.0 mg/ml.

3. Results

3.1. Identification and Diversity

A total 235 fungal strains were isolated from *A. clathratum* in decay at 15 sites and 89 fungal groups were determined by their morphologies and ITS analysis. Then, they were further identified at the species level based on genus-by-genus phylogenetic analyses using ITS, *act, benA, CaM,* and *tef1*. Consequently, 64 species were identified at species level, but 25 species remained unidentified species due to ambiguous phylogenetic relationship (Table 1, Figure 5-6).

Table 1. Information and enzyme activity of fungal strains isolated from *Agarum clathratum*. The number of enzyme activity indicates a diameter of clear zone of each media plates. '#' indicates the number of isolated strains

Species	Collection No. (#)	Clear zone (mm)		
		Alginase	β-glucosidase	Endoglucanase
Acremonium fuci	SFC102273 (34)	8	6.5	9
Alfaria terrestris	SFC102380 (3)	10	14	10
Alternaria broccoli-italicae	SFC102269 (1)	-	-	-
Arthrinium malaysianum	SFC102318 (1)	-	-	-
Aspergillus chevalieri	SFC102355 (1)	-	-	-
A. costaricaensis	SFC102407 (2)	6	1	6
A. fumigatus	SFC102289 (3)	-	-	-
A. insulicola	SFC102359 (1)	5	5	5
A. terreus	SFC102419 (1)	4.5	1.5	4.5
A. welwitschiae	SFC102281 (3)	-	-	-
Astromyces cruciatus	SFC102362 (3)	-	-	-
Chaetomium globosum	SFC102361 (1)	-	-	-
Chloridium sp.	SFC102453 (1)	-	4.5	3.5
Cladosporium cladosporioides	SFC102433 (5)	1	1.5	-
C. grevilleae	SFC102263 (1)	2.5	-	4

(Table 1 continued)

Species	Collection No. (#)	Clear zone (mm)		
		Alginase	β-glucosidase	Endoglucanase
C. perangustum	SFC102255 (1)	-	-	-
C. rectoides	SFC102395 (3)	2	3	1
C. sphaerospermum	SFC102352 (1)	-	-	-
Clonostachys miodochialis	SFC102394 (11)	-	-	-
C. rosea	SFC102311 (1)	1	5	5
Coniella quercicola	SFC102291 (2)	-	-	-
Corollospora gracilis	SFC102400 (26)	3	4.5	5
C. maritima	SFC102442 (2)	-	1	-
Corollospora sp.	SFC102459 (1)	3.5	-	4
Diaporthe sp.	SFC102272 (1)	-	-	-
Didymella bellidis	SFC102458 (1)	1	1	1
Didymella pomorum	SFC102377 (1)	1	-	-
Didymella sp.	SFC102337 (2)	1	-	-
Discosia artocreas	SFC102399 (1)	7	5	4
Epicoccum sorghinum	SFC102353 (1)	1	1	-
Epicoccum sp.	SFC102301 (1)	1.5	1	-

(Table 1 continued)

Species	Collection No. (#)	Clear zone (mm)		
		Alginase	β-glucosidase	Endoglucanase
Eutypella scoparia	SFC102238 (1)	3.5	7	3
Fusairum equiseti complex	SFC102386 (4)	-	-	-
F. acuminatum	SFC102286 (1)	-	1.5	1.5
F. graminearum	SFC102314 (2)	-	-	-
Fusarium sp.	SFC102248 (1)	-	-	-
Galactomyces sp.	SFC102330 (1)	-	-	-
Hypocreales sp.	SFC102393 (1)	-	2	-
Hypoxylon perforatum	SFC102443 (4)	9	9	8.5
Lophiostoma sp.	SFC102275 (1)	-	-	-
Myrmecridium schulzeri	SFC102294 (1)	-	-	-
Neopestalotiopsis clavispora	SFC102316 (3)	-	-	-
Nigrospora oryzae	SFC102383 (1)	-	-	-
Paraconiothyrium fuckelii	SFC102348 (1)	1	-	2
Paradendryphiella arenariae	SFC102409 (3)	-	-	1.5
Paraphaeosphaeria sp.	SFC102461 (1)	-	-	-
P. sporulosa	SFC102388 (3)	1	-	-
Penicillium antarcticum	SFC102385 (1)	1	-	2

(Table 1 continued)

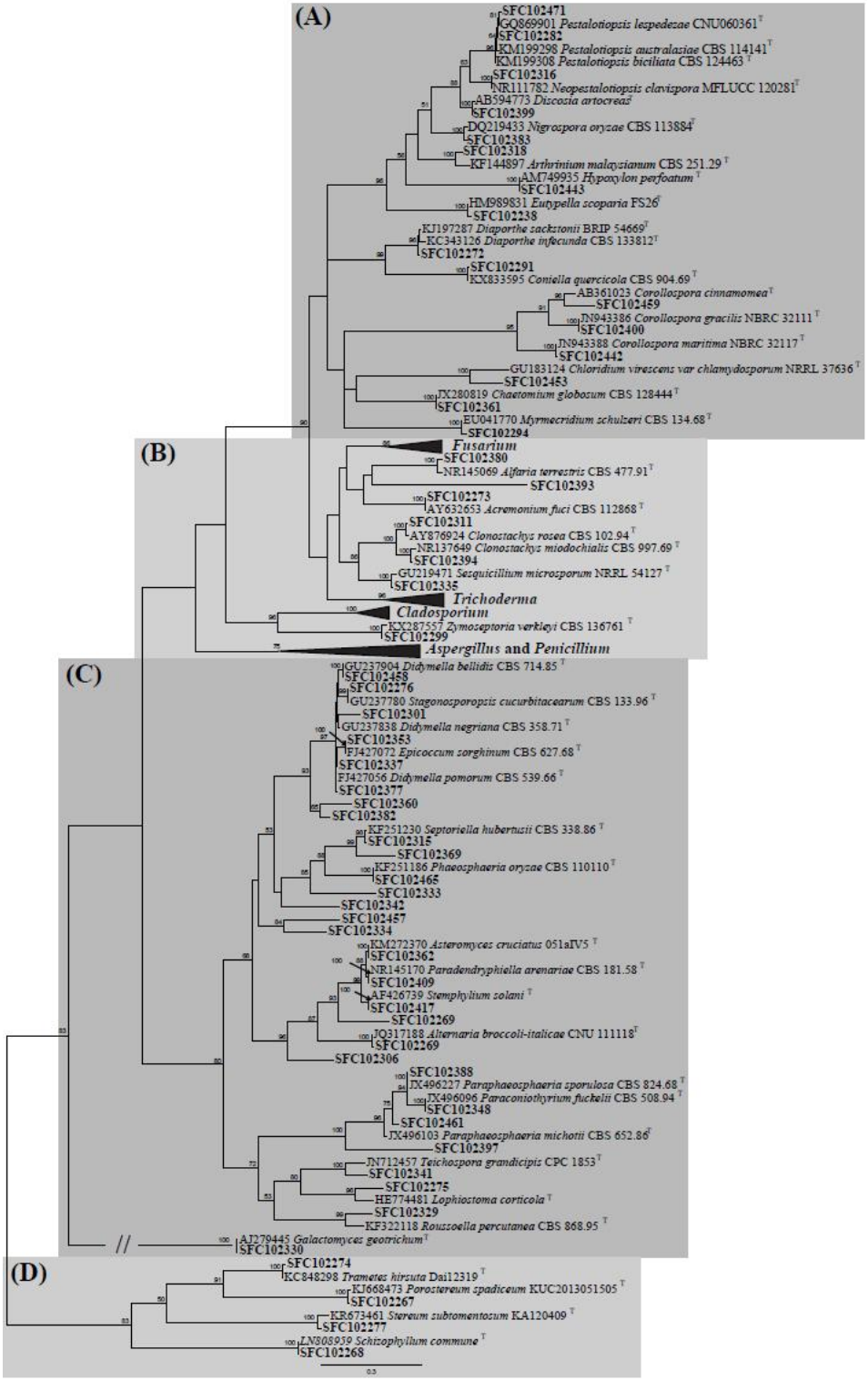
Species	Collection No. (#)	Clear zone (mm)		
		Alginase	β-glucosidase	Endoglucanase
P. aurantiogriseum	SFC102451 (1)	6	-	5
P. bialowiezense	SFC102254 (1)	2	8	5.5
P. bilaiae	SFC102288 (1)	-	-	-
P. citrinum	SFC102305 (8)	1	2	2.5
P. cremeogriseum	SFC102287 (1)	-	-	-
P. daejeonium	SFC102320 (2)	1	1	1
P. guanacastense	SFC102452 (5)	2	2	2
P. madriti	SFC102420 (2)	5	13.5	12.5
P. oxalicum	SFC102415 (1)	4.5	3	2.5
P. roseomaculatum	SFC102343 (3)	-	-	-
P. spinolusum	SFC102243 (2)	-	-	-
P. virgatum	SFC102450 (3)	4	2.5	3.5
Pestalotiopsis lespedezae	SFC102471 (1)	2	3	1
Pestalotiopsis sp.	SFC102282 (4)	3	-	2
Phaeosphaeria oryzae	SFC102465 (3)	-	-	2
Pleosporales sp. 1	SFC102360 (1)	7	8	6
Pleosporales sp. 2	SFC102369 (6)	5	10	5

(Table 1 continued)

Species	Collection No. (#)	Clear zone (mm)		
		Alginase	β-glucosidase	Endoglucanase
Pleosporales sp. 3	SFC102333 (2)	6	7	1
Pleosporales sp. 4	SFC102334 (1)	3	1	3
Pleosporales sp. 5	SFC102342 (1)	7.5	11.5	6.5
Pleosporales sp. 6	SFC102260 (1)	-	-	-
Pleosporales sp. 7	SFC102306 (1)	1.5	1	3
Pleosporales sp. 8	SFC102397 (5)	3.5	-	-
Pleosporales sp. 9	SFC102457 (1)	2	2	2
Pleosporales sp. 10	SFC102382 (1)	-	-	-
Porostereum spadiceum	SFC102267 (1)	-	1	-
Roussoella sp.	SFC102329 (2)	4.5	5	5
Schizophylum commune	SFC102268 (1)	3	1.5	-
Septoriella hubertusii	SFC102315 (1)	4.5	4	3
Sesquicillium microsporum	SFC102335 (1)	3	8.5	5.5
Stagonosporopsis cucurbitacearum	SFC102276 (1)	1.5	-	-
Stemphylium solani	SFC102417 (1)	6	7	5.5
Stereum sp.	SFC102277 (3)	1	-	-
Teichospora sp.	SFC102341 (1)	-	-	-
Trametes hirsuta	SFC102274 (2)	-	-	-

(Table 1 continued)

Species	Collection No. (#)	Clear zone (mm)		
		Alginase	β-glucosidase	Endoglucanase
Trichoderma atroviride	SFC102367 (1)	-	-	-
T. guizhouense	SFC102249 (2)	-	-	-
Trichoderma sp. 1	SFC102293 (1)	-	-	-
Trichoderma sp. 2	SFC102292 (1)	2.5	-	-
Trichoderma sp. 3	SFC102252 (6)	-	-	-
Zymoseptoria verkleyi	SFC102299 (1)	1	1	2


(A)
SFC102471
GQ869901 Pestalotiopsis leepedezae CNU060361T
SFC102282
KM199298 Pestalotiopsis australasiae CBS 114141T
KM199308 Pestalotiopsis biciliata CBS 124463T
SFC102316
NR111782 Neopestalotiopsis clavispora MFLUCC 120281T
AB594773 Discosia artocreas
SFC102399
DQ219433 Nigrospora oryzae CBS 113884T
SFC102383
SFC102318
KF144897 Arthrinium malaysianum CBS 251.29T
AM749935 Hypoxylon perfoatum T
SFC102443
HM989831 Eutypella scoparia FS26T
SFC102238
KJ197287 Diaporthe sackstonii BRIP 54669T
KC343126 Diaporthe infecunda CBS 133812T
SFC102272
SFC102291
KX833595 Coniella quercicola CBS 904.69T
AB361023 Corollospora cinnamomea T
SFC102459
JN943386 Corollospora graciliz NBRC 32111T
SFC102400
JN943388 Corollospora maritima NBRC 32117T
SFC102442
GU183124 Chloridium virescens var chlamydosporum NRRL 37636T
SFC102453
JX280819 Chaetomium globosum CBS 128444T
SFC102361
EU041770 Myrmecridium schulzeri CBS 134.68T
SFC102294
(B)
Fusarium
SFC102380
NR145069 Alfaria terrestris CBS 477.91T
SFC102393
SFC102273
AY632653 Acremonium fuci CBS 112868T
SFC102311
AY876924 Clonostachys rosea CBS 102.94T
NR137649 Clonostachys miodochialis CBS 997.69T
SFC102394
GU219471 Sesquicillium microsporum NRRL 54127T
SFC102335
Trichoderma
Cladosporium
KX287557 Zymoseptoria verkleyi CBS 136761T
SFC102299
Aspergillus and Penicillium
(C)
GU237904 Didymella bellidis CBS 714.85T
SFC102458
SFC102276
GU237780 Stagonosporopsis cucurbitacearum CBS 133.96T
SFC102301
GU237838 Didymella negriana CBS 358.71T
SFC102353
FJ427072 Epicoccum sorghinum CBS 627.68T
SFC102337
FJ427056 Didymella pomorum CBS 539.66T
SFC102377
SFC102360
SFC102382
KF251230 Septoriella hubertusii CBS 338.86T
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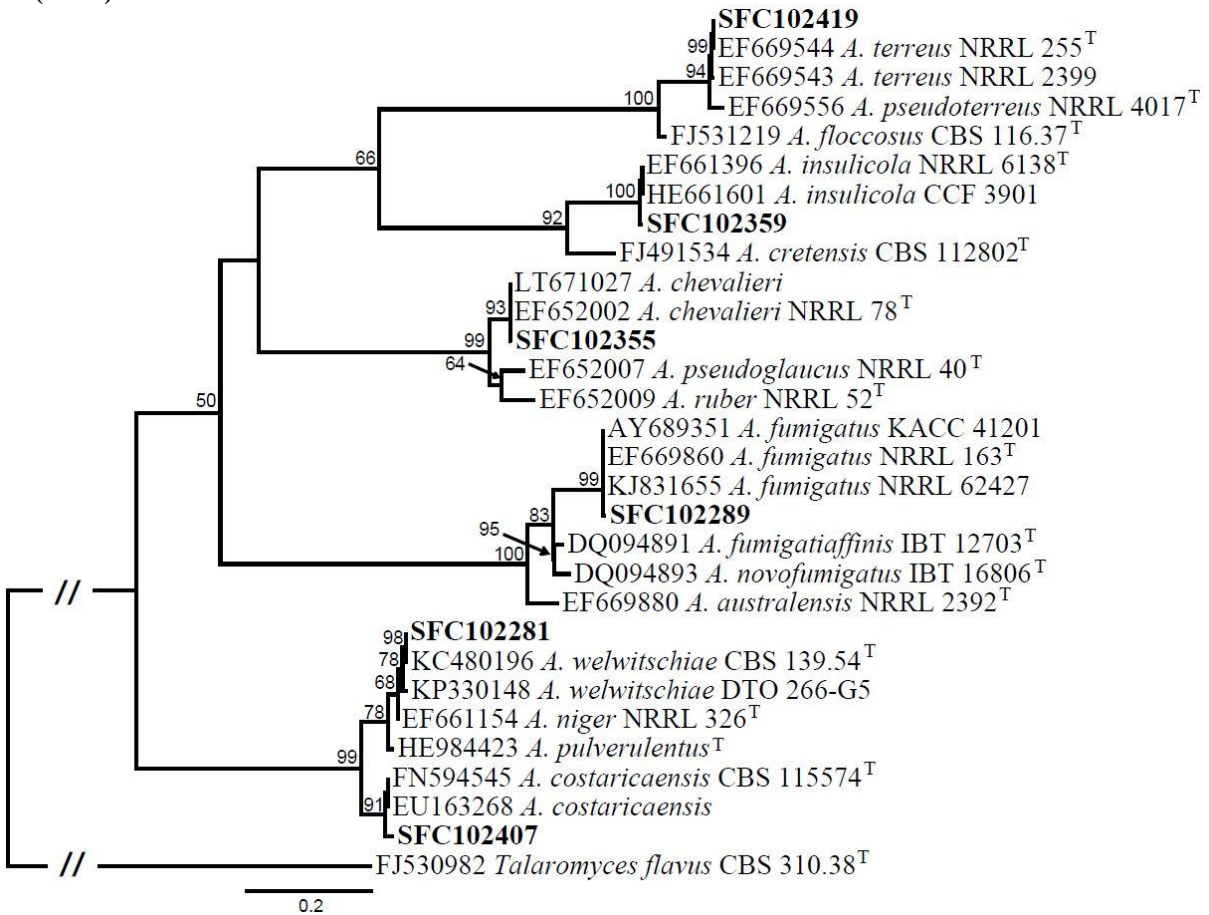
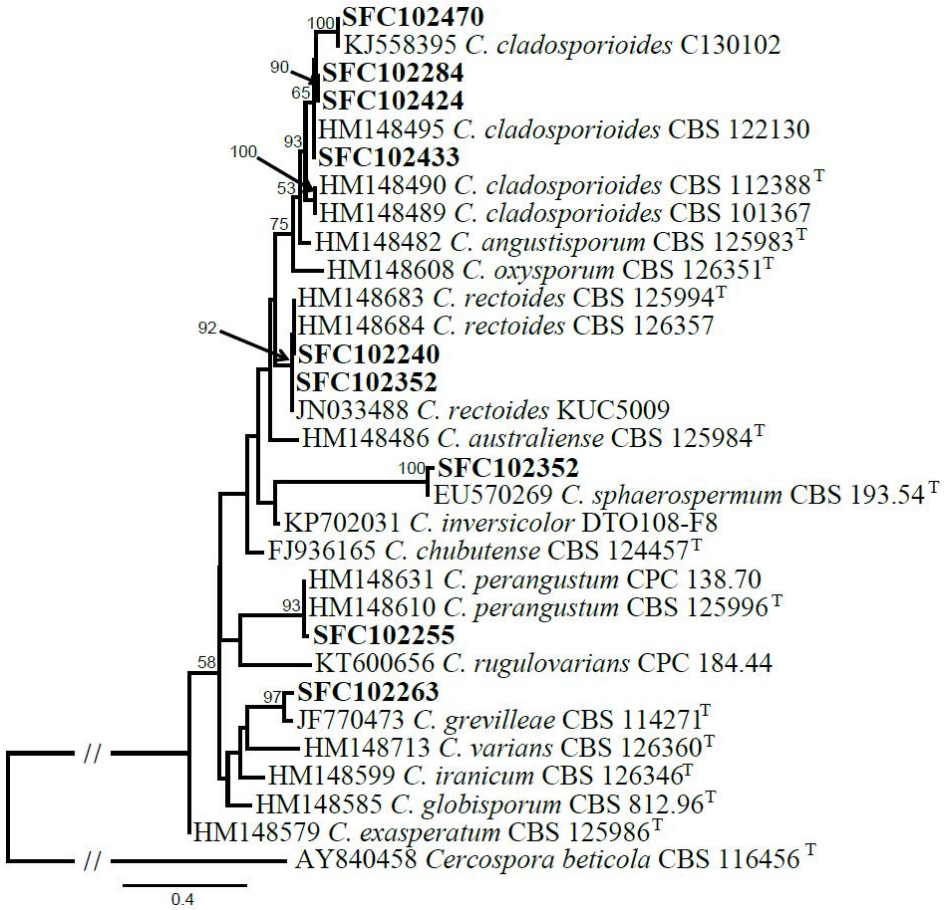
KF251186 Phaeosphaeria oryzae CBS 110110T
SFC102465
SFC102333
SFC102342
SFC102457
SFC102334
KM272370 Asteromyces cruciatus 051aIV5T
SFC102362
NR145170 Paradendryphiella arenariae CBS 181.58T
SFC102409
AF426739 Stemphylium solani T
SFC102417
SFC102269
JQ317188 Alternaria broccoli-italicae CNU 111118T
SFC102269
SFC102306
SFC102388
JX496227 Paraphaeosphaeria sporulosa CBS 824.68T
JX496096 Paraconiothyrium fuckelii CBS 508.94T
SFC102348
SFC102461
JX496103 Paraphaeosphaeria michotii CBS 652.86T
SFC102397
JN712457 Teichospora grandicipis CPC 1853T
SFC102341
SFC102275
HE774481 Lophiostoma corticola T
SFC102329
KF322118 Rousseoella percutanea CBS 868.95T
AJ279445 Galactomyces geotrichum T
SFC102330
(D)
SFC102274
KC848298 Trametes hirsuta Dai12319T
KJ668473 Porostereum spadiceum KUC2013051505T
SFC102267
KR673461 Stereum subtomentosum KA120409T
SFC102277
LN308959 Schizophyllum commune T
SFC102268
0.3

Figure 5. Maximum likelihood phylogenetic tree of fungal species from *A. clathratum* based on ITS sequences "^{T}" indicates the ex-type strains. Bootstrap scores of >50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. Order Diaporthales, Microscales, Sordariales, Trichosphaeriales and Xylariales (A), order Caponodiales, Eurotiales, and Hypocreales (B), order Pleosporales and Saccarotomycetales (C), and order Agaricales, Polyporales, and Russulales (D).

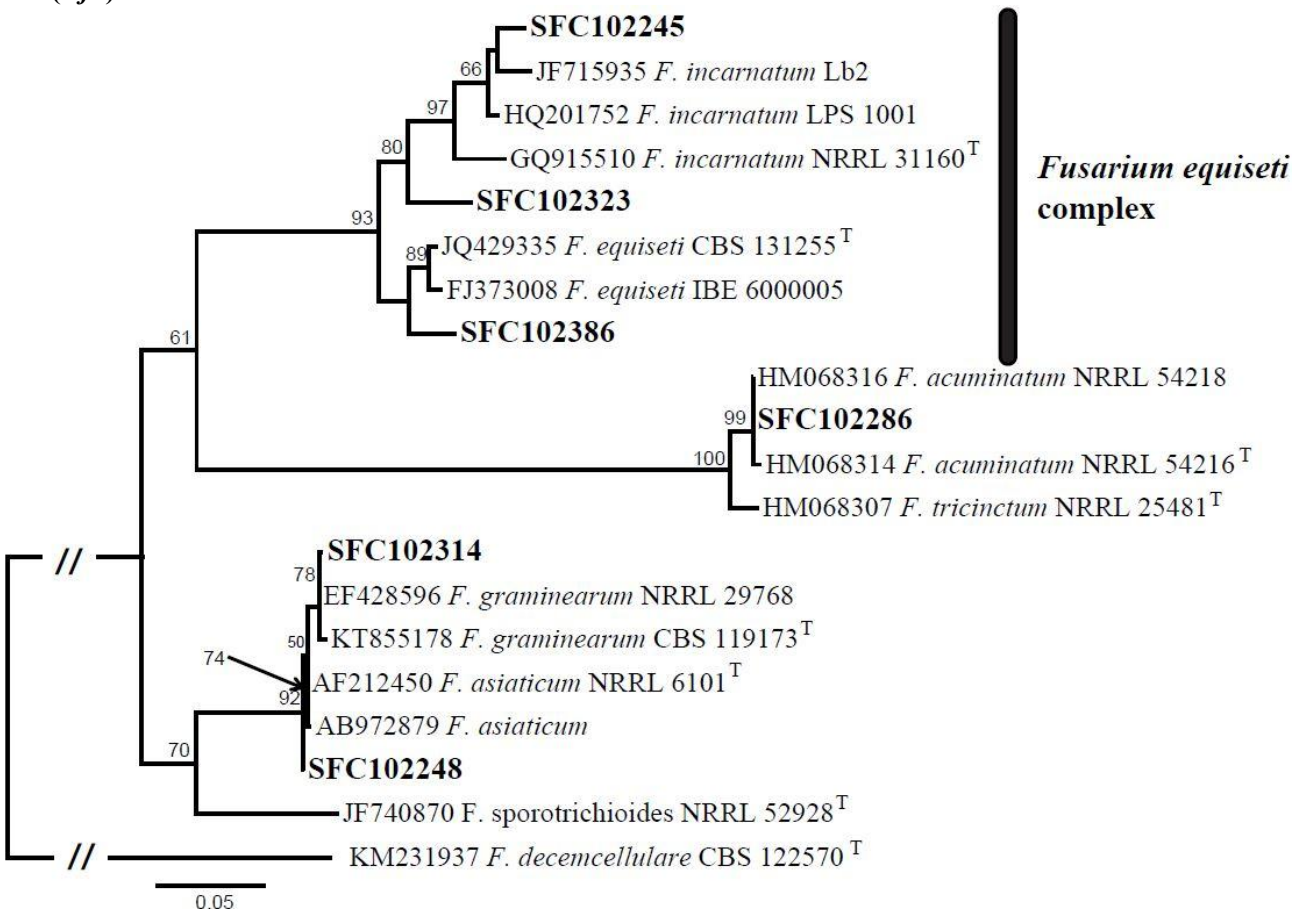
(A) *Aspergillus* (*CaM*)



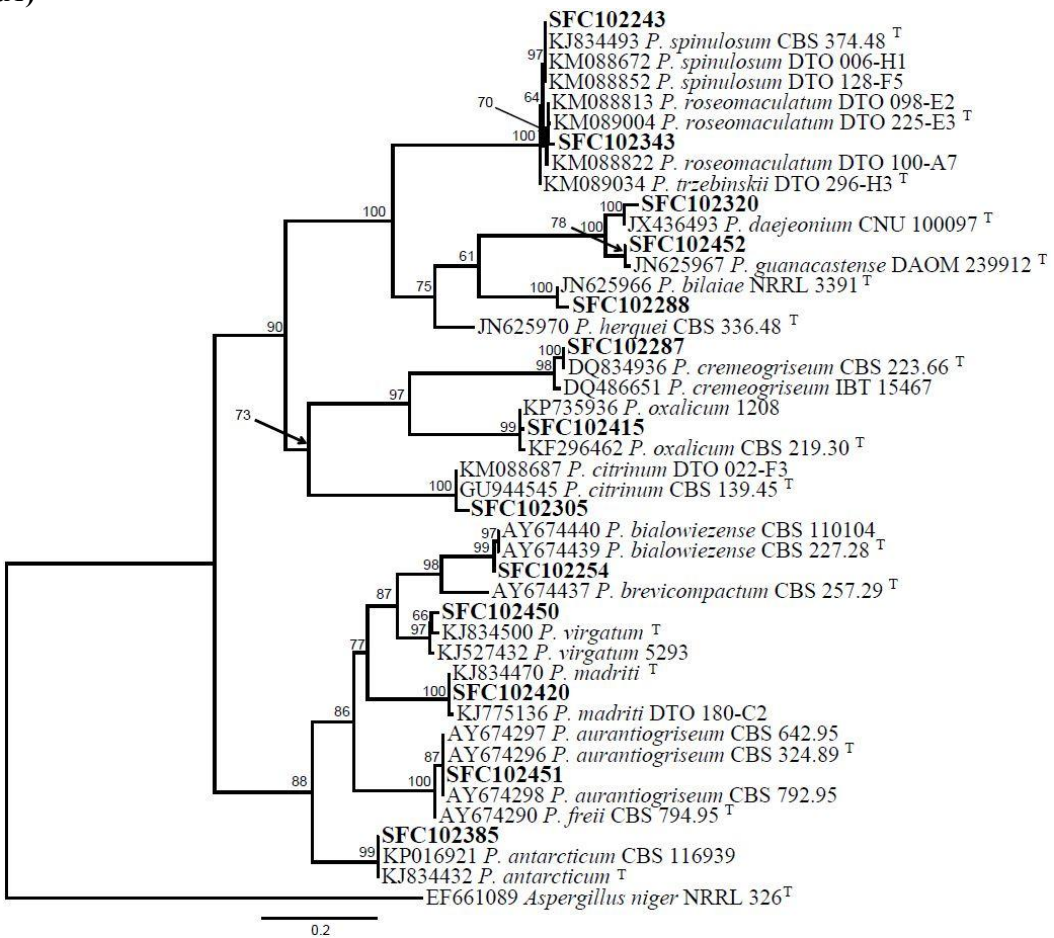
(B) *Cladosporium* (*act*)



(C) *Fusarium* (*tef1*)



(D) *Pencillium* (*benA*)



(E) *Trichoderma (tef1)*

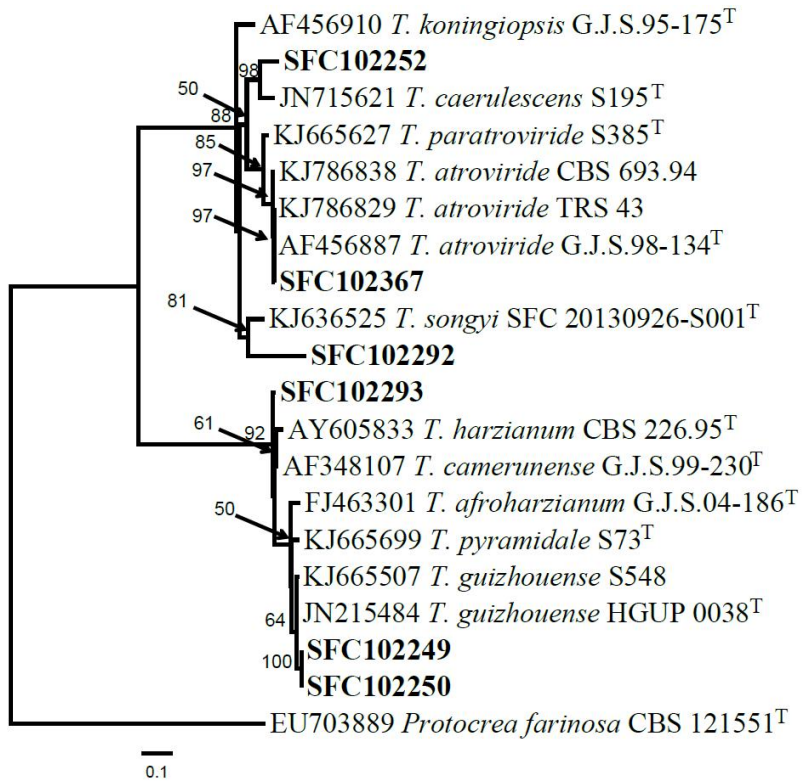


Figure 6. Maximum likelihood phylogenetic tree of *Aspergillus* (*CaM*) (A), *Cladosporium* (*act*) (B), *Fusarium* (*tef1*) (C), *Penicillium* (*benA*) (D), and *Trichoderma* (*tef1*) (E). "T" indicates the ex-type strains. Bootstrap scores of >50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site.

The isolated strains were categorized into two phyla, five classes, 14 orders, 42 genera, and 89 species. 97.4% of strains (229 strains) were Ascomycota and 2.6% of them (6 strains) were Basidiomycota. (Figure 5-6, Table 1). At order level, 30% were occupied to Hypocreales, followed by Pleosporales (21%), Eurotiales (18%), Microscales (12%), and Xylariales (7%). At genus level, over one third of strains belonged to genus *Acremonium* (15%), *Penicillium* (13%), or *Corollospora* (12%), followed by *Aspergillus* (5%), *Cladosporium* (5%), *Clonostachys* (5%), and *Trichoderma* (5%) (Figure 7-8). The most diverse genus was *Penicillium* which contained 13 species followed by *Aspergillus* (5 species) and *Cladosporium* (5 species). At species level, *Acremonium fuci* was shown as the most dominant species (34 strains) and found in most sites, followed by *Corollospora gracillis* (26 strains), *Clonostachys miodochialis* (11 strains), and *Penicillium citrinum* (8 strains) (Figure 7).

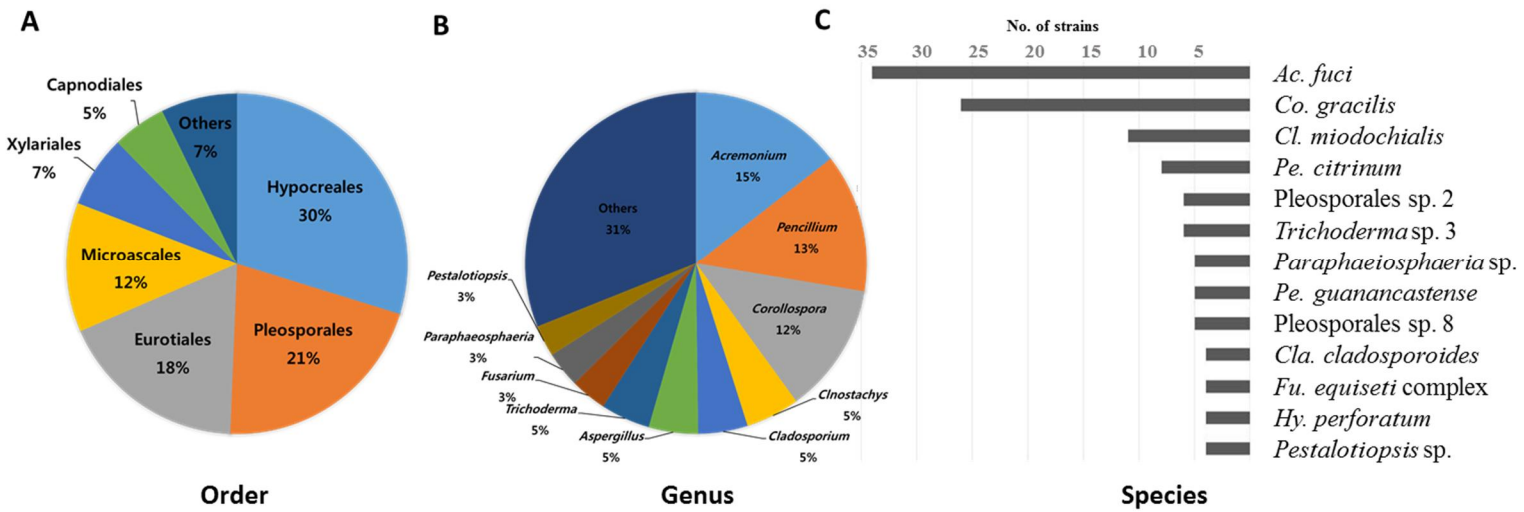


Figure 7. Composition of the dominant marine fungi isolated from *A. clathratum* at the order level (A), genus level (B), and species level (C).

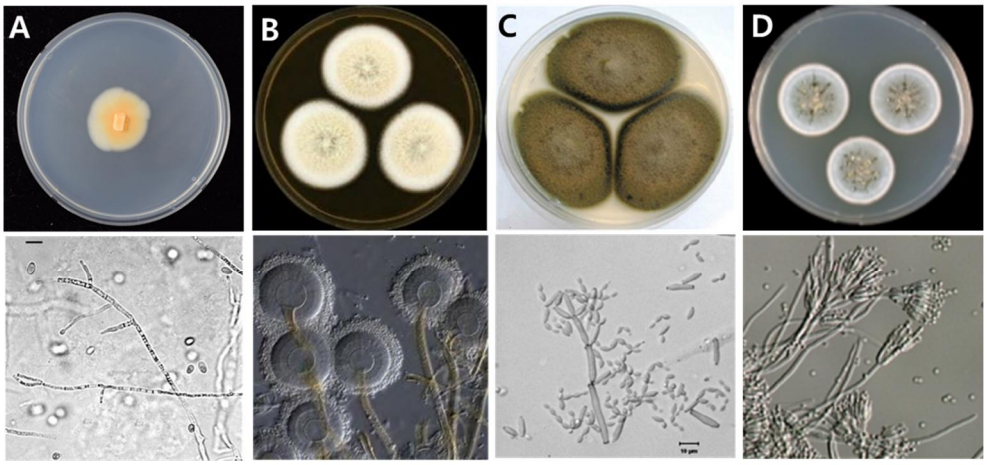


Figure 8. Macro- (top) and micro-morphology (bottom) of genus of fungi which were isolated abundantly. From left to right, *Acremonium* (A), *Aspergillus* (B), *Cladosporium* (C), and *Penicillium* (D).

3.2. Halo-tolerance and Enzyme activity

To investigate halo-tolerance of 89 species, each species was inoculated on PDA plates supplemented with ASW and without ASW and their growth were shown differently depending on salinity. Among 89 species, 49 species (55.1%) grew faster on PDA plates without ASW while 40 species (44.9%) exhibited faster growth rate under saline condition (Table 2). Species in Basidomycota such as *Porostereum. spadiceum* showed huge growth difference depending on saline condition compared to species in Ascomycota such as *Acremonium fuci* or *Penicillium. madriti* (Figure 9)

Table 2. Halo-tolerance of each fungal species. Each species was incubated at 25°C for 3-5 days on PDA plates supplemented with ASW and without ASW. Diameter of each colony was measured in mm. Boldic indicates species which were used for enzyme quantification

Collection No.	Species	With ASW	Without ASW
SFC102273	*Acremonium fuci*	20.7±1.2	14.3 ±0.6
SFC102380	*Alfaria terrestris*	33.7±1.2	33.7±1.2
SFC102269	*Alternaria broccoli-italicae*	44.3±1.5	40.0±4.0
SFC102318	*Arthrinium malaysianum*	27.7±0.6	26.3±0.6
SFC102355	*A. chevalieri*	34.0±2.6	24.7±1.5
SFC102407	*A. costaricaensis*	24.3±0.6	31.3±2.1
SFC102289	*A. fumigatus*	35.7±0.6	43.3±1.5
SFC102359	*A. insulicola*	14.7±1.2	16.7±1.5
SFC102419	*A. terreus*	50.0±0	42.7±1.5
SFC102281	*A. welwitschiae*	28.0±2.6	18.7±1.5
SFC102362	*Astromyces cruciatus*	35.7±3.2	47.7±1.5
SFC102361	*Chaetomium globosum*	39.3±0.6	33.0±1.0
SFC102453	*Chloridium* sp.	6.3±1.5	14.3±0.6
SFC102433	*Cladosporium cladosporioides*	18.3±0.6	2.02±0
SFC102263	*C. grevilleae*	18.7±1.2	18.0±1.0
SFC102255	*C. perangustum*	22.0±1.0	22.3±1.5
SFC102395	*C. rectoides*	14.0±1.0	16.3±1.2
SFC102352	*C. sphaerospermum*	42.0±2.0	49.3±0.6
SFC102394	*Clonostachys miodochialis*	25.7±1.2	25.3±1.5
SFC102311	*C. rosea*	29.0±6.1	18.3±1.5
SFC102291	*Coniella quercicola*	40.7±1.2	49.3±0.6
SFC102400	*Corollospora gracilis*	50.0±0	37.0±1.7
SFC102442	*C. maritima*	17.3±0.6	21.0±1.7
SFC102459	*Corollospora* sp.	9.3±0.6	10.3±1.5
SFC102272	*Diaporthe* sp.	50.0±0	49.0±1.0

(Table 2 continued)

Collection No.	Species	With ASW	Without ASW
SFC102458	*Didymella bellidis*	32.7±0.6	39.3±1.5
SFC102377	*D. pomorum*	16.3±2.1	20.3±2.3
SFC102337	*Didymella* sp.	11.0±0	11.3±0.6
SFC102399	*Discosia artocreas*	21.3±1.2	10.3±1.5
SFC102353	*Epicoccum sorghinum*	35.3±1.5	16.7±0.6
SFC102301	*Epicoccum* sp.	40.0±1.7	21.7±0.6
SFC102238	*Eutypella scoparia*	18.0±1.0	29.7±3.1
SFC102386	*Fusairum equiseti* complex	17.0±0	21.7±1.2
SFC102286	*F. acuminatum*	22.7±2.3	16.7±0.6
SFC102314	*F. graminearum*	21.3±0.6	24.3±0.6
SFC102248	*Fusarium* sp.	27.3±1.5	14.7±0.6
SFC102330	*Galactomyces* sp	18.0±0	20.7±1.2
SFC102393	Hypocreales sp.	17.3±1.2	20.3±1.5
SFC102443	*Hypoxylon perforatum*	13.3±1.2	11.7±0.6
SFC102275	*Lophiostoma* sp.	13.7±1.5	13.0±0
SFC102294	*Myrmecridium schulzeri*	2.3±0.6	18.3±2.9
SFC102316	*Neopestalotiopsis clavispora*	39.3±3.2	40.7±2.3
SFC102383	*Nigrospora oryzae*	42.3±2.5	25.0±2.6
SFC102348	*Paraconiothyrium fuckelii*	25.0±1.0	16.3±0.6
SFC102409	*Paradendryphiella arenariae*	46.0±1.7	37.0±1.0
SFC102461	*Paraphaeosphaeria* sp.	10.7±1.2	20.7±0.6
SFC102388	*P. sporulosa*	21.7±0.6	20.3±1.5
SFC102385	*Penicillium. antarcticum*	50.0±0	44.0±1.0
SFC102451	*P. aurantiogriseum*	21.7±1.5	16.3±0.6
SFC102254	*P. bialowiezense*	18.7±0.6	17.3±1.5
SFC102288	*P. bilaiae*	21.3±0.6	24.3±0.6
SFC102305	*P. citrinum*	39.7±1.5	24.0±1.7
SFC102287	*P. cremeogriseum*	21.0±1.0	18.0±0
SFC102320	*P. daejeonium*	48.7±1.2	49.0±1.7
SFC102452	*P. guanacastense*	29.3±2.3	29.0±1.0
SFC102420	*P. madriti*	24.0±1.0	26.7±0.6

(Table 2 continued)

Collection No.	Species	With ASW	Without ASW
SFC102415	*P. oxalicum*	46.7±0.6	37.7±2.5
SFC102343	*P. roseomaculatum*	19.3±0.6	24.0±1.7
SFC102243	*P. spinolusum*	33.7±0.6	3.03±1.0
SFC102450	*P. virgatum*	21.7±0.6	14.3±0.6
SFC102471	*Pestalotiopsis lespedezae*	44.3±0.6	50.0±0.0
SFC102282	*Pestalotiopsis* sp.	48.7±1.2	36.0±1.0
SFC102465	*Phaeosphaeria oryzae*	23.7±1.2	20.3±1.5
SFC102382	Pleosporales sp. 1	44.7±1.5	34.0±1.7
SFC102360	Pleosporales sp. 2	19.7±0.6	20.3±0.6
SFC102369	Pleosporales sp. 3	28.7±0.6	36.0±1.0
SFC102333	Pleosporales sp. 4	26.3±0.6	18.7±0.6
SFC102334	Pleosporales sp. 5	16.7±0.6	19.7±0.6
SFC102342	Pleosporales sp. 6	40.3±2.1	32.3±0.6
SFC102306	Pleosporales sp. 7	14.3±0.6	23.7±1.2
SFC102397	Pleosporales sp. 8	15.7±1.2	19.7±1.2
SFC102457	Pleosporales sp. 9	28.3±1.2	33.0±3.0
SFC102267	Pleosporales sp. 10	10.7±0.6	20.3±0.6
SFC102329	*Porostereum spadiceum*	15.7±0.6	44.3±0.6
SFC102268	*Roussoella* sp.	39.3±0.6	47.0±1.0
SFC102315	*Schizophylum commune*	26.3±1.2	36.0±2.6
SFC102335	*Septoriella hubertusii*	40.7±1.5	44.7±0.6
SFC102276	*Sesquicillium microsporum*	23.0±1.0	30.7±1.2
SFC102417	*Stagonosporopsis cucurbitacearum*	45.3±0.6	50.3±0.6
SFC102277	*Stemphylium solani*	41.3±0.6	38.7±1.2
SFC102341	*Stereum* sp.	38.0±0	49.3±0.6
SFC102274	*Teichospora* sp.	12.3±1.2	15.7±1.2
SFC102367	*Trametes hirsuta*	12.0±1.7	33.0±2.6
SFC102249	*Trichoderma atroviride*	13.0±0	16.0±1.7
SFC102306	*T. guizhouense*	32.0±0	50.3±0.6
SFC102293	*Trichoderma* sp. 1	32.7±3.2	50.7±0.6

(Table 2 continued)

Collection No.	Species	With ASW	Without ASW
SFC102292	*Trichoderma* sp. 2	10.0±1.0	42.0±2.6
SFC102252	*Trichoderma* sp. 3	39.3±1.5	51.3±0.6
SFC102299	*Zymoseptoria verkleyi*	21.0±1.0	20.7±1.5

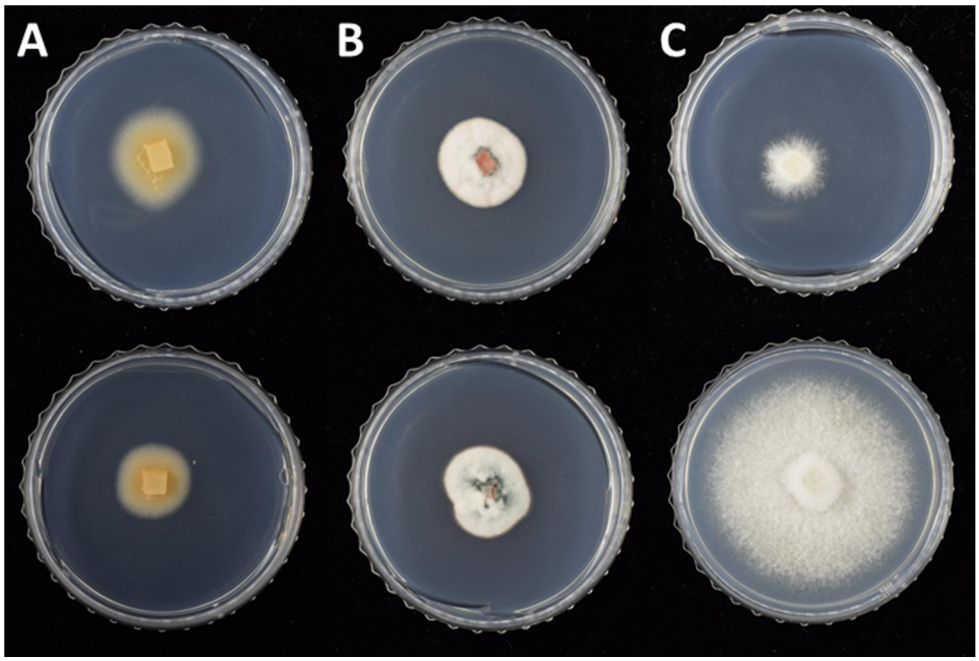


Figure 9. Colony on PDA plates supplemented with ASW (Top) and without ASW (Bottom). From left to right, *Acremonium fuci* (A), *Penicillium madriti* (B), and *Porostereum spadiceum* (C).

For screening of cellulase (β-glucosidase and endoglucanase) and alginase, 89 representative strains were chosen from each species and they were inoculated on plate containing different carbon substrate for plate screening assays. Among them, 57 species showed enzyme activity of either cellulase or alginase (Table 1, Figure 10).

42 species of isolated fungal species showed β-glucosidase activity and especially, three species (*Alfaria terrestris,* Pleosporales sp. 5, and *Penicillium madriti*) were top 3 species showing the highest β -glucosidase activity. On the other hand, 44 species had endoglucanase and in particular, *Acremonium fuci, Alfaria terrestris* and *Penicillium madriti* were top 3 species showing showed the highest activity for endoglucanase. 52 species had alginase activity and only *Alfaria terrestris, Hypoxylon perforatum,* and Pleosporales sp. 5 showed higher activity than other species (Table. 3). Three species showing the highest enzyme activity were selected for enzyme quantification, and total five species were selected including overlapped species (*Acremonium fuci, Alfaria terrestris, Hypoxylon perforatum, Penicillum madriti,* and Pleosporales sp. 5) (Figure 11).

All strains in these species were screened additionally to confirm variation of enzyme activity between strains except Pleosporales sp. 5 which had only a strain. The difference of activity was shown slightly between each strain, but all strains showed enzyme activities. Particularly, strain SFC102273 from *Acremonium fuci,* SFC102380 from *Alfaria terrestris*, SFC102443 from *Hypoxylon perforatum*, SFC102420 from *Penicillium madriti,* and SFC102342 from Pleosporales sp. 5 exhibited the highest enzyme activity (Table 2-3).

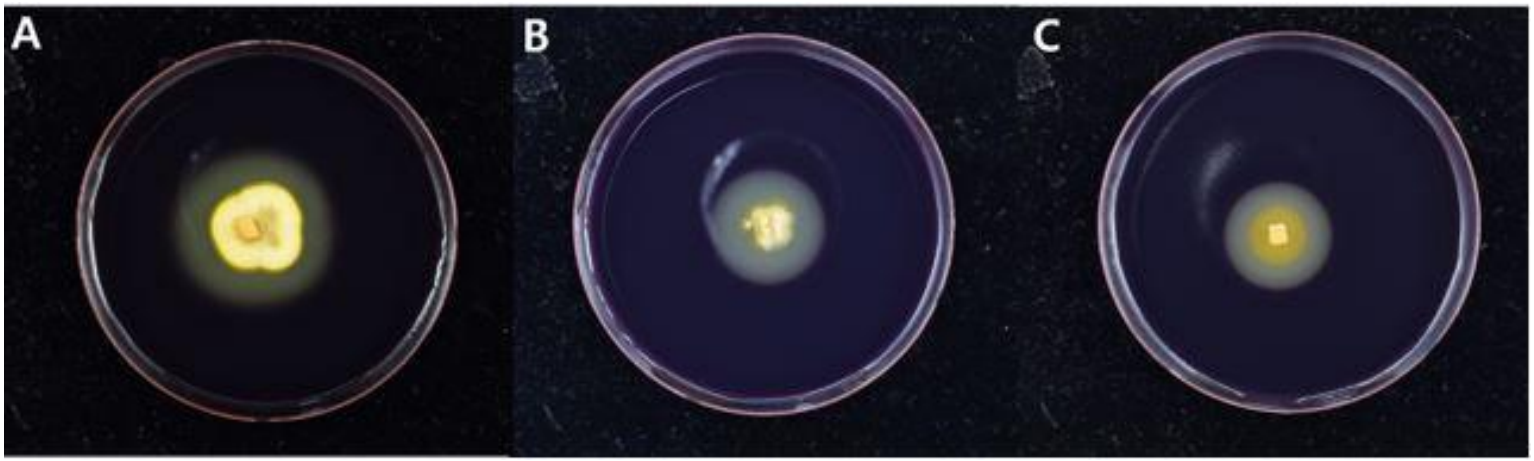


Figure 10. Plate screening assays of *Alfaria terrestris* (SFC102380) using Gram's Iodine method. From left to right, alginase (A), β-glucosidase (B), and endoglucanase

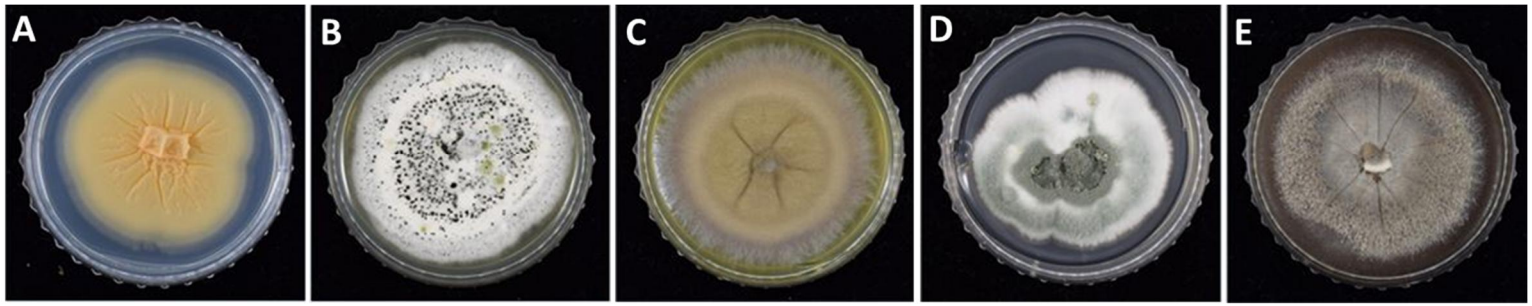


Figure 11. Colony of five selected fungal species showing higher enzyme activity. From left to right, *Acremonium fuci* (A), *Alfaria terrestris* (B), *Hypoxylon perforatum* (C), *Penicillium madriti* (D), and Pleosporales sp. 5 (E).

Table 3. Strain variation of *Acremonum fuci, Alfaria terrestris, Hypoxylon perforatum,* and *Penicillium madriti* based on plate assays. Boldic indicates a strain which exhibited the highest enzyme activity

Species	Collection No.	Alginase	β-glucosidase	Endoglucanase
Acremonium fuci	SFC102462	2	1.5	4
	SFC102468	5.5	2.9	9
	SFC102262	3	1	6
	SFC102264	7	3	8
	SFC102271	4.5	2.5	6
	SFC102273	8	6.5	9
	SFC102279	5	2	7.5
	SFC102280	4	2	5.5
	SFC102285	3	2.5	5.5
	SFC102290	5	2.5	7
	SFC102309	5	2	4
	SFC102317	4.5	2	6
	SFC102319	8	5	7.5
	SFC102322	8	3.5	8
	SFC102328	5	2	5.5
	SFC102336	4	1	6
	SFC102340	5	5	7
	SFC102344	6	5	7
	SFC102347	3	1	6
	SFC102351	4	2	6
	SFC102354	4.5	2.5	6
	SFC102356	3	1	7.5
	SFC102357	5	2.5	7.5
	SFC102358	5	2	6.5
	SFC102364	3	2	6
	SFC102366	5	2.5	6.5
	SFC102373	5.5	2	7.5

(Table 3 continued)

Species	Collection No.	Alginase	β-glucosidase	Endoglucanase
	SFC102378	6	3	8
	SFC102381	4	2	6.5
	SFC102384	4	2	5
	SFC102391	8	3	7
	SFC102401	3	2	4
	SFC102402	7.5	3	7
	SFC102441	4	2	5.5
Alfaria terrestris	SFC102374	5	5.5	5
	SFC102376	6	9	5
	SFC102380	10	13.5	9.5
Hypoxylon perforatum	SFC102456	8	8	7.5
	SFC102296	6.5	6	5
	SFC102443	9	9	8.5
	SFC102449	6.5	9	7.5
Penicillium madriti	SFC102416	2.5	8	6
	SFC102420	4.5	13.5	12.5

To investigate the specific fungal alginase and cellulase activity, enzyme quantification was performed using modified DNS methods. When single carbon substrate was given, *Hypoxylon perforatum* has the best β-glucosidase followed by *Penicillium madriti, Alfaria terrestris,* and *Acremonium fuci*. In endoglucanase activity, *Penicillium madiriti* had the highest activity followed by *Alfaria terrestris, Acremonium fuci* and *Hypoxylon perforautm.* Pleosporales sp. 5 relatively low activity compared to others while it showed high enzyme activity on plate screening. For alginase, *Penicillium madriti* and *Alfaria terrestris* exhibited higher activity than other species (Table 4).

When *A. clathratum* was given as carbon source, the enzyme activity was shown differently. All species showed much higher endoglucanase activity than β-glucosidase and *Penicillium madriti* exhibited the highest enzyme activity for all enzymes (Table 5).

Table 4. The enzyme quantification comparison of selected species by measuring produced reducing sugar when single substrate was given, respectively.

Collection No.	Species	Produced reducing sugar (mg/ml)		
		Alginase	**β-glucosidase**	**Endoglucanase**
SFC102273	*Acremonium fuci*	N/A	0.280	0.017
SFC102380	*Alfaria terrestris*	0.022	0.314	0.027
SFC102443	*Hypoxylon perforatum*	0.015	0.536	0.011
SFC102420	*Penicillium madriti*	0.032	0.406	0.031
SFC102342	Pleosporales sp. 5	0.003	0.132	0.010
FU42*	*Penicillium crysogenum*	0.004	0.234	0.017

'*' indicates positive control which showed good fungal enzyme activity in previous study (Lee et al., 2015)

Table 5. The enzyme quantification comparison of selected species when *A. clathratum* was given.

Collection No.	Species	Enzyme activity (U/ml)			
		β-glucosidase	**β-xylosidase**	**Cellobiohydrolase**	**Endoglucanase**
SFC102273	*Acremonium fuci*	0.046	0.022	0.008	0.158
SFC102380	*Alfaria terrestris*	0.096	0.021	0.008	0.232
SFC102443	*Hypoxylon perforatum*	0.068	0.019	0.009	0.269
SFC102420	*Penicillium madriti*	0.229	0.024	0.022	0.532
SFC102342	Pleosporales sp. 5	0.057	0.024	0.008	0.454
FU42*	*Penicillium crysogenum*	0.107	0.025	0.009	0.158

'*' indicates positive control which showed good fungal enzyme activity in previous study (Lee et al., 2015)

4. Discussion

Macroalgae are the second largest group for marine fungi, numerous fungal species have been reported, such as *Aspergillus, Cladosporium,* and *Penicillium* (Bugni and Ireland, 2004; Jones and Pang, 2012). Up to date, studies about fungal diversity of macroalgae have been conducted in worldwide. These algae associated fungi participate in degrading organic substrates as decomposers and they are thought to be potential bioresources due to their various bioactive compounds and enzymes (Bugni and Ireland 2004; Godinho et al., 2013; Flewelling et al., 2013; Furbino et al., 2014; Bonugli-santos et al., 2015). There was only a study about fungal diversity and enzyme activity from *A. clathratum* and it focused on only *Penicillium* species (Park et al., 2017).

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

In this study, a total 235 strains were isolated from 15 sites along eastern coast of Korea and they were identified to 89 species. Although isolation was conducted from single substrate, *A. clathratum,* various fungal species were detected at species level because of improving their phylogenetic resolution by both ITS and additional DNA markers. In this study, most of them accounted for phylum Ascomycota whereas few species in Basidiomycota and this result corresponded to previous

study which showed most marine fungi belonged to Ascomycota (Jones and Pang 2012). Pleosporales are known as one of the most dominant group from marine environment, on the other hand, species in Hypocreales are not common in habitants, except Bionectriaceae where *Acremonium* belongs (Jones and Pang, 2012). In this study, species in Hypocreales were found abundantly which contained *Acremonium fuci* mostly. In Pleosporales, there were several unidentified species and they remained at order level (Table 1, Figure 6). For this species, it could not be identified at species level accurately. In general, species in this order have similar morphology features and enough database for this order are not established, therefore many species remained as being unidentified (Jones et al., 2009; Suetrong et al., 2009).

The abundant genera showed several different patterns. In particular, two major patterns were shown; one is occupied diverse species, and the other contains single species. *Aspergillus, Penicillium* and *Trichoderma* accounted for the first case, and they were commonly found not only in terrestrial environments, but also in marine environments such as macroalgae, coral, and sea sands (Jones and Pang, 2012; Jones et al., 2015). In general, their diversity has been reported much higher from brown algae than either red or green algae due to different components ratio (Suryanarayanan 2010; Popper et al., 2011; Jones and Pang, 2012; Furbino et al., 2014; Raghukumar, 2017). Especially, *Penicillium* diversity from macroalgae including *A. clathratum* was reported in previous studies (Park et al., 2016; Park et al., 2017).

The most abundant species were *Acremonium fuci* and *Corollospora gracilis* which are known as marine obligate fungi (Figure 7). In particular, *Acremonium fuci* is found within marine-derived clade and commonly isolated brown seaweed. Zuccaro et al. (2004) explained that the reason is because this species can do conidial germination when macroalgae exists only. Although *Acremonium fuci* is one of the most representative marine fungi, their ecological role in marine environment is still far from clear (Zuccaro et al., 2004). *Corollospora* species are commonly found from sand grains, shell fragments, or algal thalli as obligate marine fungi (Kohlmeyer, 1973; Hsieh et al., 2007; Zuccaro et al., 2008), and species in this genus are known for producing antibacterial metabolites which is called as corollosporine (Ohzeki and Mori, 2001). Especially, *Corollospora gracilis* was studied about its modified ascomycetous pores which can prevent from flow of seawater into centrum to adapt aquatic habitat (Hsieh et al., 2007).

4.2. Halo-tolerance and fungal enzyme activity

The tolerances of species isolated from *A. clathratum* were shown differently depending on species (Table 2). According to Jones (2000), salinity is one of the main factors which influence fungal growth and it is shown quite differently in terrestrial fungi. In this study, two different growth patterns were shown for salinity; similar growth regardless salinity or better growth without salinity In general,

marine fungi generally have less growth variation in a range of salinity while non-marine fungi exhibited repressed growth. (Jones and Jennings, 1964). We could confirm that most isolated species which were reported as marine fungi had similar growth rate regardless salinity while species which were reported as terrestrial fungi such as *Stereum* or *Porostereum* had significant different growth (Table 2). During sampling, *A. clathratum* samples were in decay and collected on sea sands, therefore, it is thought that both terrestrial and marine origin fungi coexisted and were isolated.

Macroalgae is composed large amount of polysaccharides, especially mainly cellulose and alginate (Mabeau and Fleurence, 1993). Because they are very stable polysaccharides, it is essential for microbial enzymes to convert to monomers by degrading in nutrient cycling of ecosystem (Bugni and Ireland, 2004; Jones and Pang, 2012). Marine fungi play major role of carbon nutrient cycle in marine environment by degrading cell walls and transporting nutrients using their depolymerizing enzymes (Jones and Pang, 2012; Richards et al., 2012; Arnosti et al., 2013; Raghukumar, 2017). Especially, species in Dothideomycetes which contains order Pleosporales are found commonly from macroalgae as symbionts or saprobes, and known for participating in degrading macroalgae (Suetrong, 2009).

Cellulase includes several sub-enzymes such as β-glucosidase and endoglucanase which can hydrolyze cellobiose and endoglucan, respectively and they are known for major cellulose degrading enzymes (Coughlan, 1991; Hyde et al., 1999). Species in Pleosporales, Eurotiales and Hypocreales have been reported for their capacity of degrading cellulose, especially species in *Aspergillus* and

Penicillium are known as superior fungi (Jones and Pang, 2012). Given single carbon substrate, both endoglucanase and β-glucosidase activity were higher than *P. crysogenum* which was reported as good cellulase activity, except Pleosporales sp. 5 (Table 4). It is thought that enzyme activity might be influenced by different culture methods in plate assay or quantification. On the other hand, when *A. clathratum* was given as substrate, endoglucanase activity was much higher than β-glucosidase activity and all species exhibited higher endoglucanase activity compared to endoglucanase activity of *P. crysogenum.* It is thought because secretion of cellulolytic enzymes are influenced by proportion of cellulose components, but also induced or repressed by other enzymes. Especially, β-glucosidase is known for inducing endoglucanase synthesis (Kubicek, 1987), so it might lead more endoglucanase secretion under *A. clathratum* was given compared to only single carbon source was given. These cellulolytic enzymes are commonly found in other fungi from different substrates such as sediments, and sponges, but their activity were shown quite different depending on their substrates (Burtseva et al., 2010). In particular, endoglucanase has been exhibited from diverse marine fungal species and they have displayed other cellulolytic enzyme such as cellobiohydrolase for utilization of cellulose (Hyde et al., 1999). Both β-glucosidase and endoglucanase are used as alternative method in paper and detergent industry which require cellulose hydrolysis, replacing chemical process (Dincer and Telefoncu, 2007; Tebeka et al., 2009). In this study, *Penicillium madriti* (SFC102420) which exhibited the highest cellulase activity and it is expected for potential candidate as bio-resources.

Alginase comprises two major groups; endotype and exotype alginase and this enzyme has moderate and low processivity and generally co-works with other lysases (Wong et al., 2000). Up to date, only species in *Asteromyces cruciatus, Corollospora intermedia,* and *Dendryphiella salina* are known for good alginase producers, but study for fungal alginase was less conducted compared to other fungal enzymes (Schaumann and Weide, 1990). In this study, alginase activity was shown less compared to either β-glucosidase or endoglucanase activity in all top 5 representative species, especially, *Penicillium* species had the least alginase activity (Table 1, 3). Especially, significant results could not be obtained from enzyme quantification comparing to control (Table 4-5). In alginate degradation process, alginate is degraded rapidly by endotype alginase first and then mono-uronic acids such as glucuronic acids are converted by exotype alginase through several steps (Schaumann and Weide, 1990). Because exotype alginase are produced very slowly, alginate decomposing is known as the last stage of seaweed degradation with microorganisms (Tang et al., 2008). In this study, fungal alginase activity was confirmed on plate assay, but it was not able to detect in quantification because very little amount of reducing sugar was produced. To quantify their alginase activity, new cultural method for inducing fungal alginase is necessary in further.

Through this study, we could not find significant relationship between fungal dominance and their enzyme activities. This discordance in marine environments between microbial abundance and biological activity was reported previously (Campbell, et al., 2011; Panno et al., 2013). In nature, the biological activities of fungi such as producing extracellular enzymes are influenced by several

factors including interactions with other microbial communities, available organic matters, and other environmental factors (Arnosti et al., 2014). Most isolates which showed enzyme activity belong to ubiquitous genera common to terrestrial environments and it may suggest that they are terrestrial origin. Due to unique condition of marine environments, it seems that facultative fungi should produce more metabolites and enzymes to adapt extreme environments rather than growing on their hosts (Holler et al., 2000; Panno et al., 2013).

5. Conclusions

Marine fungi have received attention in recent years, especially interests for fungi associated seaweed have risen. These fungi play important role in marine ecosystem as decomposers using their enzymes cellulase and alginase and these enzymes are considered as important method to solve faced environmental problem caused by seaweed waste. In this study, 89 fungal species were identified from 235 strains associated with macroalgae, *A. clathratum,* and confirmed fungal enzyme activity of approximate half of isolate fungi; alginase, β-glucosidase, and endoglucanase. Among selected species, *Penicillium madriti* exhibited the highest enzyme activity. This study is expected to provide basic information for further studies of fungi associated with *A. clathratum* and their enzymes for industrial applications by suggesting potential candidates.

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7. Abstract in Korean

구멍쇠 미역(*A. clathratum*) 에서 분리한 진균의 다양성과 효소활성

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초 록

구멍쇠미역은 갈조류의 한 종류로써 최근, 한국에서는 기후변화, 부영양화, 등으로 인한 급격한 개체수의 증가로 해안가로 떠밀려와 쌓이면서 새로운 환경문제의 원인으로써 대두되고 있다. 이러한 문제를 해결하기 위한 방법으로 구멍쇠미역을 분해할 수 있는 유용 균주를 발굴하기 위해, 본 연구에서는 구멍쇠미역으로부터 진균을 분리하여 그들의 다양성을 살펴보고, 구멍쇠미역의 주요성분인 알긴산과 셀룰로오즈를 분해할 수 있는 균의 효소활성을 조사하였다. 그 결과, 구멍쇠미역으로부터 총 235균주를 분리하였고, 균의 형태적인 특징과 함께 분자적인 방법에 기반하여 동정한 결과 총

91종의 균을 동정하였다. 89 종의 균 중 가장 우점을 하고 있는 속으로 *Acremonium, Penicillium,* 그리고, *Corollospora* 가 확인이 되었고, 그 중 *Acremonium fuci* 와 *Corollospora gracilis* 가 가장 우점을 하고 있는 종으로 나타났다. 균의 효소활성을 조사하기 위해 alginase, β-glucosidase, 그리고 endoglucanase을 Plate screening assay를 통해 확인하여, 각 효소별로 가장 활성이 좋았던 상위 세 균주를 선발 한 결과, *Acremonium fuci, Alfaria terrestris, Hypoxylon perforatum, Penicillium madriti,* 그리고 Pleosporales sp. 5 이 다섯 균주가 가장 활성이 좋은 것으로 확인되었다. 기존에 효소활성이 좋았던 것으로 보고되었던 균주와 함께 정량실험을 한 결과, 선발되었던 균주의 대부분이 기존에 보고가 되었던 균주보다 활성이 더 좋은 것으로 확인이 되었고, 특히 *Alfaria terrestris* 와 *Penicillium madriti* 가 효소활성이 월등하게 높은 것이 확인이 되었다. 이 같은 우리의 연구결과는 향후, 해초로 인한 환경문제를 해결할 수 있는 유용 진균의 정보를 제공할 것으로 기대되는 바이다.

주 요 어: 알기나아제 · 셀룰라아제 · 효소 · 해양균 · 해초

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