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이학석사 학위논문

**Plastic-Inhabiting Fungi in Marine  
Environments and PCL Degradation  
Analysis**

해양 플라스틱에서의 균류 다양성 분석 및  
PCL을 이용한 플라스틱 분해능력 조사

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# Plastic-Inhabiting Fungi in Marine Environments and PCL Degradation Analysis

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# **Plastic-Inhabiting Fungi in Marine Environments and PCL Degradation Analysis**

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## **Abstract**

Plastic waste has a negative impact on marine ecosystems and the quantity of this source of anthropogenic pollution continues to increase. Several studies have investigated microorganisms on plastics to alleviate the ecological damage induced by plastic wastes. However, current studies focused on either diversity research, or plastic degradation test using a few strains. In order to fully understand interaction among fungi in plastisphere and their degradation, the previous two analyses need to be performed simultaneously. In this study, fungi from polyethylene terephthalate (PET) waste on Korean seacoasts were isolated and their ability to degrade plastic was evaluated by comparing the diameters of the clear zones they formed on polycaprolactone (PCL) agar. 262 strains were isolated from 47 plastic waste sources and 108 fungal species were identified via molecular methods, majority of species being included in order Pleosporales, Eurotiales, and Cladosporiales. The PCL agar assay revealed that 87 species presented with varying degrees of PCL degradation capacity. Among them, certain fungal species were strong PCL degraders. The majority of species were revealed to be non/weak PCL degrading species. According to previous references, these weaker fungi might be either secondary

colonizers which feeds on primarily degraded polymer substances, or fungicolous fungi which acquire nutrition from other plastic-degrading fungi. In conclusion, the present study demonstrated the possibility that some fungi inhabiting plastic could potentially degrade it in the marine environment. It is expected that the discoveries made herein lay theoretical and practical foundations for the development of novel bioremediation systems for marine plastispheres and help mitigate the environmental pollution issues related to plastic wastes.

**Keyword:** fungal diversity; marine fungi; phylogenetic analysis; plastic degradation; polycaprolactone

**Student Number:** 2021-25203

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# **1. Introduction**

## **1.1. Background**

Plastic products are extensively and widely used, and the plastic polymer compositions vary depending on the intended applications of the products they are used to fabricate. Common plastics include high-density polyethylene (HDPE), low-density polyethylene (LDPE), and polyethylene terephthalate (PET) (Plastics Europe, 2021). As of 2020, approximately 367 million tonnes of plastic products were produced. The plastic production volume has substantially increased since the 1990s (Plastics Europe, 2021). However, only about 9% of all plastic waste is recycled, and as much as 60% of it is buried in a landfill or discarded as litter in terrestrial environments (Geyer et al., 2017). Most marine plastic waste consists of improperly disposed terrestrial plastic waste that has entered the oceans via rivers, wastewater outflows, wind, and tides. In 2010, 4–12 million tonnes of marine plastic waste originated from land (Jambeck et al., 2015). Additionally, about 1.15–2.41 million tonnes of marine plastic waste originated from river (Lebreton et al., 2017).

The accumulation of plastic wastes has had a direct and indirect negative impact on marine ecosystems. Plastic debris are ingested by marine animals and damage their internal organs (Ahrendt et al., 2020; Wright et al., 2013). Furthermore, wave action, weathering, and other processes break down plastic pieces into microplastics which are the plastic wastes that were degraded into smaller fragments and fibers (Thompson et al., 2004), causing other more hazards. Microplastics float on ocean surfaces and contain

Persistent Organic Pollutants (POPs), which are endocrine disruptors that hinder the survival rate of marine organisms (Rios et al., 2007). Microplastics also transport microbial pathogens and alien species, hazardous to marine ecosystems in other regions (Arias-Andres et al., 2018; Beloe et al., 2022; Bowley et al., 2021). Thus, plastic in marine ecosystems has become a serious environmental issue. However, there are no policies or solutions in place that effectively mitigate the plastic waste problem. Recent studies have explored chemical degradation, recycling, and biodegradation as potential marine plastic waste remediation measures.

Much research attention has been directed toward plastic waste degradation by microorganisms. Plastic waste has persisted in natural environments for decades. Plastic debris that is inhabited and partially decomposed by the microbial community is now referred to as the “plastisphere” (Zettler et al., 2013) and numerous different microorganisms are found (Hirota et al., 2021; Amaral-Zettler et al., 2020). Some of them were reported to degrade plastic by various kinds of tests (Badahit et al., 2018; Sangeetha Devi et al., 2019; Hou et al., 2022; Kumari et al., 2019; Muonja et al., 2018; Yamada-Onodera et al., 2001); and enzymatic activities involved in plastic degradation have been investigated extensively (Temporiti et al., 2022). Nevertheless, prior research has focused mainly on plastic-decomposing bacteria. Fungi comprise only about 3% of all eukaryotic organisms in the plastisphere, although they play a vital role as decomposers in the environment (Rogers et al., 2020). Numerous plastic-degrading fungi have been detected and identified in the landfill (terrestrial) plastisphere including *Aspergillus* spp. (Cosgrove et al., 2007; Muonja et al., 2018; Zahra et al., 2010), *Fusarium* spp. (Kanelli et al., 2015; Zahra et al., 2010), and *Penicillium simplissimum*

(Yamada-Onodera et al., 2001). Previous studies on plastic-degrading fungi in marine environments concentrated primarily on several specific taxa such as *Aspergillus* sp. (Sarkhel et al., 2019) and *Zalerion maritimum* (Paço et al., 2017).

Previously, my lab team isolated different fungi from various substrates in marine environments such as sailfin eggs (Park et al., 2018) and microalgae (Lee et al., 2019; Park et al., 2016). Many of these fungi had high enzymatic activity (Lee et al., 2019; Park et al., 2015a; Park et al., 2019). Since these studies detected the fungal ability to degrade complex organic matter, it was expected that plastic-isolated fungi could decompose plastic substrates. A metabarcoding analysis revealed that a wide array of fungi survived on plastics collected from seawater (Lacerda et al., 2020, Davidov et al., 2020) and the sea floor (De Tender et al., 2017).

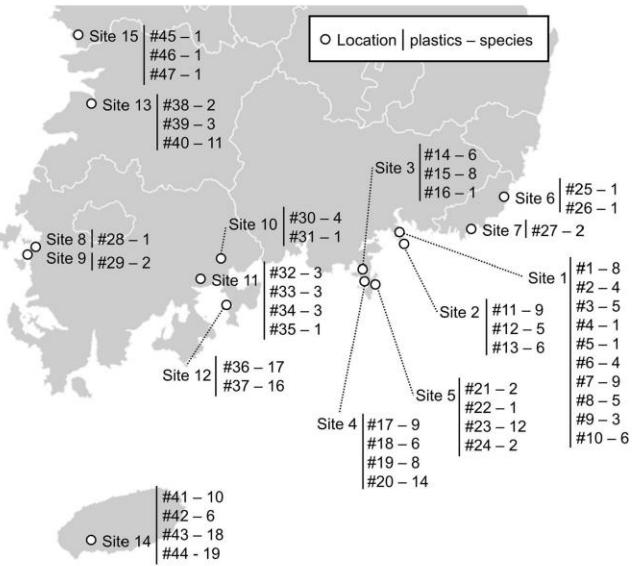
## **1.2. Objective**

The objective of this study is to both analyze the fungal diversity in marine plastisphere, and test their plastic degradation abilities, providing background information for future degradation research, of which diversity data would be important for understanding fungal interaction and degradation dynamics. Hypothesis was established that different fungi can inhabit plastic waste and most of them actively participate in plastic degradation. In the present study, therefore, fungal diversity in PET waste collected from seacoasts was investigated and a polycaprolactone (PCL) degradation assay was used to evaluate their capacity to degrade plastic. PCL is a biopolymer that has been extensively used in biodegradation research as a surrogate for non-degradable polymers. Its usage in fungal incubation varies from film/sheet form (Benedict et al., 1983; Fukushima et al., 2010), or agar from as emulsified substance (Lee et al., 2021).

## **2. Materials and Methods**

### **2.1. Sampling locations and sampling strategy**

Forty-seven PET wastes such as PET bottles and PET cups were collected from 15 sites along the western and southern sea coast of the Republic of Korea in April, 2018 (Figure 1). PET bottles and PET cups with intact shape were collected to prevent wrong sample collection. The PET surfaces were cleansed of debris by washing with artificial seawater (ASW; purified from seawater in South Korea, salinity = 3.5%). Each PET waste sample was cut with sterilized scissors into 27 pieces each 1 cm<sup>2</sup> in area. To isolate the fungi, nine pieces per sample were placed in dichloran rose bengal chloramphenicol agar (DRBC; Difco, Sparks, MD, USA), glycerol yeast extract agar (GYA; 1 g L<sup>-1</sup> glucose, 0.1 g L<sup>-1</sup> yeast extract, 0.5 g L<sup>-1</sup> peptone, and 15 g L<sup>-1</sup> agar), and potato dextrose agar (PDA; Difco, Sparks, MD, USA) supplemented with ASW. The plates were incubated at 25 °C for 7–14 d. Pure fungal colonies were then transferred to new PDA + ASW plates. Pure fungal strains were stored in 20% (v/v) glycerol at –80°C and deposited in the Seoul National University Fungus Collection (SFC).

**A****B**

**Figure 1. Data for plastic waste and fungi collected along Korean seacoasts (A). Data for plastic waste collected from mudflats and sand (B).**

**Table 1. Collected PET wastes and information of their sampling location.**

Site	Mud/ Sand	Location	Coordinates (Latitude, Longitude)	Number of PET wastes
Site 1	Sand	Gwanpo-ri, Jangmok-myeon, Geoje-si, Gyeongsangnam-do	34.9904, 128.6950	10
Site 2	Mud	Jangmok-ri, Jangmok-myeon, Geoje-si, Gyeongsangnam-do	34.9916, 128.6810	3
Site 3	Mud	Dongdal-ri, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do	34.8701, 128.4551	3
Site 4	Sand	Wonpyeong-ri, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do	34.9005, 128.4561	4
Site 5	Mud	Jangpyeong-ri, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do	34.8808, 128.4689	4
Site 6	Sand	Samsung-ri, Ilgwang-myeon, Gijang-gun, Busan	35.2632, 129.2353	2
Site 7	Mud	Dadae-dong, Saha-gu, Busan	35.0473, 128.9721	1
Site 8	Mud	Masan-ri, Hyeongyeong-myeon, Muan-gun, Jeollanam-do	35.0654, 126.3423	1
Site 9	Mud	Songjeong-ri, Hyeongyeong-myeon, Muan-gun, Jeollanam-do	35.0317, 126.3906	1
Site 10	Mud	Jangyang-ri, Beolgyo-eup, Boseong-gun, Jeollanam-do	34.8364, 127.3694	2
Site 11	Mud	Masan-ri, Byeollyang-myeon, Suncheon-si, Jeollanam-do	34.8372, 127.4476	4
Site 12	Mud	Boksan-ri, Sora-myeon, Yeosu-si, Jeollanam-do	34.7735, 127.5725	2
Site 13	Mud	Jinseo-ri, Jinseo-myeon, Buan-gun, Jeollabuk-do	35.5950, 126.6033	3
Site 14	Sand	Jungmun-dong, Seogwipo-si, Jeju-do	33.2427, 126.4188	4
Site 15	Mud	Songseok-ri, Maseo-myeon, Seocheon-gun, Chungcheongnam-do	36.0816, 126.6250	3

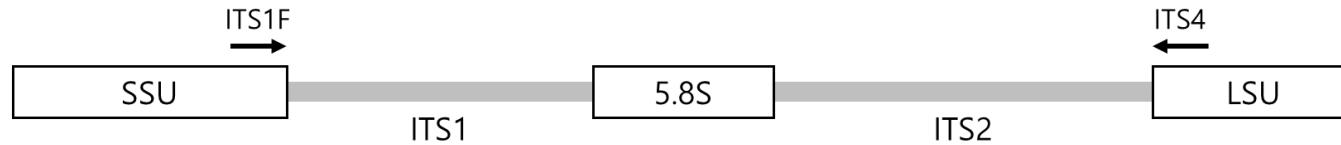
## **2.2. Molecular identification**

The fungal isolates on PDA were grouped according to their morphological characteristics such as texture, color, colony size, and sporulation. At least one strain was selected from each group for molecular identification. Genomic DNA was extracted by a modified cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich, 1994). PCR amplification of the internal transcribed spacer (ITS) region was performed using ITS1F/ITS4 primers (Gardes and Bruns, 1993; White et al., 1990) and AccuPower PCR Master Premix (Bioneer Co., Daejeon, Republic of Korea). All representative strains were identified down to the genus level based on their ITS sequences. The strains within certain genera were identified to the species level using various protein-coding gene analyses and different primer sets. Actin (*act*) was amplified using ACT-512F/ACT-783R (Carbone and Kohn, 1999) or ACT1Fd/ACT1Rd (Aveskamp et al., 2009; Groenewald et al., 2013) whilst  $\beta$ -tubulin (*Beta*) was amplified using Bt2a/Bt2b (Glass and Donaldson, 1995). PCR was performed in a C1000 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) under previously described conditions (Park et al., 2015b). PCR amplicons were checked with 1% agarose gel and purified with an Expi™ PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea) according to the manufacturer's instructions. DNA was sequenced at Macrogen (Seoul, Republic of Korea) in an ABI PRISM 3700 Genetic Analyzer (Life Technologies, Gaithersburg, MD, USA).

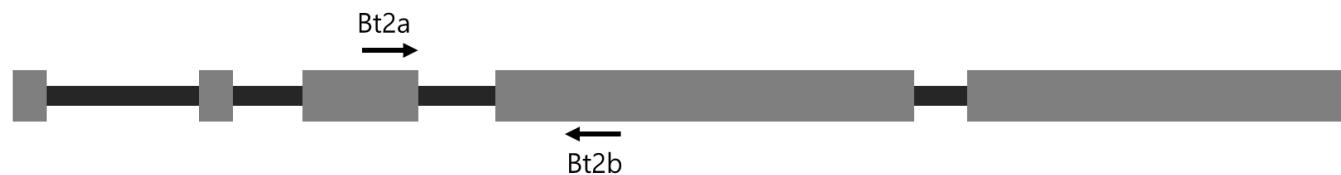
All sequences were proofread and edited with MEGA7 (Kumar et al., 2016) and deposited in GenBank (Supplementary Table 2). For the phylogenetic analysis, the type sequences of the reference species were retrieved from GenBank (Supplementary Table

3) and aligned with the sample sequences for each locus with MAFFT v. 7 (Katoh and Standley, 2013) using the default parameters. Maximum likelihood phylogenetic analyses were performed on each gene (ITS, *act*, *BenA*) using RAxML (Stamatakis, 2006), the GTRGAMMA evolution model, and 1,000 bootstrap replicates.

### Internal Transcriber Space (ITS)



### Beta-tubulin (*Bt**A*)



### Actin (*act*)



Figure 2. Schematic diagram of fungal genes and the location of primers used for PCR.

### **2.3. PCL agar assay**

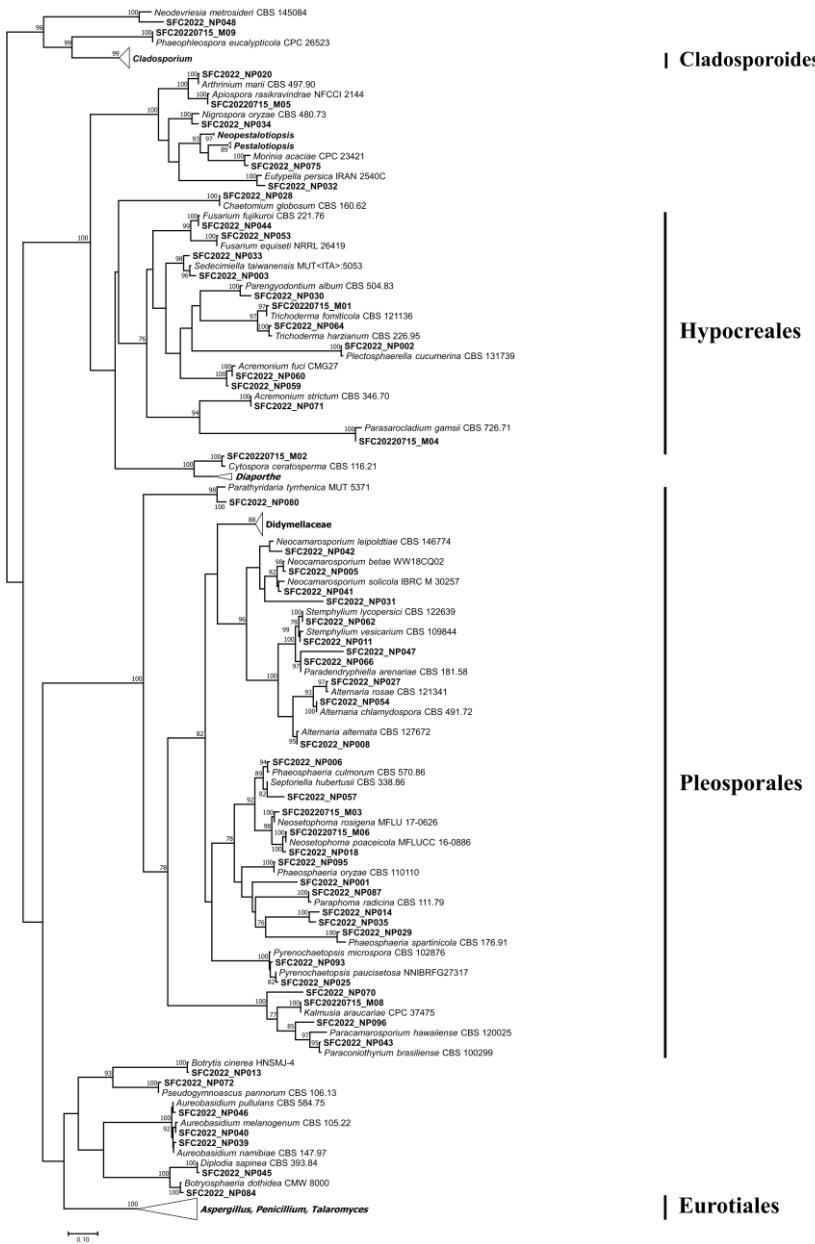
The PET-degrading ability was determined for one to nine representative strains of each fungal species (Table 1) by measuring the clear zones formed in polycaprolactone (PCL) media. The PCL agar was prepared according to a previously reported method (Lee et al., 2021) and consisted of a 1% (w/v) emulsified PCL suspension (pellet form, 3 mm in diameter; Sigma-Aldrich., St. Louis, MO, USA) in acetone plus distilled water (10% of acetone volume). The PCL suspension was added to an autoclaved medium comprising a 0.8% (w/v) yeast nitrogen base (Difco-Becton Dickinson, Broken Bow, NE, USA), 1.5% (w/v) agar, and distilled water, then poured into 90 mm-Petri dishes.

Representative strains of each fungal species identified were inoculated with a 4-mm hole punch at the center of each agar plate. Clear zone formation was evaluated by measuring the distance between the margin of the clear (transparent) zone and that of the colony after 7 d incubation at 25°C. All clear zones were measured in triplicate and averaged. PCL degradation by each species was determined from the averages of the clear zone lengths of all representative strains of the same species. For species with multiple tested strains, the standard deviations of the average clear zone lengths of all strains within the same species were also calculated.

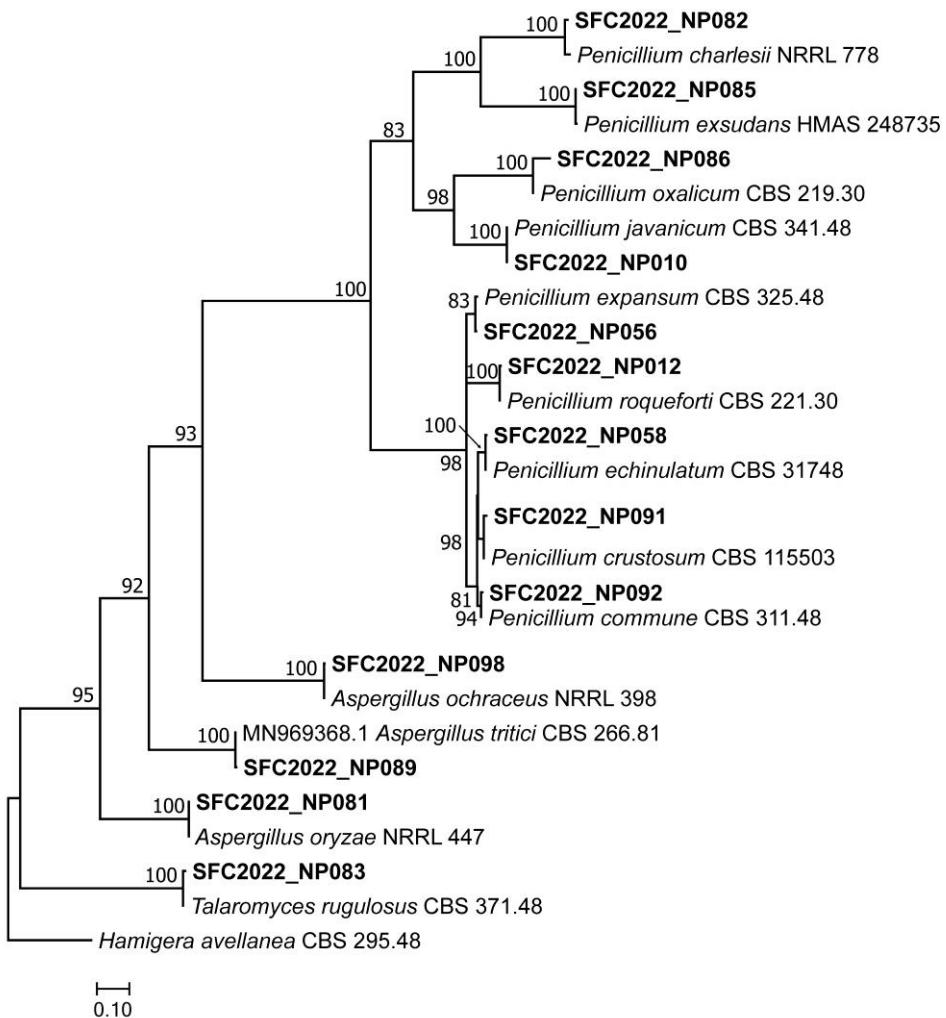
### **3. Results**

#### **3.1. Identification results**

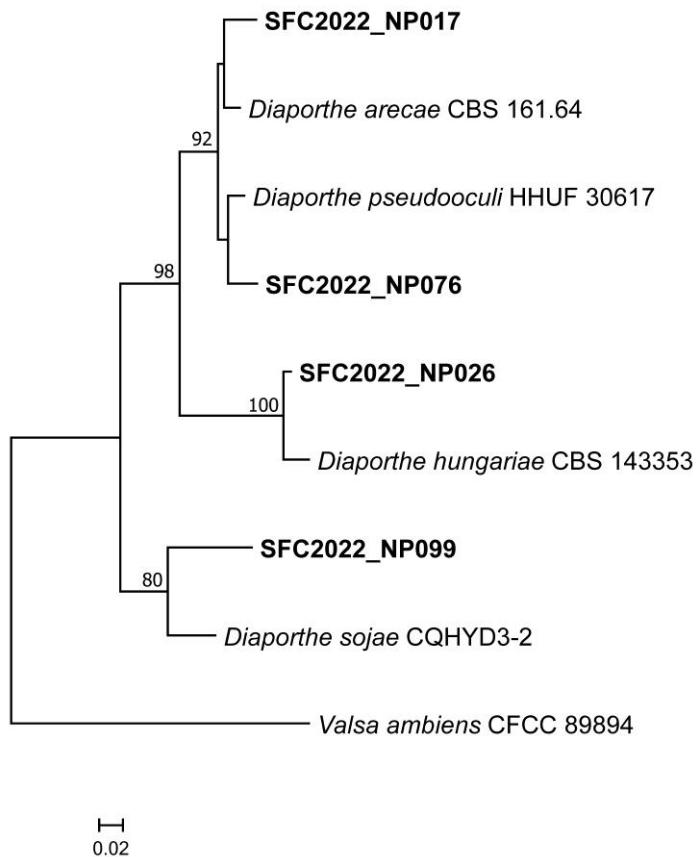
A total of 262 fungal strains were isolated from 47 PET wastes. Multiple strains of the same species derived from a single PET waste were treated as a single strain. One to nineteen fungal strains were isolated per PET. Depending on the isolation medium used (DRBC, GYA, or PDA), different numbers of fungal strains were isolated from the same PET waste (Figure 1, Supplementary Table S1). All fungal strains were grouped into 108 taxa based on their morphological features and ITS sequencing results (Figure 2). Forty-seven taxa were identified to the species level based on their protein-coding genes. The actin gene was used to identify *Cladosporium* species whilst the β-tubulin gene was used to identify *Aspergillus*, *Diaporthe*, *Didymella*, *Epicoccum*, *Juxtaphoma*, *Neodidymeliopsis*, *Nothophoma*, *Penicillium*, *Pestalotiopsis*, *Remotididymella*, and *Talaromyces* species (Table 1, Supplementary Figure S1). Based on the ITS sequences alone, 47 taxa were confirmed to the species level whilst 14 others were identified to the genus, family, and order levels.



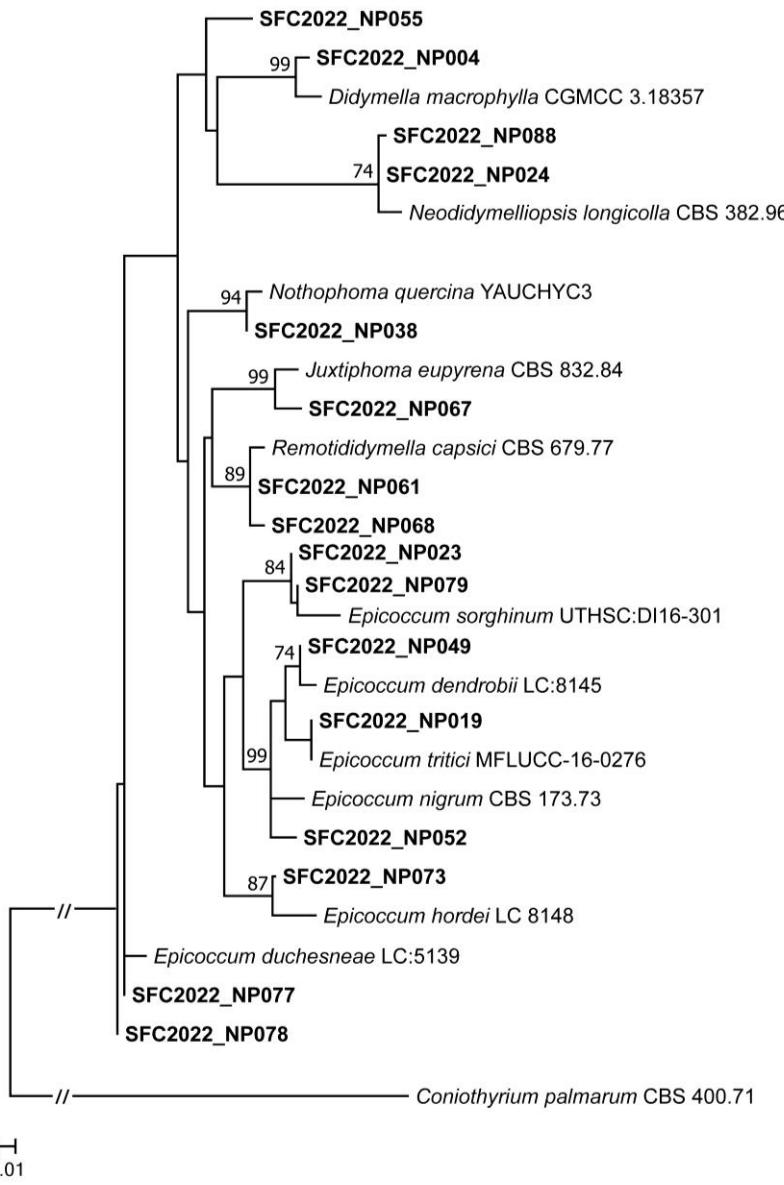
**Figure 3. Phylogenetic tree of fungi isolated from marine plastic waste based on ML analysis of ITS.** Bootstrap scores > 70 are presented at nodes. Scale bar indicates the number of nucleotide substitutions per site. Representative strains of each taxon based on ITS sequences are shown in bold font.



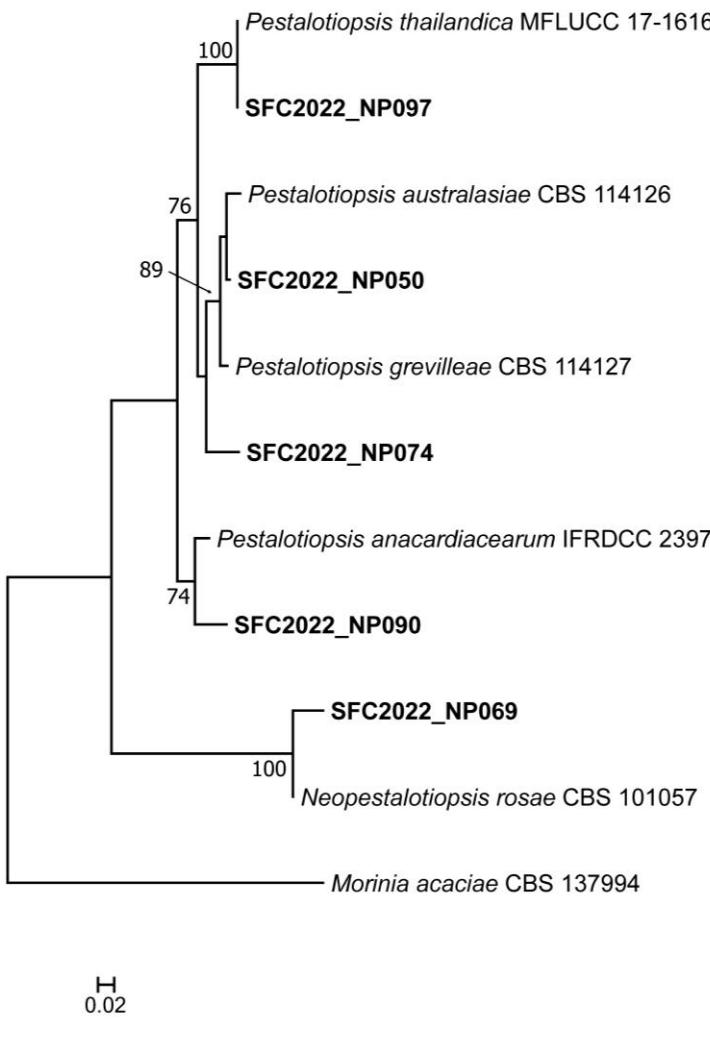
**Figure 4. Phylogenetic tree of order Eurotiales isolated from marine plastic waste based on ML analysis of *BenA*.** Bootstrap scores > 70 are presented at nodes. Scale bar indicates the number of nucleotide substitutions per site. Representative strains of each taxon are shown in bold font.



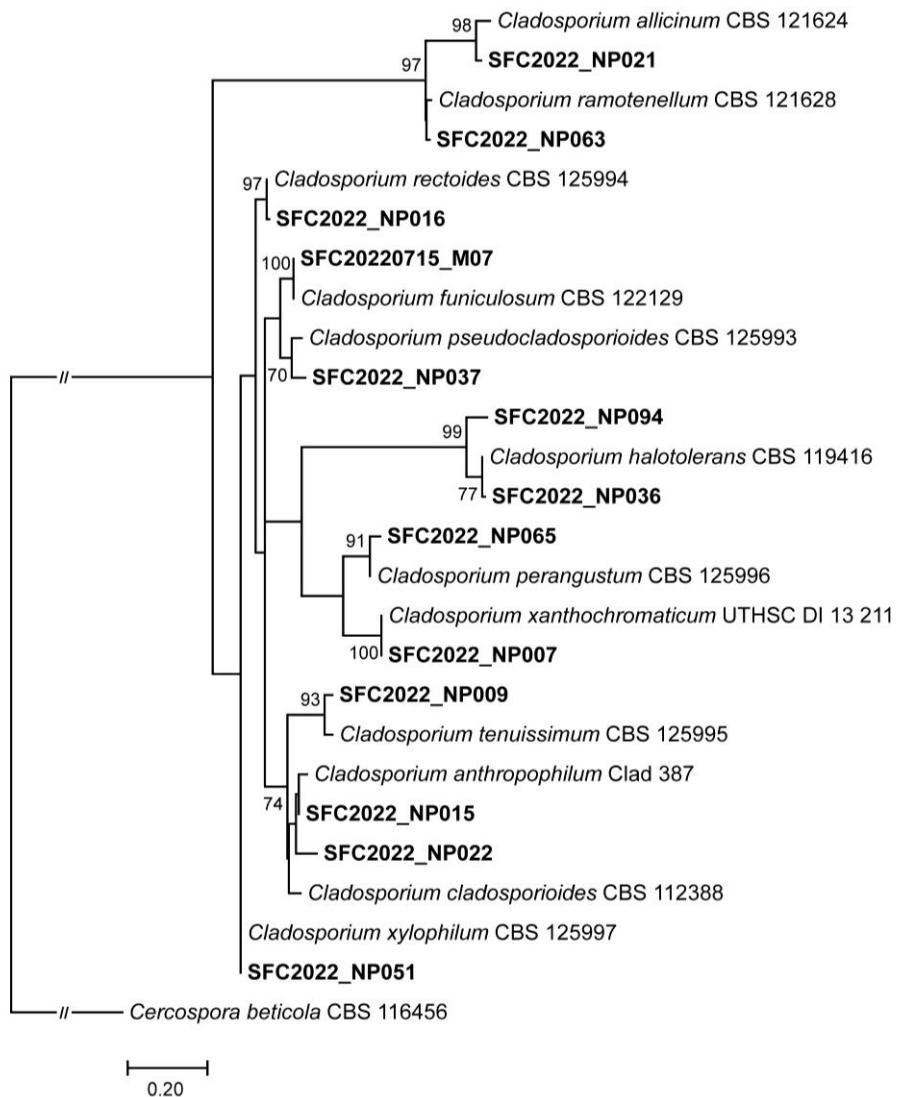
**Figure 5. Phylogenetic tree of genus *Diaporthe* isolated from marine plastic waste based on ML analysis of *BenA*.** Bootstrap scores > 70 are presented at nodes. Scale bar indicates the number of nucleotide substitutions per site. Representative strains of each taxon are shown in bold font.



**Figure 6. Phylogenetic tree of family Didymellaceae isolated from marine plastic waste based on ML analysis of BenA.** Bootstrap scores > 70 are presented at nodes. Scale bar indicates the number of nucleotide substitutions per site. Representative strains of each taxon are shown in bold font.



**Figure 7. Phylogenetic tree of genus *Pestalotiopsis* and *Neopestalotiopsis* isolated from marine plastic waste based on ML analysis of *BenA*.** Bootstrap scores > 70 are presented at nodes. Scale bar indicates the number of nucleotide substitutions per site. Representative strains of each taxon are shown in bold font.



**Figure 8. Phylogenetic tree of genus *Cladosporium* isolated from marine plastic waste based on ML analysis of *act*.** Bootstrap scores > 70 are presented at nodes. Scale bar indicates the number of nucleotide substitutions per site. Representative strains of each taxon are shown in bold font.

**Table 2. Collected PET wastes and isolated fungal species.** Representative strains and cultures whence fungal species were isolated are also indicated.

Site	PET code	Species name	Culture
Site 1	1	<i>Acremonium cf. fuci</i>	PDA
		<i>Acremonium fuci</i>	PDA
		<i>Alternaria alternata</i>	GYA
		<i>Cladosporium ramotenellum</i>	GYA/PDA
		<i>Cladosporium tenuissimum</i>	DRBC
		<i>Cladosporium xylophilum</i>	PDA
		<i>Neodidymelopsis cf. longicolla</i>	DRBC
		<i>Paradendryphiella arenariae</i>	DRBC
	2	<i>Acremonium cf. fuci</i>	GYA
		<i>Acremonium fuci</i>	GYA
2	3	<i>Cladosporium anthropophilum</i>	GYA
		<i>Cladosporium rectoides</i>	GYA
		<i>Alternaria alternata</i>	GYA/PDA
		<i>Alternaria chlamydospora</i>	PDA
	3	<i>Cladosporium ramotenellum</i>	PDA
		<i>Paradendryphiella arenariae</i>	GYA
		<i>Stemphylium vesicarium</i>	GYA
		<i>Pleosporales sp. 1</i>	GYA
		<i>Pleosporaceae sp. 2</i>	DRBC
		<i>Alternaria alternata</i>	GYA
3	4	<i>Cladosporium ramotenellum</i>	GYA
		<i>Cladosporium tenuissimum</i>	GYA
		<i>Pleosporales sp. 1</i>	PDA
		<i>Alternaria alternata</i>	DRBC/GYA/PDA
		<i>Cladosporium pseudocladosporioides</i>	PDA
		<i>Cladosporium ramotenellum</i>	GYA/PDA
		<i>Diaporthe cf. hungariae</i>	PDA
		<i>Epicoccum dendrobii</i>	PDA
4	5	<i>Epicoccum sp.</i>	PDA
		<i>Parasarocladium cf. gamsii</i>	GYA
		<i>Pestalotiopsis cf. australasiae</i>	DRBC
		<i>Septoriella cf. hubertusii</i>	GYA/PDA
		<i>Alternaria alternata</i>	PDA
		<i>Cladosporium anthropophilum</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA
		<i>Pleosporales sp. 1</i>	PDA
5	6	<i>Pleosporales sp. 2</i>	PDA
		<i>Alternaria alternata</i>	GYA/PDA
		<i>Cladosporium ramotenellum</i>	PDA
	7	<i>Pleosporales sp. 1</i>	PDA
		<i>Cladosporium ramotenellum</i>	GYA/PDA
6	8	<i>Cladosporium ramotenellum</i>	PDA
		<i>Pleosporales sp. 1</i>	PDA
	9	<i>Cladosporium ramotenellum</i>	PDA
		<i>Pleosporales sp. 1</i>	PDA
7	10	<i>Cladosporium ramotenellum</i>	GYA/PDA

**Table 2.** Continued.

<b>Site</b>	<b>PET code</b>	<b>Species name</b>	<b>Culture</b>
Site 1	10	<i>Cladosporium xylophilum</i>	PDA
		<i>Fusarium equiseti</i>	DRBC/GYA
		<i>Neocamarosporium betae</i>	PDA
		<i>Paradendryphiella arenariae</i>	DRBC/PDA
		Pleosporales sp. 1	PDA
Site 2	11	<i>Acremonium fuci</i>	GYA
		<i>Alternaria alternata</i>	GYA/PDA
		<i>Apiospora rasikravindrae</i>	PDA
		<i>Aureobasidium pullulans</i>	GYA
		<i>Cladosporium anthropophilum</i>	PDA
		<i>Cladosporium funiculosum</i>	GYA
		<i>Cladosporium ramotenellum</i>	GYA/PDA
		<i>Cladosporium tenuissimum</i>	PDA
		Didymosphaeriaceae sp. 2	GYA
		<i>Cladosporium ramotenellum</i>	DRBC
Site 3	12	<i>Cladosporium rectoides</i>	DRBC
		<i>Cladosporium xylophilum</i>	PDA
		<i>Diaporthe cf. hungariae</i>	DRBC
		<i>Neodidymelopsis cf. longicolla</i>	DRBC
		<i>Acremonium cf. fuci</i>	GYA
		<i>Acremonium fuci</i>	GYA
		<i>Cladosporium ramotenellum</i>	GYA
		<i>Diaporthe cf. hungariae</i>	DRBC/PDA
		<i>Paraconiothyrium brasiliense</i>	DRBC
		<i>Paradendryphiella arenariae</i>	DRBC/GYA
Site 4	14	<i>Acremonium cf. fuci</i>	GYA
		<i>Cladosporium ramotenellum</i>	GYA/PDA
		<i>Cladosporium xanthochromaticum</i>	GYA
		<i>Epicoccum dendrobii</i>	PDA
		<i>Paradendryphiella arenariae</i>	PDA
		<i>Parasarocladium cf. gamsii</i>	PDA
		<i>Acremonium cf. fuci</i>	GYA
		<i>Alternaria alternata</i>	DRBC
		<i>Cladosporium anthropophilum</i>	PDA
		<i>Fusarium equiseti</i>	DRBC
Site 4	15	<i>Neodidymelopsis longicolla</i>	GYA
		<i>Paradendryphiella arenariae</i>	DRBC/GYA
		<i>Remotididymella cf. capsici</i>	DRBC/PDA
		<i>Sphaeropsis sapinea</i>	GYA
		<i>Paradendryphiella arenariae</i>	GYA
Site 4	17	<i>Acremonium cf. fuci</i>	PDA
		<i>Acremonium fuci</i>	GYA

**Table 2.** Continued.

<b>Site</b>	<b>PET code</b>	<b>Species name</b>	<b>Culture</b>
Site 4	17	<i>Alternaria alternata</i>	GYA
		<i>Cladosporium ramotenellum</i>	GYA/PDA
		<i>Epicoccum duchesneae</i>	PDA
		<i>Neodevriesia cf. metrosideri</i>	PDA
		<i>Neodidymellopsis cf. longicolla</i>	GYA
		<i>Paradendryphiella arenariae</i>	DRBC/GYA/PDA
		Pleosporales sp. 1	GYA
		<i>Alternaria alternata</i>	GYA
		<i>Cladosporium allicinum</i>	GYA
		<i>Cladosporium anthropophilum</i>	GYA
18	18	<i>Diaporthe cf. arecae</i>	GYA
		<i>Fusarium equiseti</i>	GYA
		<i>Paradendryphiella arenariae</i>	GYA
		<i>Acremonium cf. fuci</i>	GYA
		<i>Alternaria alternata</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA
		<i>Epicoccum tritici</i>	GYA
		<i>Fusarium fujikuroi</i>	PDA
		<i>Neodidymellopsis cf. longicolla</i>	PDA
		<i>Paradendryphiella arenariae</i>	GYA/PDA
19	19	<i>Remotididymella cf. capsici</i>	PDA
		<i>Acremonium cf. fuci</i>	PDA
		<i>Acremonium fuci</i>	GYA
		<i>Aureobasidium pullulans</i>	DRBC
		<i>Cladosporium perangustum</i>	GYA
		<i>Cladosporium tenuissimum</i>	GYA
		<i>Cladosporium xylophilum</i>	PDA
		<i>Diaporthe cf. hungariae</i>	PDA
		<i>Fusarium equiseti</i>	PDA
		<i>Neosetophoma poaceicola</i>	GYA
20	20	<i>Paradendryphiella arenariae</i>	DRBC/GYA/PDA
		Pleosporales sp. 1	PDA
		<i>Remotididymella cf. capsici</i>	DRBC/PDA
		<i>Stemphylium lycopersici</i>	DRBC
		<i>Trichoderma harzianum</i>	DRBC
		<i>Alternaria chlamydospora</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA
		<i>Acremonium cf. fuci</i>	PDA
		<i>Alternaria alternata</i>	PDA
Site 5	21	<i>Alternaria chlamydospora</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA
22	22	<i>Acremonium cf. fuci</i>	PDA
		<i>Alternaria alternata</i>	PDA
		<i>Alternaria chlamydospora</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA
23	23	<i>Cladosporium ramotenellum</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA
		<i>Cladosporium ramotenellum</i>	PDA

**Table 2.** Continued.

<b>Site</b>	<b>PET code</b>	<b>Species name</b>	<b>Culture</b>
Site 5	23	<i>Remotididymella</i> sp.	PDA
		<i>Juxtiphoma</i> cf. <i>eupyrena</i>	PDA
		<i>Neocamarosporium betae</i>	PDA
		<i>Penicillium commune</i>	PDA
		<i>Penicillium crustosum</i>	PDA
		<i>Penicillium echinulatum</i>	PDA
		<i>Penicillium expansum</i>	PDA
		Pleosporales sp. 1	PDA
		<i>Alternaria alternata</i>	GYA
		<i>Paradendryphiella arenariae</i>	GYA
Site 6	25	<i>Alternaria alternata</i>	PDA
	26	<i>Alternaria alternata</i>	GYA
Site 7	27	<i>Acremonium</i> cf. <i>fuci</i>	GYA
		<i>Paradendryphiella arenariae</i>	GYA
Site 8	28	<i>Neocamarosporium solicola</i>	DRBC
Site 9	29	<i>Chaetomium globosum</i>	GYA
		<i>Phaeosphaeria spartinicola</i>	GYA
		<i>Alternaria alternata</i>	DRBC
Site 10	30	Pleosporales sp. 1	DRBC/GYA
		Pleosporales sp. 2	GYA
		Pleosporales sp. 4	DRBC/GYA
	31	<i>Cladosporium halotolerans</i>	PDA
Site 11	32	<i>Alternaria alternata</i>	DRBC
		<i>Cladosporium anthropophilum</i>	DRBC
		<i>Nigrospora</i> cf. <i>oryzae</i>	GYA
		<i>Eutypella</i> cf. <i>persica</i>	PDA
	33	<i>Nigrospora</i> cf. <i>oryzae</i>	DRBC
		<i>Cytospora ceratosperma</i>	PDA
		<i>Plectosphaerella cucumerina</i>	GYA
		Pleosporales sp. 4	PDA
		<i>Sedecimiella taiwanensis</i>	DRBC
Site 12	34	<i>Trichoderma fomiticola</i>	GYA
		<i>Acremonium</i> cf. <i>fuci</i>	PDA
		<i>Alternaria alternata</i>	DRBC/GYA/PDA
		<i>Apiospora marii</i>	GYA
		<i>Botrytis cinerea</i>	PDA
	35	<i>Cladosporium allicinum</i>	GYA
		<i>Cladosporium anthropophilum</i>	DRBC/GYA
		<i>Cladosporium</i> cf. <i>cladosporioides</i>	GYA
		<i>Cladosporium pseudocladosporioides</i>	GYA
		<i>Cladosporium rectoides</i>	DRBC
		<i>Cladosporium tenuissimum</i>	PDA

**Table 2.** Continued.

<b>Site</b>	<b>PET code</b>	<b>Species name</b>	<b>Culture</b>
Site 12	36	<i>Diaporthe</i> cf. <i>hungariae</i>	DRBC
		<i>Didymella</i> cf. <i>macrophylla</i>	DRBC
		<i>Epicoccum tritici</i>	DRBC/PDA
		<i>Neosetophoma</i> cf. <i>poaceicola</i>	DRBC
		<i>Penicillium javanicum</i>	PDA
		<i>Penicillium roqueforti</i>	PDA
		<i>Pyrenophaetopsis paucisetosa</i>	DRBC
		<i>Stemphylium vesicarium</i>	PDA
		<i>Acremonium</i> cf. <i>fuci</i>	DRBC/GYA
		<i>Alternaria alternata</i>	DRBC
	37	<i>Cladosporium perangustum</i>	DRBC
		<i>Cladosporium rectoides</i>	DRBC
		<i>Cladosporium tenuissimum</i>	DRBC
		<i>Cladosporium xanthochromaticum</i>	DRBC
		<i>Diaporthe</i> cf. <i>arecae</i>	GYA
Site 13	38	<i>Epicoccum</i> cf. <i>sorghinum</i>	DRBC
		<i>Epicoccum sorghinum</i>	DRBC
		<i>Neocamarosporium betae</i>	DRBC/GYA/PDA
		<i>Neocamarosporium</i> sp.	DRBC
		<i>Neodidymelliopsis longicolla</i>	DRBC
		<i>Paradendryphiella arenariae</i>	DRBC/GYA
		<i>Phaeosphaeria culmorum</i>	PDA
		<i>Pleosporales</i> sp. 1	GYA
		<i>Hypocreales</i> sp.	DRBC
		<i>Parengyodontium album</i>	GYA
	39	<i>Alternaria alternata</i>	GYA
		<i>Alternaria</i> cf. <i>rosae</i>	GYA
		<i>Neocamarosporium</i> sp.	DRBC/GYA/PDA
		<i>Alternaria alternata</i>	PDA
		<i>Alternaria</i> cf. <i>rosae</i>	DRBC/PDA
Site 14	41	<i>Aureobasidium melanogenum</i>	DRBC/PDA
		<i>Aureobasidium namibiae</i>	DRBC
		<i>Cladosporium pseudocladosporioides</i>	DRBC
		<i>Cladosporium rectoides</i>	GYA
		<i>Neocamarosporium solicola</i>	DRBC
		<i>Neocamarosporium</i> sp.	GYA
		<i>Neodidymelliopsis</i> cf. <i>longicolla</i>	PDA
		<i>Neosetophoma rosigena</i>	DRBC/PDA
		<i>Parathyridaria</i> cf. <i>tyrrhenica</i>	GYA
		<i>Acremonium fuci</i>	DRBC
		<i>Aspergillus ochraceus</i>	GYA/PDA
		<i>Cladosporium tenuissimum</i>	GYA

**Table 2.** Continued.

Site	PET code	Species name	Culture
Site 14	41	<i>Diaporthe</i> cf. <i>pseudooculi</i>	GYA
		<i>Didymosphaeriaceae</i> sp. 1	DRBC
		<i>Epicoccum</i> cf. <i>duchesneae</i>	GYA
		<i>Parathyridaria</i> cf. <i>tyrrhenica</i>	GYA
		<i>Penicillium charlesii</i>	PDA
		<i>Penicillium crustosum</i>	DRBC
		<i>Phaeophleospora eucalypticola</i>	GYA
		<i>Cladosporium</i> cf. <i>halotolerans</i>	PDA
		<i>Epicoccum</i> cf. <i>duchesneae</i>	PDA
		<i>Morinia</i> cf. <i>acaciae</i>	GYA
	42	<i>Penicillium commune</i>	GYA/PDA
		<i>Pseudogymnoascus pannorum</i>	PDA
		<i>Pyrenophaetopsis microspora</i>	PDA
		<i>Acremonium fuci</i>	GYA
		<i>Diaporthe</i> cf. <i>arecae</i>	GYA/PDA
	43	<i>Diaporthe</i> cf. <i>sojae</i>	DRBC
		<i>Epicoccum</i> cf. <i>duchesneae</i>	PDA
		<i>Epicoccum duchesneae</i>	PDA
		<i>Epicoccum sorghinum</i>	DRBC
		<i>Kalmusia araucariae</i>	DRBC
		<i>Neodidymelliosis</i> cf. <i>longicolla</i>	DRBC
		<i>Neopestalotiopsis</i> sp.	DRBC
		<i>Paraphoma radicina</i>	DRBC
		<i>Penicillium charlesii</i>	DRBC
		<i>Penicillium commune</i>	GYA
		<i>Pestalotiopsis</i> sp.	DRBC
		<i>Phaeophleospora eucalypticola</i>	DRBC
		<i>Phaeosphaeria oryzae</i>	PDA
		<i>Pleosporales</i> sp. 1	PDA
44	44	<i>Pseudogymnoascus pannorum</i>	DRBC
		<i>Talaromyces rugulosus</i>	DRBC
		<i>Aspergillus oryzae</i>	GYA
		<i>Aspergillus tritici</i>	DRBC
		<i>Botryosphaeria dothidea</i>	GYA
		<i>Cladosporium</i> cf. <i>halotolerans</i>	DRBC
		<i>Epicoccum</i> cf. <i>hordei</i>	PDA
		<i>Epicoccum duchesneae</i>	GYA
		<i>Neopestalotiopsis</i> sp.	PDA
		<i>Penicillium commune</i>	DRBC/PDA
		<i>Penicillium crustosum</i>	DRBC/GYA/PDA
		<i>Penicillium exsudans</i>	PDA
		<i>Penicillium oxalicum</i>	DRBC

**Table 2.** Continued.

Site	PET code	Species name	Culture
Site 14	44	<i>Pestalotiopsis</i> cf. <i>anacardiacearum</i>	PDA
		<i>Pestalotiopsis</i> cf. <i>australasiae</i>	GYA/PDA
		<i>Pestalotiopsis</i> sp.	DRBC/PDA
		<i>Pestalotiopsis thailandica</i>	GYA
		Pleosporales sp. 1	GYA
		Pleosporales sp. 3	PDA
		<i>Pyrenophaetopsis microspora</i>	GYA
		<i>Sarocladium strictum</i>	DRBC/GYA
Site 15	45	<i>Nothophoma quercina</i>	PDA
	46	Pleosporaceae sp.	DRBC
	47	Pleosporales sp. 2	DRBC

### **3.2. Fungal composition**

All 108 species detected belonged to the Ascomycota and were classified into 15 orders and 46 genera (Table 1, Figure 2). Pleosporales was the dominant order and included 44 species. It was followed by Cladosporiales and Eurotiales with 13 species each (Figure 3A). Cladosporiales only included the genus *Cladosporium* whereas Eurotiales comprised the genera *Penicillium*, *Aspergillus*, and *Talaromyces*. The latter two included three and one species, respectively, and nine *Penicillium* species were identified (Figure 3B, Table 1). Eleven different species were isolated from at least five PET wastes (Figure 3C). *Alternaria alternata* was isolated from 21 different PET waste sources followed by *Cladosporium ramotenerellum* (16 PET wastes) and *Paradendryphiella arenariae* (14 PET wastes).

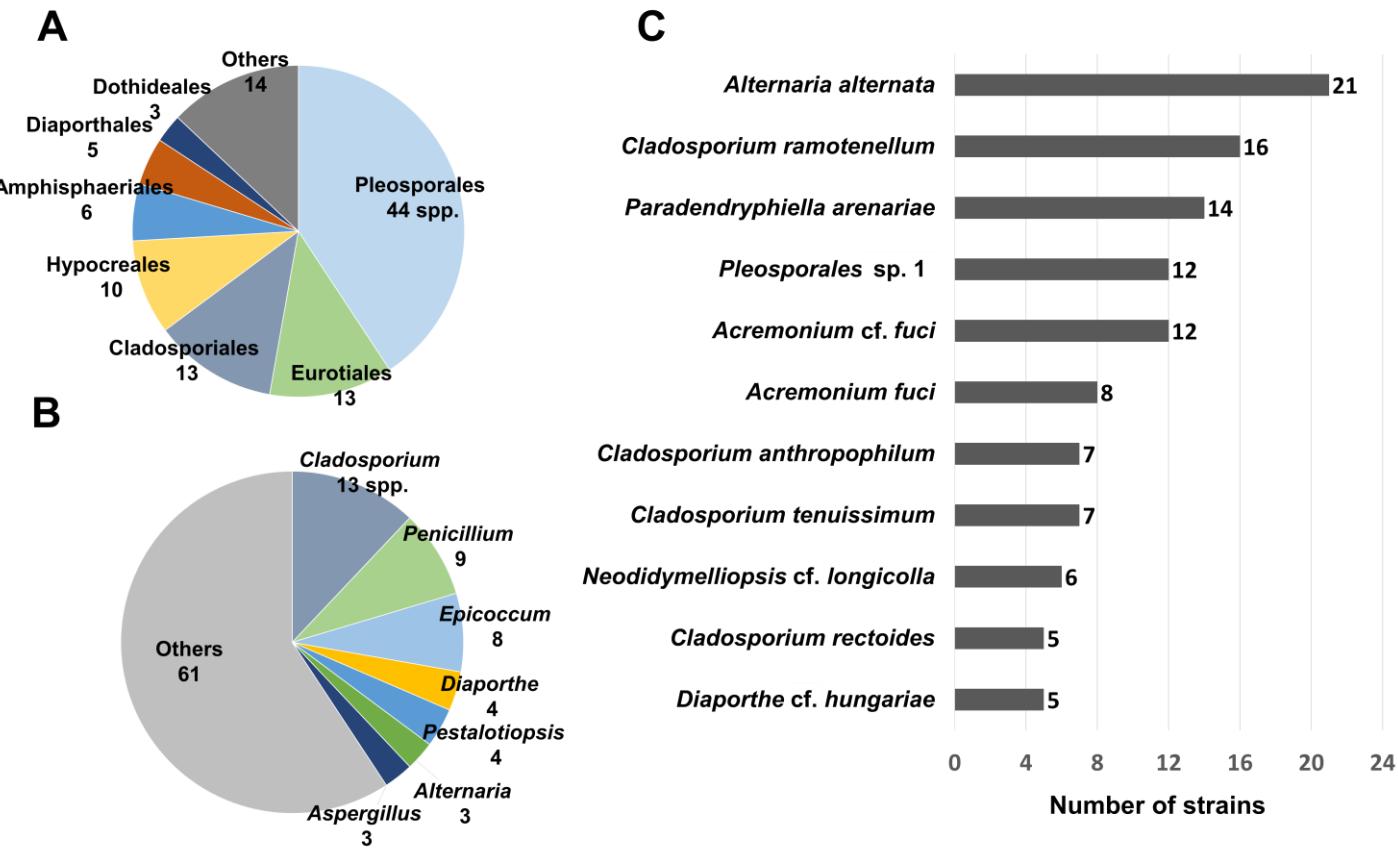


Figure 9. Proportions of fungi isolated from plastic waste. Order (A), genus (B), and species (C) levels.

### 3.3. PCL assay results

A PCL degradation test was performed on 146 representative strains of 108 species (Supplementary Table S3). The clear zone lengths of the fungal strains were in the range of 0–13.96 mm. Based on the average clear zone lengths, fungal PCL degradation ability was categorized into four levels, namely, no degradation (0 mm: 0), weak ( $0 < (+) \leq 5$  mm), moderate ( $5 < (++) \leq 10$  mm), and strong ( $10 < (+++) \leq 15$  mm) (Figure 4). Five species exhibited strong PCL degradation, 18 species showed moderate PCL degradation, 64 species presented with weak PCL degradation, and 21 species did not degrade PCL at all (Table 1). There was also intraspecies variation. The PCL degradation capacities of eight *Alternaria alternata* strains ranged from 12.88 mm to 3.32 mm. In most cases, however, all tested strains of the same species were similar in terms of their PCL degradation ability.

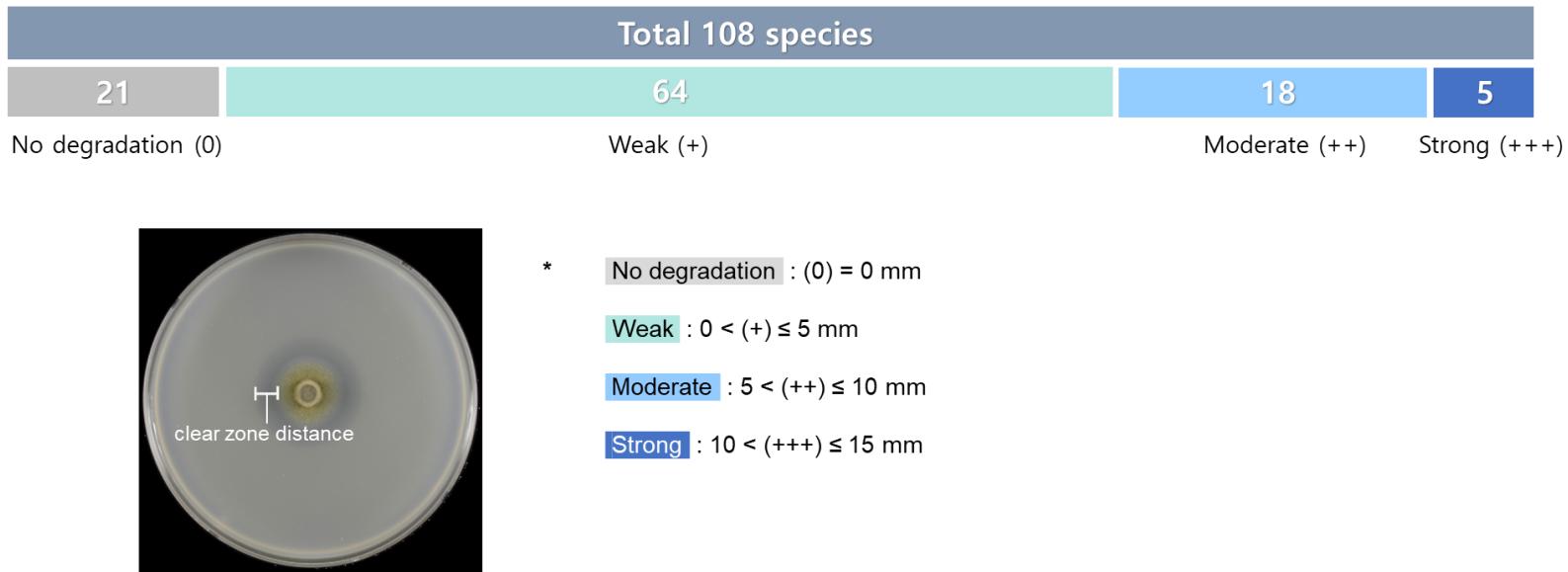
*Phaeophleospora eucalypticola* had the strongest PCL degradation ability (clear zone length = 13.96 mm). Four *Cladosporium* species also showed strong PCL degradation activity. *Cladosporium allicinum* had the widest clear zone (13.92 mm) followed by *C. xanthochromaticum* (11.37 mm), *C. rectoides* (10.34 mm), and *C. tenuissimum* (10.21 mm). Seventeen species of moderate PCL-degrading fungi were classified into ten genera including two *Alternaria* spp., two *Aureobasidium* spp., two *Phaeosphaeria* spp., five *Cladosporium* spp., and each one species of *Cytospora*, *Epicoccum*, *Neodevriesia*, *Nothophoma*, *Sarocladium*, *Sphaeropsis* (Table 1). *Cladosporium* species showed relatively high PCL degradation activity among the moderate PCL degraders.

Weak PCL-degrading fungi included 64 species. They were classified as 30

genera (Table 1). *Didymella*, *Epicoccum*, and *Remotiodidymella* (Didymellaceae) showed relatively weak PCL degradation as no strain produced a clear zone wider than 5 mm. The species in the Eurotiales exhibited very weak PCL degradation ability. None of the *Penicillium* strains produced clear zones wider than 1 mm (Figure 4, Table 1). *Aspergillus* and *Talaromyces* showed higher PCL-degrading activity than *Penicillium*. The lengths of the average clear zones produced by *Aspergillus* and *Talaromyces* were 2.30 mm and 3.31 mm on average, respectively. Most species in the Order Amphisphaeraiales were weak PCL degraders (Table 1).

Whereas most fungi could degrade PCL, certain species isolated from 27 PET waste sources failed to form clear zones on PCL agar. Most of them were isolated along with other PCL-degrading fungi (Figure 5). Weak and moderate PCL-degrading fungi were detected in most samples. Weakly PCL-degrading fungi were particularly abundant in PET samples Nos. 20, 36, 37, 43, and 44. By contrast, strong PCL degrading fungi did not predominate in any PET samples and always co-occurred either with weak or moderate PCL-degrading fungi or with those that did not degrade PCL at all.

Min: 0 mm, Max: 13.96 mm



**Figure 10. Summary of PCL agar assay results.** The degradation level of each species is labeled according to their clear zone distances.

**Table 3. Fungi isolated from PET wastes and their PCL degradation assay results.**

Fungal orders are in bold font. Averages and SD of clear zone lengths are presented and categorized into four levels.

Species	Total No. strains	PCL Tested Strains	Clear Zone Length (mm)	Degradation Level*
<b>Amphisphaerales</b>				
<i>Morinia</i> cf. <i>acaciae</i>	1	1	1.55	(+)
<i>Neopestalotiopsis</i> sp.	2	1	0	0
<i>Pestalotiopsis</i> cf. <i>anacardiacearum</i>	1	1	0.88	(+)
<i>Pestalotiopsis</i> cf. <i>australasiae</i>	2	1	0.34	(+)
<i>Pestalotiopsis</i> sp.	2	1	0.9	(+)
<i>Pestalotiopsis thailandica</i>	1	1	1.35	(+)
<b>Botryosphaerales</b>				
<i>Botryosphaeria dothidea</i>	1	1	0.37	(+)
<i>Sphaeropsis sapinea</i>	1	1	6.94	(++)
<b>Capnodiales</b>				
<i>Neodevriesia</i> cf. <i>metrosideri</i>	1	1	6.08	(++)
<b>Cladosporiales</b>				
<i>Cladosporium allicinum</i>	2	1	13.92	(+++)
<i>Cl. anthropophilum</i>	7	1	9.74	(++)
<i>Cl. cf. halotolerans</i>	2	1	8.6	(++)
<i>Cl. cf. cladosporioides</i>	1	1	8.22	(++)
<i>Cl. funiculosum</i>	1	1	1.66	(+)
<i>Cl. halotolerans</i>	1	1	2.46	(+)
<i>Cl. perangustum</i>	2	2	6.76 ( $\pm 4.19$ )	(++)
<i>Cl. pseudocladosporioides</i>	3	2	7.74 ( $\pm 6.84$ )	(++)
<i>Cl. ramotenellum</i>	16	6	4.85 ( $\pm 2.17$ )	(+)
<i>Cl. rectoides</i>	5	2	10.34 ( $\pm 3.28$ )	(+++)
<i>Cl. tenuissimum</i>	7	3	10.21 ( $\pm 0.82$ )	(+++)
<i>Cl. xanthochromaticum</i>	2	1	11.37	(+++)

\* 0 mm: (0), 0 < (+)  $\leq$  5 mm, 5 < (++)  $\leq$  10 mm, 10 < (++)  $\leq$  15 mm

**Table 3.** Continued.

<b>Species</b>	<b>Total No. strains</b>	<b>PCL Tested Strains</b>	<b>Clear Zone Length (mm)</b>	<b>Degradation Level*</b>
<i>Cl. xylophilum</i>	4	2	0.57 ( $\pm 0.11$ )	(+)
<b>Diaporthales</b>				
<i>Diaporthe cf. arecae</i>	3	2	1.33 ( $\pm 0.4$ )	(+)
<i>Diaporthe cf. hungariae</i>	5	2	2.48 ( $\pm 0.95$ )	(+)
<i>Diaporthe cf. pseudooculi</i>	1	1	2.18	(+)
<i>Diaporthe cf. sojae</i>	1	1	1.09	(+)
<i>Cytospora ceratosperma</i>	1	1	6.17	(++)
<b>Dothideales</b>				
<i>Aureobasidium melanogenum</i>	1	1	8.28	(++)
<i>Au. namibiae</i>	1	1	6.24	(++)
<i>Au. pullulans</i>	2	1	0.56	(+)
<b>Eurotiales</b>				
<i>Aspergillus ochraceus</i>	1	1	2.19	(+)
<i>As. oryzae</i>	1	1	4.8	(+)
<i>As. tritici</i>	1	1	2.03	(+)
<i>Penicillium charlesii</i>	2	1	0	0
<i>Pe. commune</i>	4	2	0 ( $\pm 0$ )	0
<i>Pe. crustosum</i>	3	1	0	0
<i>Pe. echinulatum</i>	1	1	0.37	(+)
<i>Pe. expansum</i>	1	1	0	0
<i>Pe. exsudans</i>	1	1	0	0
<i>Pe. javanicum</i>	1	1	0	0
<i>Pe. oxalicum</i>	1	1	0	0
<i>Pe. roqueforti</i>	1	1	0.07	(+)
<i>Talaromyces rugulosus</i>	1	1	3.31	(+)
<b>Glomerellales</b>				
<i>Plectosphaerella cucumerina</i>	1	1	0.39	(+)

\* 0 mm: (0),  $0 < (+) \leq 5$  mm,  $5 < (++) \leq 10$  mm,  $10 < (++) \leq 15$  mm

**Table 3.** Continued.

Species	Total No. strains	PCL Tested Strains	Clear Zone Length (mm)	Degradation Level*
<b>Helotiales</b>				
<i>Botrytis cinerea</i>	1	1	1.03	(+)
<b>Hypocreales</b>				
<i>Acremonium cf. fuci</i>	12	3	0 ( $\pm 0$ )	0
<i>Ac. fuci</i>	8	2	0 ( $\pm 0$ )	0
<i>Fusarium equiseti</i>	4	2	0.63 ( $\pm 0.21$ )	(+)
<i>Fusarium fujikuroi</i>	1	1	0.73	(+)
<i>Hypocreales sp.</i>	1	1	2.43	(+)
<i>Parasarocladium cf. gamsii</i>	2	2	3.38 ( $\pm 4.08$ )	(+)
<i>Parengyodontium album</i>	1	1	0	0
<i>Sarocladium strictum</i>	1	1	7.63	(++)
<i>Trichoderma harzianum</i>	1	1	0.41	(+)
<i>Tr. fomiticola</i>	1	1	0.3	(+)
<b>Mycosphaerellales</b>				
<i>Phaeophleospora eucalypticola</i>	2	1	13.96	(++++)
<b>Pleosporales</b>				
<i>Alternaria alternata</i>	21	8	6.14 ( $\pm 3.28$ )	(++)
<i>Al. cf. rosae</i>	2	1	5.82	(++)
<i>Al. chlamydospora</i>	3	1	1.7	(+)
<i>Didymella cf. macrophylla</i>	1	1	1.28	(+)
<i>Didymosphaeriaceae sp. 1</i>	1	1	6.1	(++)
<i>Didymosphaeriaceae sp. 2</i>	1	1	3.33	(+)
<i>Epicoccum cf. duchesneae</i>	3	1	3.58	(+)
<i>Epicoccum cf. hordei</i>	1	1	0	0
<i>Epicoccum cf. sorghinum</i>	1	1	2.65	(+)
<i>Epicoccum dendrobii</i>	2	1	6.26	(++)
<i>Epicoccum duchesneae</i>	3	2	0.43 ( $\pm 0.61$ )	(+)
<i>Epicoccum sorghinum</i>	2	1	1.5	(+)

\* 0 mm: (0), 0 < (+)  $\leq$  5 mm, 5 < (++)  $\leq$  10 mm, 10 < (+++)  $\leq$  15 mm

**Table 3.** Continued.

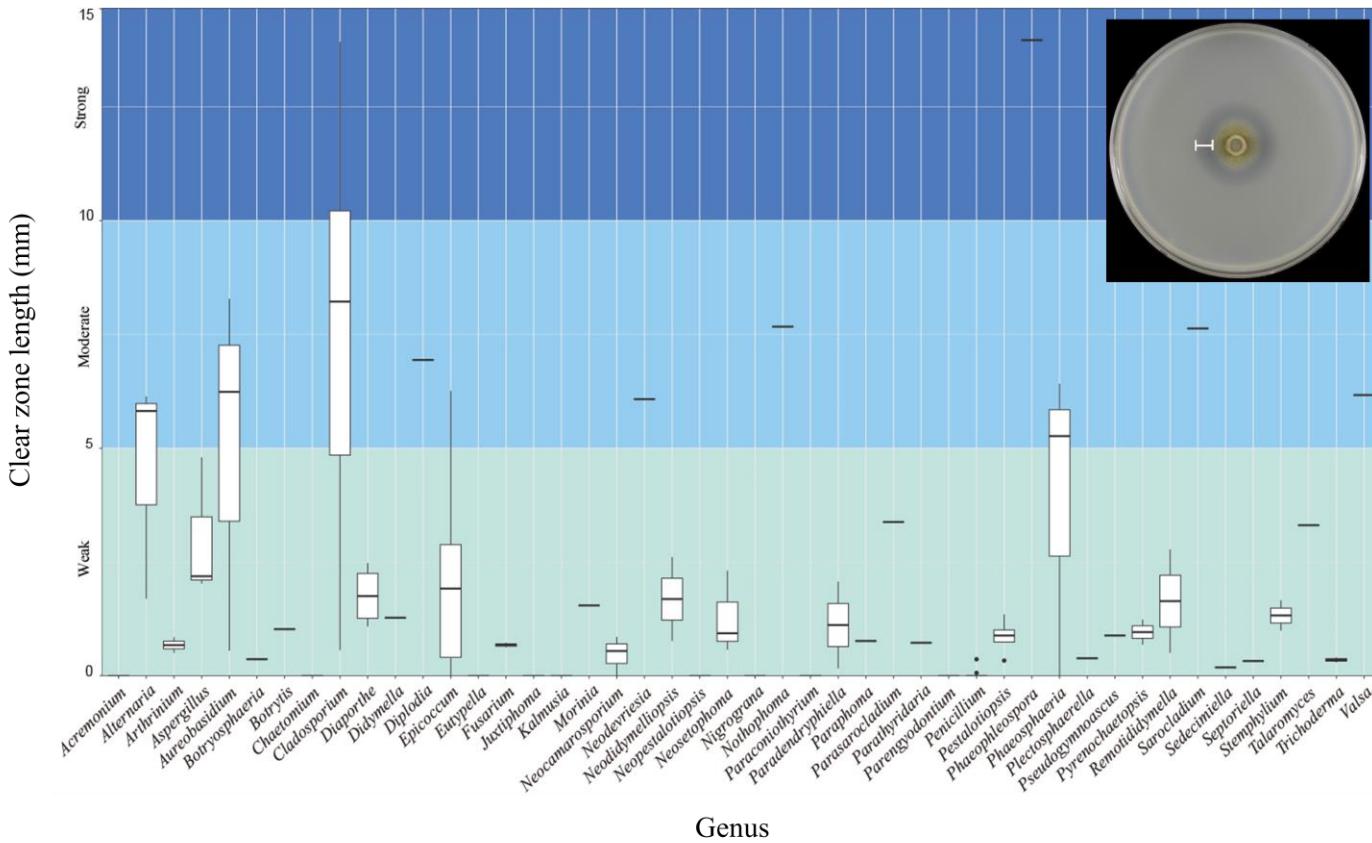
Species	Total No. strains	PCL Tested Strains	Clear Zone Length (mm)	Degradation Level*
<i>Epicoccum</i> sp.	1	1	0.35	(+)
<i>Epicoccum tritici</i>	2	1	2.34	(+)
<i>Juxtiphoma</i> cf. <i>eupyrena</i>	1	1	0	0
<i>Kalmusia araucariae</i>	1	1	0	0
<i>Neocamarosporium betae</i>	3	1	0	0
<i>Neocamarosporium solicola</i>	2	1	0.55	(+)
<i>Neocamarosporium</i> sp.	3	2	0.86 ( $\pm 0.72$ )	(+)
<i>Neodidymelliopsis</i> cf. <i>longicolla</i>	6	3	2.61 ( $\pm 3.37$ )	(+)
<i>Neodidymelliopsis longicolla</i>	2	1	0.77	(+)
<i>Neosetophoma</i> cf. <i>poaceicola</i>	1	1	2.31	(+)
<i>Neosetophoma poaceicola</i>	1	1	0.94	(+)
<i>Neosetophoma rosigena</i>	1	1	0.58	(+)
<i>Nothophoma quercina</i>	1	1	7.67	(++)
<i>Paraconiothyrium brasiliense</i>	1	1	0	0
<i>Paradendryphiella arenariae</i>	14	3	0.17 ( $\pm 0.3$ )	(+)
<i>Paraphoma radicina</i>	1	1	0.77	(+)
<i>Parathyridaria</i> cf. <i>tyrrhenica</i>	2	2	0.73 ( $\pm 1.03$ )	(+)
<i>Phaeosphaeria culmorum</i>	1	1	0	0
<i>Phaeosphaeria spartinicola</i>	1	1	5.27	(++)
<i>Phaeosphaeria oryzae</i>	1	1	6.42	(++)
Pleosporaceae sp. 1	1	1	0.42	(+)
Pleosporaceae sp. 2	1	1	2.07	(+)
Pleosporales sp. 1	12	2	0 ( $\pm 0$ )	0
Pleosporales sp. 2	3	2	1.33 ( $\pm 1.15$ )	(+)
Pleosporales sp. 3	1	1	0.93	(+)
Pleosporales sp. 4	2	2	3.37 ( $\pm 1.52$ )	(+)
<i>Pyrenophaetopsis microspora</i>	2	1	1.24	(+)
<i>Pyrenophaetopsis paucisetosa</i>	1	1	0.69	(+)

\* 0 mm: (0), 0 < (+)  $\leq$  5 mm, 5 < (++)  $\leq$  10 mm, 10 < (+++)  $\leq$  15 mm

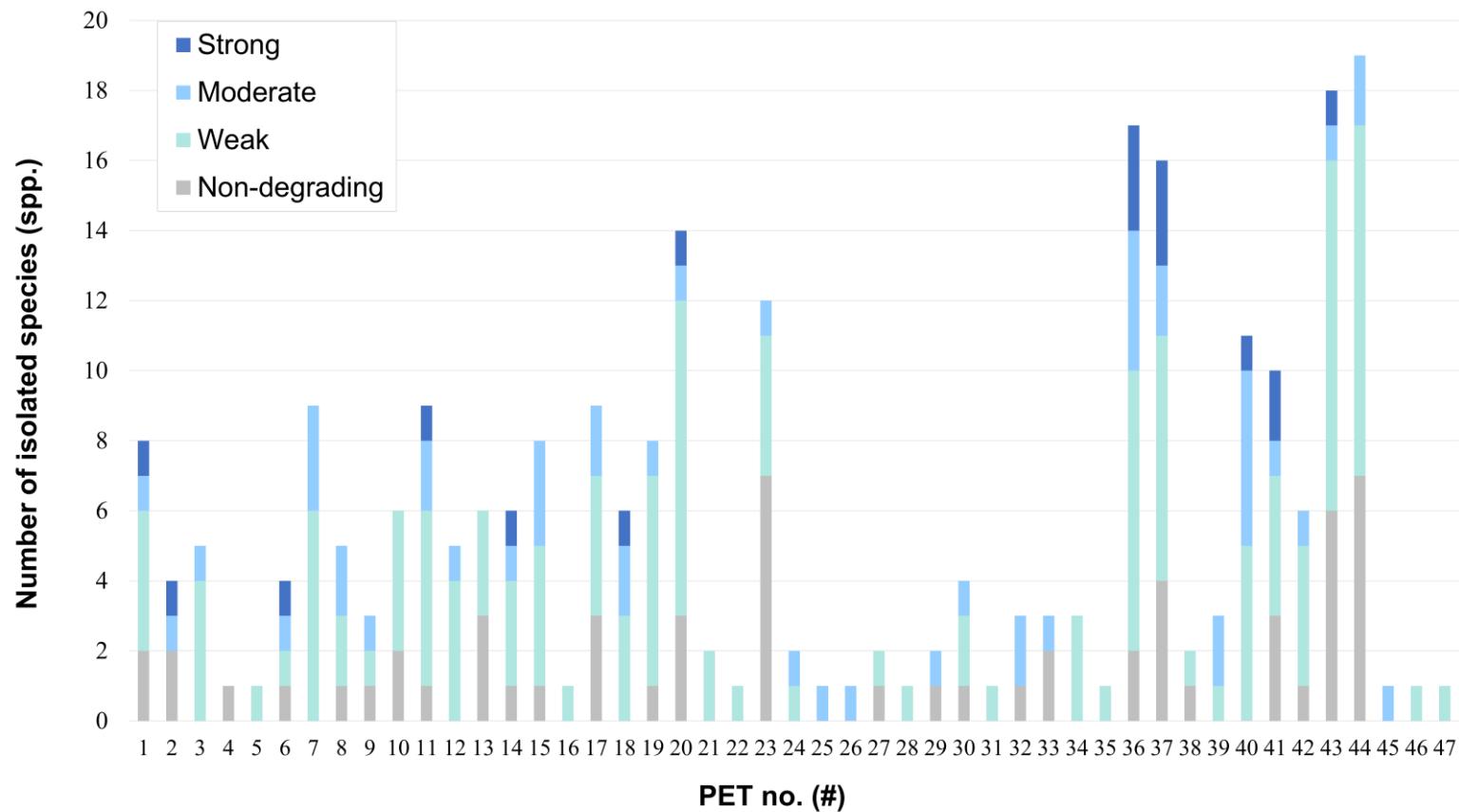
**Table 3.** Continued.

<b>Species</b>	<b>Total No. strains</b>	<b>PCL Tested Strains</b>	<b>Clear Zone Length (mm)</b>	<b>Degradation Level*</b>
<i>Remotididymella cf. capsici</i>	3	2	0.51 ( $\pm 0.72$ )	(+)
<i>Remotididymella</i> sp.	1	1	2.78	(+)
<i>Stemphylium lycopersici</i>	1	1	1	(+)
<i>St. vesicarium</i>	2	2	1.66 ( $\pm 0.07$ )	(+)
<b>Sordariales</b>				
<i>Chaetomium globosum</i>	1	1	0.93	(+)
<b>Thelebolales</b>				
<i>Pseudogymnoascus pannorum</i>	2	1	0.89	(+)
<b>Xylariales</b>				
<i>Eutypella cf. persica</i>	1	1	0.69	(+)
<b>Others (incertae sediae)</b>				
<i>Apiospora marii</i>	1	1	0.51	(+)
<i>Ap. rasikravindrae</i>	1	1	0.85	(+)
<i>Nigrospora cf. oryzae</i>	2	1	0	0
<i>Sedecimiella taiwanensis</i>	1	1	0.19	(+)
<i>Septoriella cf. hubertusii</i>	1	1	0.33	(+)

\* 0 mm: (0),  $0 < (+) \leq 5$  mm,  $5 < (++) \leq 10$  mm,  $10 < (+++) \leq 15$  mm



**Figure 11. Boxplot of PCL-degrading ability of all tested strains with the genera detected in the present study.** Boxplot constructed based on average clear zone length of each species. Inset: photograph of *Cladosporium rectoides* (SFC2022\_NP016) culture.



**Figure 12. Fungal species isolated from collected plastic wastes.** Color intensity is commensurate with PCL degradation level.

## **4. Discussion**

Fungi from marine PET wastes were collected and went under PCL degradation test to discover competent plastic-degrading fungi in the marine plastisphere and determined whether they could degrade PET. Numerous fungi were isolated despite the limited number of PET wastes examined here. They resembled that reported in a previous study on fungal diversity in the marine environment (Jones et al., 2015; Kwon et al., 2021). *Acremonium fuci* was isolated from seaweed in Europe and North America (Zuccaro et al., 2004), and several *Cladosporium* species, such as *C. perangustum*, *C. tenuissimum*, and *C. xanthochromaticum* were found in marine sediments (Lou et al., 2020). *Paradendryphiella arenariae* were reported from various microalgae in Europe (Dela Cruz et al., 2006), and a number of other fungal species including *Fusarium equiseti*, *Nigrospora oryzae*, *Penicillium oxalicum*, and *Trichoderma harzianum* were found in sea sand, mudflats, and seaweeds (Heo et al., 2019; Park et al., 2019). The fungal species detected in this research were also similar to those in other marine and terrestrial plastisphere. Many species in Pleosporales were detected in plastisphere of the Antarctic Ocean (Lacerda et al., 2020) and the North Sea (De Tender et al., 2017). The *Aspergillus*, *Chaetomium*, *Epicoccum*, *Fusarium*, and *Trichoderma* species were identified in the terrestrial plastisphere (Kemona and Piotrowska, 2016; Ye et al., 2020).

Several putative plastic-degrading fungi were identified by the PCL agar degradation test. Approximately 81% of all identified species formed clear zones and were, therefore, potential plastic biodegraders. PET degradation-associated enzyme activity was detected in PCL-degrading fungus (Nyyssölä et al., 2013). Hence, the

species identified here could conceivably decompose PET and other plastics as well. *Cladosporium* included 13 PCL-degrading species of which four and nine had strong and moderate PCL-degrading activity, respectively. Prior research confirmed that several *Cladosporium* strains effectively degraded other substrates, such as polyurethane (Bonhomme et al., 2003; Brunner et al., 2018; Srikanth et al., 2022). Therefore, *Cladosporium* species could degrade plastic wastes in the marine environment. *Aureobasidium pullulans*, which displayed relatively good polyurethane degradation were also reported previously (Crabbe et al., 1994). *Phaeophleospora eucalypticola* showed the strongest PCL-degrading activity, but this species has not been given much attention to its degrading abilities. Further research on *P. eucalypticola* may reveal its full potential for the degradation of plastics. It is reported that various enzymes, such as cutinase, laccase, and esterase from fungi were used in degradation of PET (Anbalagan et al., 2022; Khan et al., 2022), and this may explain the high biodiversity in relatively small number of plastic samples.

In this study, most of the highly abundant species showed low levels of PCL degradation ability. *Paradendryphiella arenariae*, Pleosporales sp.1, and *Acremonium cf. fuci* were very abundant but relatively weak PCL degraders. In contrast, the fungi with the strongest degradation ability were far less abundant. *Phaeophleospora eucalypticola* and *C. xanthochromaticum* showed strong PCL degradation capacity, but only two strains were isolated from 47 plastic wastes. Each PET waste had many fungal species with low level of PCL degradation but generally possessed only one fungal species that was highly effective. This result allowed us to infer that varying fungal species on plastic waste performed different roles. The abundant weak plastic degraders may grow on plastic

wastes to utilize materials primarily degraded by mechanical or biological process. The initial breakdown mechanism of plastics may include polymer oxidation, which increases the hydrophilicity of plastics, weakens their bonds and mechanical structures, and facilitates secondary colonizer access (Oberbeckmann and Labrenz, 2020). Microorganisms also gradually degrade other complex molecules such as lignin (Janusz et al., 2017) and anthropogenic (synthetic) polymers (Chen et al., 2020). Hence, the colonizing mechanisms of various microbes strongly influence fungal diversity on plastic wastes. Species with weak or no PCL-degrading ability may have been isolated from the primary colonizers as fungicolous fungi. The inhabitation of fungal species on other plastic-colonizing microorganisms has previously been reported (Webb, 2000). Some species in order Hypocreales and Pleosporales are known to obtain their nutrients either commensally or parasitically from other fungi (Sun et al., 2019). Most species in Hypocreales and Pleosporales showed low PCL degradation ability, proving that they get their nutrients from alternative sources.

## **5. Conclusions**

The results of this study showed that numerous fungi inhabit PET wastes in the marine environment. Certain fungal taxa including *Phaeophleospora eucalypticola*, *Alternaria* spp., *Aureobasidium* spp., and *Cladosporium* spp. have strong PCL degrading activity. Fungi with low level of PCL degrading ability were abundant and co-occurred with one of strong PCL degrader. The wide diversity and ranges of abundance, and plastic-degrading capacity of fungi even on small quantities of PET suggest that the various fungal taxa play different roles in marine plastic waste decomposition.

Fungal diversity in plastisphere has been relatively understudied, compared to other known environments. In future research, therefore, marine fungal diversity can be extended further by investigating on many other kinds of plastic substrates. Also, applied research such as crude enzyme extraction and degradation gene identification could be established on the data from this study. Clarifying the functions of each of these fungal taxa would be the next objective in order to develop a strategy for effective and efficient plastic waste degradation in the marine environment.

**Supplementary Table S1. Strains and their GenBank accession numbers used for phylogenetic analyses in this study.**

Species	Representative strain	ITS	act	BenA
<i>Acremonium fuci</i>	CMG27	MK986700	-	-
	<b>SFC2022_NP060</b>	<b>OP070793</b>	-	-
<i>Acremonium cf. fuci</i>	<b>SFC2022_NP059</b>	<b>OP070792</b>	-	-
<i>Alternaria alternata</i>	CBS 127672	MH864614	-	-
	<b>SFC2022_NP008</b>	<b>OP070736</b>	-	-
<i>Alternaria chlamydospora</i>	CBS 491.72	NR_136039	-	-
	<b>SFC2022_NP054</b>	<b>OP070787</b>	-	-
<i>Alternaria rosae</i>	CBS 121341	NR_136017	-	-
<i>Alternaria cf. rosae</i>	<b>SFC2022_NP027</b>	<b>OP070755</b>	-	-
<i>Apiospora marii</i>	CBS 497.90	NR_166043	-	-
	<b>SFC2022_NP020</b>	<b>OP070748</b>	-	-
<i>Apiospora rasikravindrae</i>	NFCCI 2144	NR_119932	-	-
	<b>SFC20220715_M05</b>	<b>OP070781</b>	-	-
<i>Aspergillus ochraceus</i>	NRRL 398	NR_077150	-	EF661322
	<b>SFC2022_NP098</b>	<b>OP070834</b>	-	<b>OP022418</b>
<i>Aspergillus oryzae</i>	NRRL 447	NR_135395	-	EF661483
	<b>SFC2022_NP081</b>	<b>OP070817</b>	-	<b>OP022410</b>
<i>Aspergillus tritici</i>	CBS 266.81	NR_135414	-	MN969368
	<b>SFC2022_NP089</b>	<b>OP070825</b>	-	<b>OP022415</b>
<i>Aureobasidium melanogenum</i>	CBS 105.22	NR_159598	-	-
	<b>SFC2022_NP040</b>	<b>OP070770</b>	-	-
<i>Aureobasidium namibiae</i>	CBS 147.97	NR_147362	-	-
	<b>SFC2022_NP039</b>	<b>OP070769</b>	-	-
<i>Aureobasidium pullulans</i>	CBS 584.75	NR_144909	-	-
	<b>SFC2022_NP046</b>	<b>OP070776</b>	-	-
<i>Botryosphaeria dothidea</i>	CMW 8000	NR_111146	-	-
	<b>SFC2022_NP084</b>	<b>OP070820</b>	-	-
<i>Botrytis cinerea</i>	HNSMJ-4	MW820601	-	-

**Supplementary Table S1.** Continued.

Species	Representative strain	ITS	act	BenA
<i>Botrytis cinerea</i>	<b>SFC2022_NP013</b>	<b>OP070741</b>	-	-
<i>Cercospora beticola</i>	CBS 116456	-	AY840458	-
<i>Chaetomium globosum</i>	CBS 160.62	NR_144851	-	-
	<b>SFC2022_NP028</b>	<b>OP070756</b>	-	-
<i>Cladosporium allicinum</i>	CBS 121624	NR_152266	EF679502	-
	<b>SFC2022_NP021</b>	<b>OP070749</b>	<b>OP022373</b>	-
<i>Cladosporium anthropophilum</i>	Clad 387	-	MZ695001	-
	<b>SFC2022_NP015</b>	<b>OP070743</b>	<b>OP022371</b>	-
<i>Cladosporium cladosporioides</i>	CBS 112388	NR_119839	HM148490	-
<i>Cladosporium cf. cladosporioides</i>	<b>SFC2022_NP022</b>	<b>OP070750</b>	<b>OP022374</b>	-
<i>Cladosporium funiculosum</i>	CBS 122129	NR_119845	HM148583	-
	<b>SFC20220715_M07</b>	<b>OP070802</b>	<b>OP022380</b>	-
<i>Cladosporium halotolerans</i>	CBS 119416	NR_119605	EF101397	-
	<b>SFC2022_NP036</b>	<b>OP070766</b>	<b>OP022375</b>	-
<i>Cladosporium cf. halotolerans</i>	<b>SFC2022_NP094</b>	<b>OP070830</b>	<b>OP022381</b>	-
<i>Cladosporium perangustum</i>	CBS 125996	NR_119851	HM148610	-
	<b>SFC2022_NP065</b>	<b>OP070798</b>	<b>OP022379</b>	-
<i>Cladosporium pseudocladosporioides</i>	CBS 125993	NR_119852	HM148647	-
	<b>SFC2022_NP037</b>	<b>OP070767</b>	<b>OP022376</b>	-
<i>Cladosporium ramotenellum</i>	CBS 121628	NR_119658	EF679538	-
	<b>SFC2022_NP063</b>	<b>OP070796</b>	<b>OP022378</b>	-
<i>Cladosporium rectoides</i>	CBS 125994	NR_111539	HM148683	-
	<b>SFC2022_NP016</b>	<b>OP070744</b>	<b>OP022372</b>	-
<i>Cladosporium tenuissimum</i>	CBS 125995	NR_119855	HM148687	-
	<b>SFC2022_NP009</b>	<b>OP070737</b>	<b>OP022370</b>	-
<i>Cladosporium xanthochromaticum</i>	UTHSC: DI13-211	NR_148191	LN834599	-
	<b>SFC2022_NP007</b>	<b>OP070735</b>	<b>OP022369</b>	-

**Supplementary Table S1.** Continued.

Species	Representative strain	ITS	act	BenA
<i>Cladosporium xylophilum</i>	CBS 125997	NR_111541	HM148721	-
	<b>SFC2022_NP051</b>	<b>OP070783</b>	<b>OP022377</b>	-
<i>Coniothyrium palmarum</i>	CBS 400.71	MH860184	-	KT389792
<i>Cytospora ceratosperma</i>	CBS 116.21	AY347335	-	-
	<b>SFC20220715_M02</b>	<b>OP070757</b>	-	-
<i>Diaporthe arecae</i>	CBS 161.64	MH858400	-	KC344000
<i>Diaporthe cf. arecae</i>	<b>SFC2022_NP017</b>	<b>OP070745</b>	-	<b>OP022383</b>
<i>Diaporthe hungariae</i>	CBS 143353	MG281126	-	MG281299
<i>Diaporthe cf. hungariae</i>	<b>SFC2022_NP026</b>	<b>OP070754</b>	-	<b>OP022387</b>
<i>Diaporthe pseudooculi</i>	HHUF 30617	NR_161019	-	LC373519
<i>Diaporthe cf. pseudooculi</i>	<b>SFC2022_NP076</b>	<b>OP070812</b>	-	<b>OP022399</b>
<i>Diaporthe sojae</i>	CQHYD3-2	MT877050	-	MT874968
<i>Diaporthe cf. sojae</i>	<b>SFC2022_NP099</b>	<b>OP070835</b>	-	<b>OP022405</b>
<i>Didymella macrophylla</i>	CGMCC 3.18357	NR_158258	-	KY742312
<i>Didymella cf. macrophylla</i>	<b>SFC2022_NP004</b>	<b>OP070732</b>	-	<b>OP022382</b>
<i>Didymosphaeriaceae sp. 1</i>	<b>SFC2022_NP096</b>	<b>OP070832</b>	-	
<i>Didymosphaeriaceae sp. 2</i>	<b>SFC2022_NP055</b>	<b>OP070788</b>	-	<b>OP022392</b>
<i>Epicoccum dendrobii</i>	CGMCC 3.18359	NR_158261	-	KY742335
	<b>SFC2022_NP049</b>	<b>OP070780</b>	-	<b>OP022389</b>
<i>Epicoccum duchesneae</i>	CGMCC 3.18345	NR_158262	-	KY742337
	<b>SFC2022_NP077</b>	<b>OP070813</b>	-	<b>OP022400</b>
<i>Epicoccum cf. duchesneae</i>	<b>SFC2022_NP078</b>	<b>OP070814</b>	-	<b>OP022401</b>
<i>Epicoccum hordei</i>	CGMCC 3.18360	NR_158263	-	KY742339
<i>Epicoccum cf. hordei</i>	<b>SFC2022_NP073</b>	<b>OP070807</b>	-	<b>OP022397</b>
<i>Epicoccum nigrum</i>	CBS 173.73	MH860655	-	FJ427107
<i>Epicoccum sorghinum</i>	UTHSC: DI16-301	LT592948	-	LT593017
	<b>SFC2022_NP079</b>	<b>OP070815</b>	-	<b>OP022419</b>
<i>Epicoccum cf. sorghinum</i>	<b>SFC2022_NP023</b>	<b>OP070751</b>	-	<b>OP022385</b>
<i>Epicoccum sp.</i>	<b>SFC2022_NP052</b>	<b>OP070785</b>	-	<b>OP022391</b>

**Supplementary Table S1.** Continued.

Species	Representative strain	ITS	act	BenA
<i>Epicoccum tritici</i>	MFLUCC-16-0276 <b>SFC2022_NP019</b>	KX926426 <b>OP070747</b>	-	KY197979 <b>OP022384</b>
<i>Eutypella cf. persica</i>	IRAN 2540C <b>SFC2022_NP032</b>	NR_171807 <b>OP070761</b>	-	-
<i>Fusarium equiseti</i>	NRRL 26419 <b>SFC2022_NP053</b>	NR_121457 <b>OP070786</b>	-	-
<i>Fusarium fujikuroi</i>	CBS 221.76 <b>SFC2022_NP044</b>	NR_111889 <b>OP070774</b>	-	-
<i>Hamigera avellanea</i>	CBS 295.48	-	-	LC076692
Hypocreales sp.	<b>SFC2022_NP033</b>	<b>OP070762</b>	-	-
<i>Juxtiphoma eupyrena</i>	CBS 832.84	MH859842	-	MN983994
<i>Juxtiphoma cf. eupyrena</i>	<b>SFC2022_NP067</b>	<b>OP070800</b>	-	<b>OP022394</b>
<i>Kalmusia araucariae</i>	CPC 37475 <b>SFC20220715_M08</b>	NR_170054 <b>OP070808</b>	-	-
<i>Morinia acaciae</i>	CBS 137994	NR_161082	-	MH554673
<i>Morinia cf. acaciae</i>	<b>SFC2022_NP075</b>	<b>OP070811</b>	-	-
<i>Neocamarosporium betae</i>	WW18CQ02 <b>SFC2022_NP005</b>	MZ734407 <b>OP070733</b>	-	-
<i>Neocamarosporium leipoldiae</i>	CBS 146774	NR_171762	-	-
<i>Neocamarosporium solicola</i>	IBRC M 30257 <b>SFC2022_NP041</b>	KX817217 <b>OP070771</b>	-	-
<i>Neocamarosporium</i> sp.	<b>SFC2022_NP042</b>	<b>OP070772</b>	-	-
<i>Neodevriesia metrosideri</i>	CBS 145084	NR_161141	-	-
<i>Neodevriesia cf. metrosideri</i>	<b>SFC2022_NP048</b>	<b>OP070779</b>	-	-
<i>Neodidymelliopsis longicolla</i>	CBS 382.96 <b>SFC2022_NP024</b>	KT389532 <b>OP070752</b>	-	KT389830 <b>OP022386</b>
<i>Neodidymelliopsis cf. longicolla</i>	<b>SFC2022_NP088</b>	<b>OP070824</b>	-	<b>OP022402</b>
<i>Neopestalotiopsis rosae</i>	CBS 101057	NR_145243	-	KM199429
<i>Neopestalotiopsis</i> sp.	<b>SFC2022_NP069</b>	<b>OP022396</b>	-	-

**Supplementary Table S1.** Continued.

Species	Representative strain	ITS	act	BenA
<i>Neosetophoma poaceicola</i>	MFLUCC 16-0886 <b>SFC2022_06</b>	NR_165861 <b>OP070784</b>	-	-
<i>Neosetophoma cf. poaceicola</i>	<b>SFC2022_NP018</b>	<b>OP070746</b>	-	-
<i>Neosetophoma rosigena</i>	MFLU 17-0626 <b>SFC2022_03</b>	NR_157525 <b>OP070764</b>	-	-
<i>Nigrospora cf. oryzae</i>	CBS 480.73 <b>SFC2022_NP034</b>	NR_153476 <b>OP070763</b>	-	-
<i>Nothophoma quercina</i>	CBS 832.84 <b>SFC2022_NP038</b>	- <b>OP070768</b>	-	MN983992 <b>OP022388</b>
<i>Paracamarosporium hawaiiense</i>	CBS 120025	NR_154287	-	-
<i>Paraconiothyrium brasiliense</i>	CBS 100299 <b>SFC2022_NP043</b>	NR_163552 <b>OP070773</b>	-	-
<i>Paradendryphiella arenariae</i>	CBS 181.58 <b>SFC2022_NP066</b>	NR_145170 <b>OP070799</b>	-	-
<i>Paraphoma radicina</i>	CBS 111.79 <b>SFC2022_NP087</b>	NR_156556 <b>OP070823</b>	-	-
<i>Parasarocladium gamsii</i>	CBS 726.71 <b>SFC2022_04</b>	NR_159615 <b>OP070778</b>	-	-
<i>Parathyridaria tyrrhenica</i>	MUT 5371	NR_169907	-	-
<i>Parathyridaria cf. tyrrhenica</i>	<b>SFC2022_NP080</b>	<b>OP070816</b>	-	-
<i>Parengyodontium album</i>	CBS 504.83 <b>SFC2022_NP030</b>	LC092880 <b>OP070759</b>	-	-
<i>Penicillium charlesii</i>	CBS 304.48 <b>SFC2022_NP082</b>	AF033400 <b>OP070818</b>	-	JX091508 <b>OP022411</b>
<i>Penicillium commune</i>	CBS 311.48 <b>SFC2022_NP092</b>	AY213672 <b>OP070828</b>	-	MN969377 <b>OP022417</b>
<i>Penicillium crustosum</i>	CBS 115503 <b>SFC2022_NP091</b>	AF033472 <b>OP070827</b>	-	MN969379 <b>OP022416</b>

**Supplementary Table S1.** Continued.

Species	Representative strain	ITS	act	BenA
<i>Penicillium echinulatum</i>	CBS 317.48 <b>SFC2022_NP058</b>	AF033473 <b>OP070791</b>	-	AY674341 <b>OP022409</b>
<i>Penicillium expansum</i>	CBS 325.48 <b>SFC2022_NP056</b>	AY373912 <b>OP070789</b>	-	AY674400 <b>OP022408</b>
<i>Penicillium exsudans</i>	CGMCC 3.18412 <b>SFC2022_NP085</b>	KX885062 <b>OP070821</b>	-	KX885042 <b>OP022413</b>
<i>Penicillium javanicum</i>	CBS 341.48 <b>SFC2022_NP010</b>	GU981613 <b>OP070738</b>	-	GU981657 <b>OP022406</b>
<i>Penicillium oxalicum</i>	CBS 219.30 <b>SFC2022_NP086</b>	AF033438 <b>OP070822</b>	-	KF296462 <b>OP022414</b>
<i>Penicillium roqueforti</i>	CBS 221.30 <b>SFC2022_NP012</b>	HQ442347 <b>OP070740</b>	-	MN969396 <b>OP022407</b>
<i>Pestalotiopsis anacardiacearum</i>	IFRDCC 2397	NR_120255	-	KC247155
<i>Pestalotiopsis cf. anacardiacearum</i>	<b>SFC2022_NP090</b>	<b>OP070826</b>	-	<b>OP022403</b>
<i>Pestalotiopsis australasiae</i>	CBS 114126	NR_147546	-	KM199409
<i>Pestalotiopsis cf. australasiae</i>	<b>SFC2022_NP050</b>	<b>OP070782</b>	-	<b>OP022390</b>
<i>Pestalotiopsis</i> sp.	<b>SFC2022_NP074</b>	<b>OP070809</b>	-	<b>OP022398</b>
<i>Pestalotiopsis thailandica</i>	MFLUCC 17-1616 <b>SFC2022_NP097</b>	NR_164471 <b>OP070833</b>	-	MK764352 <b>OP022404</b>
<i>Phaeophleospora eucalypticola</i>	CPC 26523 <b>SFC20220715_M09</b>	NR_145123 <b>OP070810</b>	-	-
<i>Phaeosphaeria culmorum</i>	CBS 570.86 <b>SFC2022_NP006</b>	MH861992 <b>OP070734</b>	-	-
<i>Phaeosphaeria oryzae</i>	CBS 110110 <b>SFC2022_NP095</b>	MH862850 <b>OP070831</b>	-	-
<i>Phaeosphaeria spartinicola</i>	CBS 176.91 <b>SFC2022_NP029</b>	MH862249 <b>OP070758</b>	-	-
<i>Plectosphaerella cucumerina</i>	CBS 131739 <b>SFC2022_NP002</b>	NR_171712 <b>OP070758</b>	-	-

**Supplementary Table S1.** Continued.

Species	Representative strain	ITS	act	BenA
<i>Pleosporaceae</i> sp. 1	<b>SFC2022_NP031</b>	<b>OP070760</b>	-	-
<i>Pleosporaceae</i> sp. 2	<b>SFC2022_NP047</b>	<b>OP070777</b>	-	-
<i>Pleosporales</i> sp. 1	<b>SFC2022_NP014</b>	<b>OP070742</b>	-	-
<i>Pleosporales</i> sp. 2	<b>SFC2022_NP035</b>	<b>OP070765</b>	-	-
<i>Pleosporales</i> sp. 3	<b>SFC2022_NP070</b>	<b>OP070804</b>	-	-
<i>Pleosporales</i> sp. 4	<b>SFC2022_NP001</b>	<b>OP070728</b>	-	-
<i>Pseudogymnoascus pannorum</i>	CBS 106.13	MH866140	-	-
	<b>SFC2022_NP072</b>	<b>OP070806</b>	-	-
<i>Pyrenopeziza microspora</i>	CBS 102876	NR_160059	-	-
	<b>SFC2022_NP093</b>	<b>OP070829</b>	-	-
<i>Pyrenopeziza paucisetosa</i>	NNIBRFG27317	MW041623	-	-
	<b>SFC2022_NP025</b>	<b>OP070753</b>	-	-
<i>Remotidymella capsici</i>	CBS 679.77	MN973478	-	MT005578
	<b>SFC2022_NP061</b>	<b>OP070794</b>	-	<b>OP022393</b>
<i>Remotidymella</i> sp.	<b>SFC2022_NP068</b>	<b>OP070801</b>	-	<b>OP022395</b>
<i>Sarocladium strictum</i>	CBS 346.70	NR_111145	-	-
	<b>SFC2022_NP071</b>	<b>OP070805</b>	-	-
<i>Sedecimiella taiwanensis</i>	MUT<ITA>:5053	KR014368	-	-
	<b>SFC2022_NP003</b>	<b>OP070731</b>	-	-
<i>Septoriella hubertusii</i>	CBS 338.86	NR_155786	-	-
<i>Septoriella</i> cf. <i>hubertusii</i>	<b>SFC2022_NP057</b>	<b>OP070790</b>	-	-
<i>Sphaeropsis sapinea</i>	CBS 393.84	NR_152452	-	-
	<b>SFC2022_NP045</b>	<b>OP070775</b>	-	-
<i>Stemphylium lycopersici</i>	CBS 122639	NR_155002	-	-
	<b>SFC2022_NP062</b>	<b>OP070795</b>	-	-
<i>Stemphylium vesicarium</i>	CBS 109844	MH862840	-	-
	<b>SFC2022_NP011</b>	<b>OP070739</b>	-	-
<i>Talaromyces rugulosus</i>	CBS 371.48	NR_103676	-	KF984575
	<b>SFC2022_NP083</b>	<b>OP070819</b>	-	<b>OP022412</b>
<i>Trichoderma fomiticola</i>	CBS 121136	NR_134391	-	-

**Supplementary Table S1.** Continued.

Species	Representative strain	ITS	<i>act</i>	<i>BenA</i>
<i>Trichoderma fomiticola</i>	<b>SFC2022_01</b>	<b>OP070730</b>	-	-
<i>Trichoderma harzianum</i>	CBS 226.95	AY605713	-	-
	<b>SFC2022_NP064</b>	<b>OP070797</b>	-	-
<i>Valsa ambiens</i>	CFCC 89894	-	-	KU710989

**Supplementary Table S2. Clear zone data for all tested fungal strains.** The degradation abilities are categorized into four levels.

Species	Representative strains	Clear Zone Length (mm)	Degradation ability*
<i>Acremonium cf. fuci</i>	SFC2022_NP059	0.00	(0)
		0.00	(0)
		0.00	(0)
<i>Acremonium fuci</i>	SFC2022_NP060	0.00	(0)
		0.00	(0)
<i>Alternaria alternata</i>	SFC2022_NP008	5.78	(++)
		12.88	(+++)
		5.03	(++)
		9.28	(++)
		3.32	(+)
		3.76	(+)
		4.34	(+)
		4.73	(+)
<i>Alternaria cf. rosae</i>	SFC2022_NP027	5.82	(++)
<i>Alternaria chlamydospora</i>	SFC2022_NP054	1.70	(+)
<i>Apiospora marii</i>	SFC2022_NP020	0.51	(+)
<i>Apiospora rasikravindrae</i>	SFC20220715_M05	0.85	(+)
<i>Aspergillus ochraceus</i>	SFC2022_NP098	2.19	(+)
<i>Aspergillus oryzae</i>	SFC2022_NP081	0.85	(+)
<i>Aspergillus tritici</i>	SFC2022_NP089	2.03	(+)
<i>Aureobasidium melanogenum</i>	SFC2022_NP040	8.28	(++)
<i>Aureobasidium namibiae</i>	SFC2022_NP039	6.24	(++)
<i>Aureobasidium pullulans</i>	SFC2022_NP046	0.56	(+)
<i>Botryosphaeria dothidea</i>	SFC2022_NP084	0.37	(+)
<i>Botrytis cinerea</i>	SFC2022_NP013	1.37	(+)
<i>Chaetomium globosum</i>	SFC2022_NP028	0.00	(0)
<i>Cladosporium allicinum</i>	SFC2022_NP021	13.92	(+++)

\* 0 mm: (0), 0 < (+) ≤ 5 mm, 5 < (++) ≤ 10 mm, 10 < (++) ≤ 15 mm

**Supplementary Table S2.** Continued.

Species	Representative strains	Clear Zone Length (mm)	Degradation ability*
<i>Cladosporium anthropophilum</i>	SFC2022_NP015	9.74	(++)
<i>Cladosporium cf. halotolerans</i>	SFC2022_NP094	8.60	(++)
<i>Cladosporium cf. cladosporioides</i>	SFC2022_NP022	8.22	(++)
<i>Cladosporium funiculosum</i>	SFC20220715_M07	1.66	(+)
<i>Cladosporium halotolerans</i>	SFC2022_NP036	2.46	(+)
<i>Cladosporium perangustum</i>	SFC2022_NP065	3.80	(+)
		9.72	(++)
<i>Cladosporium pseudocladosporioides</i>	SFC2022_NP037	2.90	(+)
		12.57	(+++)
<i>Cladosporium ramotenellum</i>	SFC2022_NP063	5.70	(++)
		5.32	(++)
		7.26	(++)
		1.60	(+)
		2.27	(+)
		3.70	(+)
<i>Cladosporium rectoides</i>	SFC2022_NP016	8.02	(++)
		12.65	(+++)
<i>Cladosporium tenuissimum</i>	SFC2022_NP009	10.83	(+++)
		10.52	(+++)
		9.28	(++)
<i>Cladosporium xanthochromaticum</i>	SFC2022_NP007	11.37	(+++)
<i>Cladosporium xylophilum</i>	SFC2022_NP051	0.65	(+)
		0.49	(+)
<i>Cytospora ceratosperma</i>	SFC20220715_M02	6.17	(++)
<i>Diaporthe cf. arecae</i>	SFC2022_NP017	1.05	(+)
		1.61	(+)
<i>Diaporthe cf. hungariae</i>	SFC2022_NP026	3.15	(+)
		1.80	(+)

\* 0 mm: (0), 0 < (+) ≤ 5 mm, 5 < (++) ≤ 10 mm, 10 < (+++) ≤ 15 mm

**Supplementary Table S2.** Continued.

Species	Representative strains	Clear Zone Length (mm)	Degradation ability*
<i>Diaporthe</i> cf. <i>pseudooculi</i>	SFC2022_NP076	2.18	(+)
<i>Diaporthe</i> cf. <i>sojae</i>	SFC2022_NP099	1.09	(+)
<i>Didymella</i> cf. <i>macrophylla</i>	SFC2022_NP004	1.28	(+)
Didymosphaeriaceae sp. 1	SFC2022_NP096	6.10	(++)
Didymosphaeriaceae sp. 2	SFC2022_NP055	1.13	(+)
<i>Epicoccum</i> cf. <i>duchesneae</i>	SFC2022_NP078	3.58	(+)
<i>Epicoccum</i> cf. <i>hordei</i>	SFC2022_NP073	0.00	(0)
<i>Epicoccum</i> cf. <i>sorghinum</i>	SFC2022_NP023	2.65	(+)
<i>Epicoccum dendrobii</i>	SFC2022_NP049	6.26	(++)
<i>Epicoccum duchesneae</i>	SFC2022_NP077	0.86	(+)
		0.00	(0)
<i>Epicoccum sorghinum</i>	SFC2022_NP079	1.50	(+)
<i>Epicoccum</i> sp.	SFC2022_NP052	0.35	(+)
<i>Epicoccum tritici</i>	SFC2022_NP019	2.34	(+)
<i>Eutypella</i> cf. <i>persica</i>	SFC2022_NP032	0.00	(0)
<i>Fusarium equiseti</i>	SFC2022_NP053	0.48	(+)
		0.78	(+)
<i>Fusarium fujikuroi</i>	SFC2022_NP044	0.73	(+)
Hypocreales sp.	SFC2022_NP033	2.43	(+)
<i>Juxtiphoma</i> cf. <i>eupyrena</i>	SFC2022_NP067	0.00	(0)
<i>Kalmusia araucariae</i>	SFC20220715_M08	0.00	(0)
<i>Morinia</i> cf. <i>acaciae</i>	SFC2022_NP075	1.55	(+)
<i>Neocamarosporium betae</i>	SFC2022_NP005	0.00	(0)
<i>Neocamarosporium solicola</i>	SFC2022_NP041	0.55	(+)
<i>Neocamarosporium</i> sp.	SFC2022_NP042	1.37	(+)
		0.35	(+)
<i>Neodevriesia</i> cf. <i>metrosideri</i>	SFC2022_NP048	6.08	(++)
<i>Neodidymelopsis</i> cf. <i>longicola</i>	SFC2022_NP088	0.00	(0)
		6.42	(++)

\* 0 mm: (0), 0 < (+) ≤ 5 mm, 5 < (++) ≤ 10 mm, 10 < (++) ≤ 15 mm

**Supplementary Table S2.** Continued.

Species	Representative strains	Clear Zone Length (mm)	Degradation ability*
<i>Neodidymelopsis cf. longicola</i>		1.42	(+)
<i>Neodidymelopsis longicolla</i>	SFC2022_NP024	0.77	(+)
<i>Neopestalotiopsis</i> sp.	SFC2022_NP069	0.00	(0)
<i>Neosetophoma cf. poaceicola</i>	SFC2022_NP018	2.31	(+)
<i>Neosetophoma poaceicola</i>	SFC20220715_M06	0.94	(+)
<i>Neosetophoma rosigena</i>	SFC20220715_M03	0.58	(+)
<i>Nigrospora cf. oryzae</i>	SFC2022_NP034	0.00	(0)
<i>Nothophoma quercina</i>	SFC2022_NP038	7.67	(++)
<i>Paraconiothyrium brasiliense</i>	SFC2022_NP043	0.00	(0)
<i>Paradendryphiella arenariae</i>	SFC2022_NP066	0.00	(0)
		0.00	(0)
		0.52	(+)
<i>Paraphoma radicina</i>	SFC2022_NP087	0.77	(+)
<i>Parasarocladium cf. gamsii</i>	SFC20220715_M04	0.49	(+)
		6.26	(++)
<i>Parathyridaria cf. tyrrhenica</i>	SFC2022_NP080	0.00	(0)
		1.46	(+)
<i>Parengyodontium album</i>	SFC2022_NP030	0.00	(0)
<i>Penicillium charlesii</i>	SFC2022_NP082	0.00	(0)
<i>Penicillium commune</i>	SFC2022_NP092	0.00	(0)
		0.00	(0)
<i>Penicillium crustosum</i>	SFC2022_NP091	0.00	(0)
<i>Penicillium echinulatum</i>	SFC2022_NP058	0.37	(+)
<i>Penicillium expansum</i>	SFC2022_NP056	0.00	(0)
<i>Penicillium exsudans</i>	SFC2022_NP085	0.00	(0)
<i>Penicillium javanicum</i>	SFC2022_NP010	0.00	(0)
<i>Penicillium oxalicum</i>	SFC2022_NP086	0.00	(0)
<i>Penicillium roqueforti</i>	SFC2022_NP012	0.07	(+)
<i>Pestalotiopsis cf. anacardiacearum</i>	SFC2022_NP090	0.88	(+)

\* 0 mm: (0), 0 < (+) ≤ 5 mm, 5 < (++) ≤ 10 mm, 10 < (++) ≤ 15 mm

**Supplementary Table S2.** Continued.

Species	Representative strains	Clear Zone Length (mm)	Degradation ability*
<i>Pestalotiopsis cf. australasiae</i>	SFC2022_NP050	0.34	(+)
<i>Pestalotiopsis</i> sp.	SFC2022_NP074	0.90	(+)
<i>Pestalotiopsis thailandica</i>	SFC2022_NP097	1.35	(+)
<i>Phaeophleospora eucalypticola</i>	SFC20220715_M09	13.96	(+++)
<i>Phaeosphaeria culmorum</i>	SFC2022_NP006	0.00	(0)
<i>Phaeosphaeria spartinicola</i>	SFC2022_NP029	5.27	(++)
<i>Phaeosphaeria oryzae</i>	SFC2022_NP095	6.42	(++)
<i>Plectosphaerella cucumerina</i>	SFC2022_NP002	0.39	(+)
Pleosporaceae sp.	SFC2022_NP031	0.42	(+)
Pleosporaceae sp. 2	SFC2022_NP047	2.07	(+)
Pleosporales sp. 1	SFC2022_NP014	0.00	(0)
		0.00	(0)
Pleosporales sp. 2	SFC2022_NP035	0.52	(+)
		2.14	(+)
Pleosporales sp. 3	SFC2022_NP070	0.93	(+)
Pleosporales sp. 4	SFC2022_NP001	2.29	(+)
		4.44	(+)
<i>Pseudogymnoascus pannorum</i>	SFC2022_NP072	0.89	(+)
<i>Pyrenopeziza microspora</i>	SFC2022_NP093	1.24	(+)
<i>Pyrenopeziza paucisetosa</i>	SFC2022_NP025	0.69	(+)
<i>Remotiodidymella cf. capsici</i>	SFC2022_NP061	0.00	(0)
		1.02	(+)
<i>Remotiodidymella</i> sp.	SFC2022_NP068	2.78	(+)
<i>Sarocladium strictum</i>	SFC2022_NP071	7.63	(++)
<i>Sedecimiella taiwanensis</i>	SFC2022_NP003	0.19	(+)
<i>Septoriella cf. hubertusii</i>	SFC2022_NP057	0.65	(+)
<i>Sphaeropsis sapinea</i>	SFC2022_NP045	6.94	(++)
<i>Stemphylium lycopersici</i>	SFC2022_NP062	1.00	(+)
<i>Stemphylium vesicarium</i>	SFC2022_NP011	1.61	(+)

\* 0 mm: (0), 0 < (+) ≤ 5 mm, 5 < (++) ≤ 10 mm, 10 < (++) ≤ 15 mm

**Supplementary Table S2.** Continued.

Species	Representative strains	Clear Zone Length (mm)	Degradation ability*
<i>Stemphylium vesicarium</i>		1.71	(+)
<i>Talaromyces rugulosus</i>	SFC2022_NP083	3.31	(+)
<i>Trichoderma fomiticola</i>	SFC20220715_M01	0.30	(+)
<i>Trichoderma harzianum</i>	SFC2022_NP064	0.41	(+)

\* 0 mm: (0), 0 < (+) ≤ 5 mm, 5 < (++) ≤ 10 mm, 10 < (+++) ≤ 15 mm

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## 7. Abstract in Korean

### 해양 플라스틱에서의 균류 다양성 분석 및 PCL을 이용한 플라스틱 분해능력 조사

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#### 초록

플라스틱 쓰레기는 해양 생태계에 악영향을 끼칠 뿐만 아니라 인간에게 영향을 끼치고 있으며, 그 양은 점점 증가하고 있다. 이러한 환경적 문제를 해결하기 위해 플라스틱 미생물을 이용한 다양한 종류의 연구들이 진행되었다. 하지만 대부분의 연구들은 다양성 조사, 혹은 분해 실험 중 하나로만 이루어져 있다. 플라스틱 균류의 분해 능력과 그 내부의 상호작용을 온전히 이해하기 위해서는, 언급한 두 가지 분석이 동시에 진행될 필요가 있다. 이번 연구에서는 한국 해변과 갯벌에 있는 polyethylene terephthalate (PET)에서 균주를 분리하고, 그들의 분해능력을 polycaprolactone (PCL) agar에 배양하여 clear zone을 관찰하는 분석을 통해 확인하였다. 결과적으로 47개의 해양 플라스틱으로부터 262개의 균주를 분리하였으며, 문자적 동정 기법으로 108 종을 확인하였다. 대부분의 종

들이) Pleosporales, Eurotiales, Cladosporiales 목에 속하는 것을 발견하였다. PCL agar 실험 결과, 87 종은 PCL을 분해할 수 있는 것을 확인하였으며, 그 중 일부는 clear zone 거리가 10 mm가 넘는 강한 PCL 분해자들이었다. 대부분의 종들은 PCL을 약하게 분해하거나, 분해를 못하는 종들이었다. 이전 자료들을 참고하였을 때, 이런 분해력이 저조한 종들은 강한 분해자들이 플라스틱을 분해하여 만들어낸 부산물을 섭취하거나, 혹은 강한 분해자들 자체에 기생하여 사는 균들일 가능성이 있다. 결론적으로, 이번 연구를 통해 해양 PET로부터 분리한 균의 플라스틱 분해 가능성을 제시하였다.

**주요어:** 균 다양성, 해양 균류, 계통학적 분석, 플라스틱 분해,  
Polycaprolactone

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