Culturable Fungal Endophytes Isolated from the Roots of Coastal Plants Inhabiting Korean East Coast

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Abstract Twelve plant species were collected from the east coast of Korea to identify culturable endophytes present in their roots. The fungal internal transcribe spacer (ITS) region (ITS1-5.8SrRNA-ITS2) was used as a DNA barcode for identification of fungi. A total of 194 fungal strains were identified and categorized into 31 genera. The genus *Penicillium* accounted for the largest number of strains, followed by the genus *Aspergillus*. Furthermore, using 5 statistical methods, the diversity indices of the fungi were calculated at the genus level. After comprehensive evaluation, the endophytic fungal group from *Phragmites australis* ranked highest in diversity analyses. Several strains responsible for plant growth and survival (*Penicillium citrinum*, *P. funiculosum*, *P. janthinellum*, *P. restrictum*, and *P. simplicissimum*), were also identified. This study provides basic data on the symbiotic relationship between coastal plants and fungi.

Keywords Coastal plants, Fungal diversity, Fungal endophytes, Korean East Coast

The seashore, located at the boundary of the land and sea, is affected by waves and tides. It has a variety of topographical and ecological characteristics in the form of coastal sand dunes, shore cliffs, and tidal flats. Repeated erosions, sedimentations, and strong winds make the landform of this region unstable. The soil in the coastal region contains high levels of salt and the sandy soil does not efficiently collect water. Frequent rain or drought also appears to be due to influences from the Taebaek Mountains and anticyclones from the East Sea. Thus, the coastal environment is harsh for plant growth.

Despite these problems, many plants inhabit the Korean East Coast. Coastal plants play important roles in the food chain and in the natural habitat of the coastal ecosystem. In addition, coastal plants help microorganisms in the soil to degrade pollutants by providing favorable components like oxygen, bioactive substances, and nutrients. The roots of coastal plants also contribute to stabilization of sandy soils, like sand dunes, via tight anchoring to the rhizosphere.

Symbiosis between plants and microorganisms is very important for the settlement of coastal plants. Soil microorganisms are also directly connected to plant diversity and productivity [1]. Fungi interact with plants and serve as, a partner in plant growth and survival. Endophytic fungi in particular play a variety of roles in their hosts such as defense from pathogenic microorganisms, growth promotion, and solubilization of essential nutrients for host plant [2-5].

The aim of this study was to identify the distribution of endophytic fungi and to analyze their diversity in the roots of coastal plants. On cliffs, sand dunes, and gravelly fields of the Korean East Coast, the isolation of endophytic fungi from plant roots was performed. Every fungal colony from a root was subcultured for isolation of a single strain and recultured to obtain enough mycelia for extraction of genomic DNA. After amplification of the internal transcribed spacer (ITS) region (ITS1-5.8SrRNA-ITS2), the DNA was sequenced and BLASTed against the NCBI GenBank sequence database. Fungi were then categorized into groups based on a
phylogenetic classification system and the identity of the host plants. Through statistical calculation methods, the bio-diversity of each plant was then assessed.

**MATERIALS AND METHODS**

**Plant materials.** Samples from 12 plant species from the coastal cliff areas, sand dunes, and gravelly fields around Yeongdeok and Pohang were collected and used for experiments (Fig. 1). The scientific name and code of plant samples are listed in Table 1. All herbaceous plant samples were collected by more than 5 heads except for *Pinus thunbergii* Parl. et. Parlatore, which is a woody plant. Each plant sample was collected from its plant colony within 1~10 m².

**Isolation and culture of endophytic fungi.** For each plant, more than 80 pieces of root were used. The soil on the plant root samples was removed using incubation with Tween 80 for 10 min, followed by washes with distilled water. Samples were then incubated twice with 1% perchloric acid for 10 min. After preprocessing, the roots were cut into 3~4 cm pieces and dehydrated. To isolate endophytic fungi, root samples were placed on Hagem minimal media (HM) containing 80 ppm streptomycin and incubated at 25°C [6, 7]. Colonies at the tip of roots were streaked on HM and incubated again at 25°C. Fungal isolates were then transferred onto potato dextrose agar to obtain a pure culture. Pure cultures of endophytic fungi were cultivated in potato dextrose broth for 7~14 days at 25°C and 100 rpm. Finally, the samples were lyophilized and used for identification.

**DNA extraction, PCR amplification, and the identification of fungal strains.** The fungal genomic DNA was extracted using the DNeasy plant mini kit (Qiagen, Valencia, CA, USA) and identified by means of sequencing of the ITS region with the universal primers ITS-1 (5’-TCC GTA GGT GAA CCT GCG G-3’) and ITS-4 (5’-TCC TCC GCT TAT TGA TAT GC-3’). The reaction cycles consisted of initial denaturation (95°C, 2 min), 35 cycles of denaturation (95°C, 30 sec), annealing (55°C, 1 min), extension (72°C, 1 min), and final extension (72°C, 7 min). The PCR products were analyzed using agarose gel electrophoresis with ethidium bromide (EtBr) staining. The products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced by means of the ABI PRISM BigDye Terminator Cycle Sequencing Kit (PE Biosystems, Foster, CA, USA) on an ABI 310 DNA sequencer (Perkin Elmer, Foster, CA, USA). After preprocessing, the resulting DNA sequence was identified using the BLASTN tool of the National Center for Biotechnology Information (NCBI) nucleotide collection (nr/nt) database.

**Statistical analysis of fungal communities.** Fungal generic richness and diversity were analyzed at the genus

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**Fig. 1.** The geographical locations of sampling sites used for this study. Twelve plant samples from the Korean East Coast were collected in Uljin, Yeongdeok, and Pohang.

**Table 1.** The scientific name, abbreviated plant name, and taxon (family) of 12 sampled plants

| No. | Scientific name | Abbreviated plant name | Plant taxon (family) |
|-----|-----------------|------------------------|----------------------|
| 1   | *Aster sphathulifolius* Maxim. | As | Compositae/Asteraceae |
| 2   | *Cynodon dactylon* (L.) Pers. | Cd | Gramineae/Poaceae |
| 3   | *Cerastium fischerianum* Ser. | Cf | Caryophyllaceae |
| 4   | *Cnidium japonicum* Miq. | Cj | Umbelliferae/Apicae |
| 5   | *Lysimachia maculata* Larmark | Lm | Primulaceae |
| 6   | *Phragmites australis* | Pa | Gramineae/Poaceae |
| 7   | *Plantago camtschatica* Cham. | Pc | Plantaginaceae |
| 8   | *Pinus thunbergii* Parl. et. Parlatore | Pt | Pinaceae |
| 9   | *Sedum kamtschaticum* Fisch. & Mey. | Sk | Crassulaceae |
| 10  | *Spergularia marina* (L.) Griseb. | Sm | Caryophyllaceae |
| 11  | *Sedum oryzifolium* Makino | So | Crassulaceae |
| 12  | *Setaria viridis* var. pachystachys Makino & Nemoto | Sv | Gramineae/Poaceae |
level among the plant samples. Margalef’s index ($D_{mg}$) and Menhinick’s index ($D_{mn}$) were used to assess genus richness [8]. Fisher’s alpha index ($\alpha$), Shannon diversity index ($H'$), and Simpson’s index of diversity were used to evaluate genus diversity [9-11]. The formulas for all calculations method are listed in Table 2.

### Table 2. The diversity index formulas used in this study

| Diversity indices                | Formula                                                                 | Description                                                                 |
|----------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Shannon’s diversity index ($H'$) | $H' = - \sum_{i=1}^{S} p_i \ln p_i$                                   | $n_i$, the number of clones in the ith OTU                                    |
| Simpson’s index of diversity (1-D)| $D = \sum_{i=1}^{S} n_i (n_i - 1)$                                    | $N$, total number of the individuals in each sample                          |
| Menhinick’s index ($D_{mn}$)     | $D_{mn} = \frac{S}{\sqrt{N(N - 1)}}$                                  | $p_i$, $n_i$ over $N$                                                       |
| Margalef’s index ($D_{mg}$)      | $D_{mg} = \frac{(S - 1)}{\ln(N)}$                                    | $S$, the number of different genera in a sample                             |
| Fisher’s alpha index ($\alpha$)  | $\alpha = \frac{\ln(\sum_{i=1}^{R} \frac{n_i}{N})}{\ln(\frac{N}{\alpha})}$ | $\sum_{i=1}^{R} \frac{n_i}{N}$                                             |

### Table 3. Partial identification of the 194 fungal isolates using the internal transcribed spacer sequence analysis

| Fungal isolates | Closest relative based on sequence homology | Similarity (%) | GenBank accession No. |
|-----------------|---------------------------------------------|----------------|-----------------------|
| E-As-1-4        | Penicillium glabrum wuwzqm1 (JN887323)       | 100            | JX238717              |
| E-As-1-7        | Cryptosporiopsis ericae PB 061 (JN655659)    | 99             | JX238718              |
| E-As-2-2        | Leptodontidium orchidicola PA 077 (JN655581) | 99             | JX238719              |
| E-As-3-4        | Alternaria alternata C8 (JF802121)          | 100            | JX238720              |
| E-As-4-1        | Penicillium sclerotiorum NRRL 2074 (AF033404) | 97             | JX238721              |
| E-As-5-1        | Penicillium canescens NRRL 910 (AF033493)    | 99             | JX238722              |
| E-As-5-2        | Cryptosporiopsis ericae 845 (GU394585)       | 99             | JX238723              |
| E-As-6-5        | Penicillium thomii (AF034448)                | 100            | JX238724              |
| E-As-7-1        | Penicillium uralic IZ-56 (HQ637345)          | 99             | JX238725              |
| E-As-7-4        | Penicillium restrictum FRR 332 (AY373928)    | 99             | JX238726              |
| E-As-7-4-1      | Penicillium sclerotiorum FRR 1202 (AY373931) | 99             | JX238727              |
| E-As-8-2        | Ascomycota sp. MG106 (IQ775573)             | 98             | JX238728              |
| E-As-9-2        | Pseudocercosporella fraxini (GU214682)       | 100            | JX238729              |
| E-As-9-4        | Penicillium thomii (FR670338)                | 100            | JX238730              |
| E-As-10-4       | Penicillium thomii song-40 (EU910586)        | 100            | JX238731              |
| E-As-10-6       | Penicillium restrictum (GU565105)            | 99             | JX238732              |
| E-As-10-7       | Penicillium canescens NRRL 910 (AF033493)    | 100            | JX238733              |
| E-As-11-2       | Penicillium sp. 2 JJK-2011 (HM469401)        | 100            | JX238734              |
| E-As-11-5       | Penicillium glabrum 4AC2K (GU372904)         | 99             | JX238735              |
| E-As-12-1       | Cryptosporiopsis ericae voucher (AY853167)   | 100            | JX238736              |
| E-As-12-3       | Penicillium sclerotiorum FRR 1202 (AY373931)| 99             | JX238737              |
| E-As-13-3       | Leptodontidium orchidicola PA 077 (JN655581) | 99             | JX238738              |
| E-As-13-5       | Penicillium funiculosum TS08 (GU980968)      | 99             | JX238739              |
| E-Cd-1-2        | Meyerozyma guilliermondii D-171 (IQ026369)   | 100            | JX238758              |
| E-Cd-2-1        | Oidiodendron griseum UAMH9825 (AF062797)     | 97             | JX238759              |
| E-Cd-2-2        | Penicillium pinophilum (AB455516)            | 100            | JX238760              |
| E-Cd-4-1        | Penicillium chrysogenum M-10 (JQ422603)      | 100            | JX238761              |
| E-Cd-5-2-3      | Penicillium verruculosus ATT0999 (HQ607835)  | 99             | JX238762              |
| E-Cd-6-1        | Aspergillus terreus CY229 (HQ608043)         | 99             | JX238763              |
| E-Cd-8-1        | Trichoderma atroviride E (IQ808079)          | 100             | JX238764              |
| E-Cd-9-1        | Cladosporium cladosporioides (JN655298)      | 99             | JX238765              |
| E-Cd-10-1       | Aspergillus hortai (FR837962)                | 99             | JX238766              |
| E-Cd-10-2       | Aspergillus terreus DBOF49 (JQ724410)        | 99             | JX238767              |

### RESULTS AND DISCUSSION

**Identification of endophytic fungi.** The nucleotide sequences of endophytic fungi were registered in the GenBank database of the National Center for Biotechnology Information (accession Nos. JX238717~JX238739, JX238758~
| Fungal isolates | Closest relative based on sequence homology | Similarity (%) | GenBank accession No. |
|----------------|--------------------------------------------|----------------|----------------------|
| E-Cf-1-1       | Penicillium pinophilum (AB606412)           | 100            | JX238768             |
| E-Cf-1-2       | Penicillium concentricum P11.1 (EU833217)  | 99             | JX238769             |
| E-Cf-1-3       | Cladosporium sp. B11 (HQ969040)            | 100            | JX238770             |
| E-Cf-1-5       | Penicillium sp. Q102 (GU188272)            | 100            | JX238771             |
| E-Cf-3-1-1     | Penicillium sp. GZU-BCECYN45-1 (GU565140)  | 99             | JX238772             |
| E-Cf-3-1-2     | Penicillium concentricum 613 (DQ681333)    | 99             | JX238773             |
| E-Cf-3-2       | Penicillium rolfisi F3-2 (IN252126)        | 100            | JX238774             |
| E-Cf-3-3       | Penicillium sp. OTU870 (GU394555)          | 99             | JX238775             |
| E-Cf-4-2       | Lecanillicium psalliota (AB083034)         | 99             | JX238776             |
| E-Cf-4-3       | Leptosphaeria sp. OTU530 (GU394537)        | 99             | JX238777             |
| E-Cf-4-5       | Phoma radicina TMYN101 (IQ676200)          | 99             | JX238778             |
| E-Cf-5-1       | Cadophora sp. REF041 (JN859261)            | 99             | JX238779             |
| E-Cf-6-1       | Penicillium sp. 250P (EU128637)            | 100            | JX238780             |
| E-Cf-6-2       | Penicillium citrinum MA-8-14 (HQ671192)    | 99             | JX238781             |
| E-Cf-6-3       | Penicillium concentricum P11.1 (EU833217)  | 99             | JX238782             |
| E-Cj-1-1       | Penicillium canamertii QLF59 (FJ025142)    | 100            | JX238783             |
| E-Cj-1-2       | Penicillium marneffi AN5 (JN974772)        | 99             | JX238784             |
| E-Cj-2-2       | Aspergillus aculeatus G2-28 (GU134884)     | 99             | JX238787             |
| E-Cj-2-3       | Penicillium canescens P156_D3_32 (JF311262)| 99             | JX238788             |
| E-Cj-2-4       | Penicillium urticae JZ-56 (HQ637345)       | 100            | JX238789             |
| E-Cj-3-3       | Paraphoma sp. OUCMB1101068 (HQ914822)      | 97             | JX238790             |
| E-Cj-3-6       | Aurobasidium pullulans UM16 (FJ515198)     | 100            | JX238791             |
| E-Cj-4-1       | Penicillium urticae JZ-56 (HQ637345)       | 100            | JX238792             |
| E-Cj-5-1       | Penicillium aculeatum (GU565105)           | 99             | JX238793             |
| E-Cj-5-2       | Penicillium simplicissimum ATT160 (HQ607866)| 100            | JX238794             |
| E-Cj-5-3       | Penicillium sp. 19DL/L (GU066613)          | 100            | JX238795             |
| E-Cj-5-4       | Penicillium restrictum (GU565105)          | 99             | JX238796             |
| E-Cj-7-2       | Aspergillus sp. WZ002 (JQ670919)           | 100            | JX238797             |
| E-Cj-9-2       | Penicillium pinophilum (AB606412)          | 100            | JX238799             |
| E-Cj-10-2      | Penicillium pinophilum (AB606412)          | 100            | JX238799             |
| E-Cj-10-4      | Penicillium urticae JZ-56 (HQ637345)       | 100            | JX238792             |
| E-Cj-11-1      | Phialophora mustea (AB190404)              | 99             | JX238801             |
| E-Cj-12-2      | Penicillium canescens ATT146 (HQ607858)    | 100            | JX238802             |
| E-Cj-12-2-1    | Penicillium corylophilum TBG1-14 (IQ862506)| 99             | JX238803             |
| E-Cj-12-2-2    | Penicillium canescens P27_D3_32 (JF311911)| 100            | JX238804             |
| E-Cj-13-2      | Penicillium sp. TR052 (HQ608086)           | 99             | JX238805             |
| E-Cj-13-3      | Ascochyta sp. MG106 (JQ775573)             | 98             | JX238806             |
| E-Cj-13-4      | Paraphoma sp. 6265 (JN903931)              | 97             | JX238807             |
| E-Cj-13-5      | Paraphoma sp. OUCMB1101068 (HQ914822)      | 97             | JX238808             |
| E-Cj-13-6      | Paraphoma sp. OUCMB1101068 (HQ914822)      | 97             | JX238808             |
| E-Cj-14-1      | Paraphoma sp. 19DL/L (GU066613)            | 100            | JX238795             |
| E-Pa-2-1       | Exserohilum rostratum (JN089762)           | 99             | JX238885             |
| E-Pa-7-3-1     | Penicillium aculeatum (FR670338)           | 100            | JX238887             |
| E-Pa-13-1      | Cladosporium oxysporum W10-02 (IQ775499)   | 100            | JX238888             |
| E-Pa-13-2      | Penicillium aculeatum LP67 (HQ392497)      | 99             | JX238889             |
| E-Pa-13-3      | Penicillium glabrum wwsqm1 (JN887323)      | 100            | JX238890             |
| E-Pa-13-4      | Penicillium restrictum FRR 332 (AY373928)  | 99             | JX238891             |
| E-Pa-3-1-1     | Penicillium purpureascens KUC1788 (HM469419)| 100            | JX238893             |
| E-Pa-3-1       | Aspergillus niger 91718 (JN565296)         | 100            | JX238895             |
| E-Pa-6-2       | Phialophora mustea BAN-C42 (IN123359)      | 99             | JX238897             |
| Fungal isolates | Closest relative based on sequence homology | Similarity (%) | GenBank accession No. |
|-----------------|-------------------------------------------|----------------|----------------------|
| E-Pc-6-3        | Zalerion varium ATCC28878 (AF169303)       | 99             | JX238898             |
| E-Pc-8-1        | Penicillium Thomii song-40 (EU910586)      | 100            | JX238899             |
| E-Pc-8-3        | Penicillium cecidicola NRRL 35466 (DQ123648) | 100             | JX238900             |
| E-Pc-8-5        | Phialophora mustea BAN-C42 (JN123359)      | 99             | JX238901             |
| E-Pc-8-7        | Penicillium kussanovii P266_D1_26 (JF11915) | 99             | JX238902             |
| E-Pc-9-6        | Eladia succula’ HSAUP063252 (FJ914702)    | 99             | JX238903             |
| E-Pc-9-7        | Meyerozyma guilliermondii M63 (HQ857743)   | 100            | JX238904             |
| E-Pc-12-1       | Alternaria alternata C8 (JF802121)         | 100            | JX238905             |
| E-Pc-12-4       | Pleosporales sp. BESC803q (KC007199)       | 100            | KJ511463             |
| E-Pc-13-5       | Fusarium solani FJAT-9245 (JQ277277)       | 100            | JX238905             |
| E-Pt-1-1        | Penicillium aculeatum Z32 (GU384213)       | 99             | JX238909             |
| E-Pt-1-2        | Penicillium pinophilum (AB455516)          | 100            | JX238910             |
| E-Pt-1-3        | Penicillium sp. MJM1981 (HQ671193)         | 100            | JX238911             |
| E-Pt-3-1        | Penicillium ochrochloron LP73 (HQ922499)   | 100            | JX238912             |
| E-Pt-3-2        | Penicillium daleae NRRL 922 (AF033442)     | 99             | JX238913             |
| E-Pt-3-4        | Penicillium sp. CCF3828 (FJ430753)         | 97             | JX238915             |
| E-Pt-3-5        | Penicillium sp. PH66 (FJ379828)            | 97             | JX238924             |
| E-Pt-3-6        | Penicillium sp. 11 (HQ407379)              | 100            | JX238926             |
| E-Pt-4-1        | Merimbla hamicoloides NRRL35712 (GU092965) | 95             | JX238918             |
| E-Pt-4-3        | Penicillium pinophilum KUC1758 (HM469418)  | 100            | JX238919             |
| E-Pt-4-4        | Penicillium sp. CCF3828 (FJ430753)         | 97             | JX238920             |
| E-Pt-4-5        | Penicillium pinophilum SGE75 (JQ776546)    | 100            | JX238921             |
| E-Pt-5-2        | Chaetomium aureum WW05 (GU966501)          | 99             | JX238922             |
| E-Pt-5-3        | Aspergillus terreus A8 (JN129182)          | 100            | JX238923             |
| E-Pt-5-4        | Penicillium sp. FF66 (FJ379828)            | 97             | JX238924             |
| E-Pt-5-5        | Chaetomium sp. 3431 (FJ527879)             | 99             | JX238925             |
| E-Pt-6-1        | Penicillium sp. J1 (HQ407379)              | 100            | JX238926             |
| E-Pt-6-4        | Penicillium purpurogenum 119 (DQ681328)    | 97             | JX238927             |
| E-Pt-7-1        | Fusarium oxysporum K4 (JF807393)           | 100            | JX238928             |
| E-Pt-7-2        | Aspergillus terreus PH4 (JN974766)          | 99             | JX238929             |
| E-Pt-7-3        | Penicillium sp. CMV-2008d (FJ160264)       | 97             | JX238930             |
| E-Pt-7-4        | Aspergillus terreus A9 (JN129183)          | 100            | JX238931             |
| E-Pt-7-5        | Penicillium pinophilum SGE75 (JQ776546)    | 100            | JX238932             |
| E-Pt-8-1        | Aspergillus tubingensis SCGAF0163 (JN851045) | 99             | JX238933             |
| E-Pt-8-2        | Penicillium kussanovii NRRL 3381 (EF422849) | 99             | JX238934             |
| E-Pt-8-3        | Aspergillus terreus DBOF49 (JQ724410)      | 100            | JX238935             |
| E-Pt-8-4        | Penicillium verruculosum ATT281 (HQ607919) | 99             | JX238936             |
| E-Pt-8-5        | Leptosphaeria sp. OTU530 (GU934537)        | 99             | JX238937             |
| E-Pt-9-1        | Penicillium sp. JZ-11 (HQ637350)           | 100            | JX238938             |
| E-Pt-9-3        | Penicillium sp. MJM1981 (HQ671193)         | 99             | JX238939             |
| E-Pt-9-4        | Neosartorya fischeri (AF176661)            | 100            | JX238940             |
| E-Pt-9-6        | Penicillium sp. CCF3828 (FJ430753)         | 97             | JX238941             |
| E-Pt-9-7        | Penicillium sp. 2 JJK-2011 (HM469401)      | 97             | JX238942             |
| E-Pt-10-2       | Acromonium sp. TR080 (HQ608111)            | 97             | JX238943             |
| E-Pt-10-4       | Penicillium purpurigenum 119 (DQ681328)    | 96             | JX238944             |
| E-Sk-1-1        | Penicillium thomii (FR670338)              | 100            | JX238945             |
| E-Sk-1-2        | Penicillium brasiliareum KUC1433 (HM469396) | 99             | JX238946             |
| E-Sk-2-2        | Penicillium restrictum (GU565105)          | 99             | JX238947             |
| E-Sk-3-2        | Meyerozyma guilliermondii PH5 (JN974767)    | 100            | JX238948             |
| E-Sk-4-1-1      | Penicillium sp. JZ-11 (HQ637350)           | 100            | JX238949             |
| E-Sk-5-1        | Pleosporales sp. BESC803q (KC007199)       | 99             | KS11464              |
| E-Sk-5-2        | Geomyces vinacea (AJ608972)                | 99             | JX238960             |
| E-Sk-7-1-1      | Fusarium solani FIAT-9245 (JQ772777)       | 100            | JX238970             |
| E-Sk-8-1        | Penicillium purpurascens KUC1788 (HM469419) | 100            | JX238971             |
| E-Sk-9-1        | Penicillium sp. B1-35 (AB274312)          | 99             | JX238972             |

*Eladia succula* = *Penicillium succulinum.*
A total of 194 fungal isolates were confirmed from plants in the Korean East Coast and classified into 31 genera and 69 species through comparisons with sequences in GenBank.

A total of 194 strains were categorized into the phylum Ascomycota. The class Eurotiomycetes (140 strains) accounted for the highest number of strains followed by the class Dothideomycetes (25 strains), Leotiomycetes (12 strains), Sordariomycetes (11 strains), Saccharomycetes (4 strains), and unclassified Ascomycota (2 strains). At the genus level, \textit{Penicillium} accounted for the highest proportion (112 strains) followed by \textit{Aspergillus} (21 strains).

The genus of each strain was noted and the proportion of each group at the class and genus levels was analyzed (Fig. 2). Eurotiomycetes accounted the highest percentage at the class level; except for the plant Pa, Eurotiomycetes accounted for more than half of the fungi in every plant.

| Fungal isolates | Closest relative based on sequence homology | Similarity (%) | GenBANK No. |
|-----------------|-------------------------------------------|----------------|-------------|
| E-Sk-9-3        | \\textit{Cadophora} sp. REF044 (JX859264) | 99             | JX238973    |
| E-Sk-10-2       | \\textit{Penicillium} sp. FF5 (JF379811)  | 99             | JX238974    |
| E-Sk-10-3       | \\textit{Penicillium thomii} song-40 (EU910586) | 100            | JX238975    |
| E-Sk-10-5       | \\textit{Penicillium canescens} 5237 (JQ390173) | 99             | JX238976    |
| E-Sk-13-2       | \\textit{Penicillium thomii} FRR 2077 (AY373934) | 100            | JX238977    |
| E-Sm-1-1        | \\textit{Phomopsis} columnaris OTU410 (GU934562) | 99             | JX238978    |
| E-Sm-1-2        | Cryptosporiopsis ericae 845 (GU934585) | 99             | JX238979    |
| E-Sm-2-1        | \\textit{Penicillium pinophilum} SGE75 (JQ776546) | 100            | JX238980    |
| E-Sm-3-3        | \\textit{Penicillium pinophilum} SGE75 (JQ776546) | 100            | JX238981    |
| E-Sm-5-1        | Cladosporium silenes CMT48 (JQ754032) | 99             | JX238982    |
| E-Sm-6-2        | \textit{Aspergillus} fumigatus SGE57 (JQ776545) | 100            | JX238983    |
| E-Sm-6-1-2      | \textit{Aspergillus} fumigatus LH02 (GU390693) | 99             | JX238984    |
| E-Sm-7-2        | Oidiodendron echinulatum (AF062791) | 100            | JX238985    |
| E-Sm-8-1        | \textit{Aspergillus} japonicus A11 (EU833207) | 99             | JX238986    |
| E-Sm-9-1        | \textit{Aspergillus} japonicus B4 (JN676110) | 99             | JX238987    |
| E-So-1-4        | \textit{Penicillium} spinulosum TTMF2 (JN863099) | 100            | JX238988    |
| E-So-2-1-3      | \\textit{Talaromyces} trachyspermus C234 (GQ363516) | 99             | JX238989    |
| E-So-3-1        | Epicoccum nigrum HLJ_9 (IN882832) | 99             | JX238990    |
| E-So-3-9        | \textit{Aspergillus} aculeatus M9 (JQ670921) | 99             | JX238991    |
| E-So-7-1-1      | \\textit{Penicillium} purpurascens KUC1788 (HM469419) | 100            | JX238992    |
| E-So-7-5        | Alternaria alternata C8 (JF802121) | 99             | JX238993    |
| E-So-8-1-3      | \textit{Aspergillus} aculeatus M9 (JQ670921) | 99             | JX238994    |
| E-So-8-2        | \textit{Penicillium} brasiliense KUC1433 (HM469396) | 99             | JX238995    |
| E-So-9-2        | Fusarium oxysporum REF214 (IN859434) | 100            | JX238996    |
| E-So-9-6        | Alternaria alternata C5 (JF802118) | 99             | JX238997    |
| E-So-9-8        | \textit{Penicillium} decaturense KUC1922 (HM469399) | 100            | JX238998    |
| E-So-10-1       | \textit{Penicillium} sp. TR052 (HQ608086) | 99             | JX238999    |
| E-So-13-1       | \textit{Penicillium thomii} (FR670339) | 100            | JX239000    |
| E-So-15-2       | \textit{Penicillium} janthinellum A1080945 | 99             | JX239001    |
| E-Sv-1-1        | \textit{Penicillium} sp. FF12 (JF379812) | 100            | JX239019    |
| E-Sv-2-1        | \textit{Penicillium} sp. OUCMBII071031 (HQ997375) | 99             | JX239020    |
| E-Sv-3-2        | \textit{Exserohilum} pedicellatum EEB 1336 (AF229478) | 99             | JX239021    |
| E-Sv-3-4        | \textit{Paeconium} lilacinus MY683 (GU980015) | 99             | JX239022    |
| E-Sv-4-1        | \textit{Aspergillus} niger (GU951769) | 99             | JX239023    |
| E-Sv-4-2        | \textit{Penicillium} funiculosum TS08 (GU980968) | 99             | JX239024    |
| E-Sv-4-3        | \textit{Penicillium} sp. JZ-10 (HQ637349) | 99             | JX239025    |
| E-Sv-5-2        | \textit{Penicillium} pannosum CBS 126341 (IN617677) | 99             | JX239026    |
| E-Sv-6-6        | \textit{Penicillium} citrinum NSF4 (JQ712839) | 100            | JX239027    |
| E-Sv-6-3        | \textit{Capnodium} sp. OUCMBII0110100 (HQ914834) | 97             | JX239028    |
| E-Sv-8-2        | \textit{Penicillium} sclerotiorum FRR 1202 (AY373931) | 99             | JX239029    |
| E-Sv-8-3        | \textit{Aspergillus} aculeatus M9 (JQ670921) | 99             | JX239030    |
| E-Sv-8-4        | \textit{Aspergillus} japonicus Yang1 (GQ396635) | 100            | JX239031    |
| E-Sv-9-1        | \textit{Penicillium} pinophilum SGE75 (JQ776546) | 99             | JX239032    |
| E-Sv-10-1       | \textit{Penicillium} citrinum SCSAA0015 (JQ647899) | 99             | JX239033    |
| E-Sv-10-2       | \textit{Penicillium} griseofulvum 091402 (EU664471) | 99             | JX239034    |
| E-Sv-10-3       | \textit{Penicillium} sp. JZ-10 (HQ637349) | 99             | JX239035    |
sample. At the genus level, *Penicillium* was the most prevalent (57.7%), followed by *Aspergillus* (10.8%), of the total fungal isolates in plant samples (except for Sm). The rest of the genera constituted 0.5~2.6%. The distribution of endophytic fungi from roots of coastal plants in the East Coast was similar to that of Ulleung Island [12]. Several plants, including *Aster sphathulifolius*, *Plantago camtschatica*, *Sedum oryzifolium*, and *Setaria viridis* Makino & Nemoto, inhabit the Ulleung Island in the East Sea. Ulleung Island in the East Sea, which is 130 km from the Korean Peninsula. The climate and vegetation of Ulleung Island are thus very similar to the East Coast and members of the genus *Penicillium* accounted for the highest percentage there as well.

### Fungal diversity at the genus level in the sampled plants.

Depending on the plants, fungal isolates were categorized into 5 genera and 11 species from As, 6 genera and 9 species from Cd, 6 genera and 7 species from Cf, 6 genera and 14 species from Cj, 2 genera and 3 species from Lm, 5 genera and 5 species from Pa, 8 genera and 14 species from Pt, 5 genera and 8 species from Sk, 6 genera and 7 species from Sm, 6 genera and 11 species from So, and 5 genera and 11 species from Sv (Table 4).

Based on counting of genera by plant samples, generic richness and diversity were calculated (Table 5). In terms of generic richness, Pc had the highest score in Margalef’s diversity index.

### Table 4. Diversity of fungal isolates according to host plant

| Abbreviated plant name | No. of isolates | Taxon of fungi |
|------------------------|-----------------|----------------|
| As                     | 23              | 5 genera, 11 species, 2 unclassified (1 species, 1 genus) |
| Cd                     | 10              | 6 genera, 9 species |
| Cf                     | 16              | 6 genera, 7 species, 2 unclassified (species level) |
| Cj                     | 25              | 6 genera, 14 species, 7 unclassified (6 species, 1 genus) |
| Lm                     | 3               | 2 genera, 3 species |
| Pa                     | 6               | 5 genera, 5 species, 1 unclassified (species) |
| Pc                     | 19              | 8 genera, 15 species, 3 unclassified (2 species, 1 genus) |
| Pt                     | 36              | 8 genera, 14 species, 15 unclassified (species) |
| Sk                     | 15              | 5 genera, 8 species, 6 unclassified (5 species, 1 genus) |
| Sm                     | 10              | 6 genera, 7 species |
| So                     | 14              | 6 genera, 11 species, 1 unclassified (species) |
| Sv                     | 17              | 5 genera, 11 species, 5 unclassified (species) |

As, *Aster sphathulifolius* Maxim.; Cd, *Cynodon dactylon* (L.) Pers.; Cf, *Cerastium fischerianum* Ser.; Cj, *Cnidium japonicum* Miq.; Lm, *Lysimachia mauritiana* Larmark; Pa, *Phragmites australis*; Pc, *Plantago camtschatica* Cham.; Pt, *Pinus thunbergii* Parlatore; Sk, *Sedum kamtschaticum* Fisch. & Mey.; Sm, *Spergularia marina* (L.) Griseb.; So, *Sedum oryzifolium* Makino; Sv, *Setaria viridis* var. pachystachys Makino & Nemoto.
index (2.38), and Pa had the highest score in Menhinick’s index (2.04). In generic diversity, Pa exhibited the highest score according to Fisher’s \( \alpha \) (14.12) and Simpson’s index of diversity (0.93), and Pc had the highest score according to Shanon’s index (1.66). This result is likely due to the higher sensitivity of Fisher’s \( \alpha \) and Simpson’s to evenness than Shanon’s index [8]. Because the genus *Penicillium* was the dominant genus in all plant samples (except for Sm), comparison of evenness does not mean much. When looking only at results of calculations of diversity index formulas, Pa is regarded as the environment that the most diverse endophytic fungi can inhabit.

Symbiotic fungi can help plants overcome abiotic stressors like drought, heat, and salinity [13]. In particular, coastal plants are frequently exposed to salt stress from scattered seawater or via permeation of saline ground water. *P. funiculorum* and *P. janthinellum*, fungal strains identified there, reportedly promote resistance to salt stress in the host [14, 15].

*Penicillium citrinum* was isolated from Cf, Lm, and Sv. *P. citrinum* reportedly produces gibberellins for the host plant [16]. Gibberellins are essential for developmental stages, including leaf expansion, pollen maturation, seed germination, stem elongation [17], and affect the growth and settlement

| Fungal taxon                  | As | Cd | Cf | Cj | Lm | Pa | Pc | Pt | Sk | Sm | So | Sv |
|------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|
| Acremonium                   | 1  |    |    |    |    |    |    |    |    |    |    |    |
| Alternaria                   |    | 1  |    |    |    |    |    |    |    |    |    |    |
| Aspergillus                  | 1  | 2  | 1  | 1  | 5  | 4  | 2  | 3  |    |    |    |    |
| Aureobasidium                | 1  |    |    |    |    |    |    |    |    |    |    |    |
| Cadophora                    | 1  |    |    |    |    |    |    |    |    |    |    |    |
| Capnodium                    |    |    |    |    |    |    |    |    |    |    |    |    |
| Chaetomium                   |    |    |    |    |    |    |    |    | 2  |    |    |    |
| Cladosporium                 | 1  | 1  | 1  |    |    |    |    |    |    |    |    |    |
| Cryptosporiopsis             | 3  |    |    |    |    |    |    |    |    |    |    |    |
| Epicoccum                    |    |    |    |    |    |    |    |    |    |    |    | 1  |
| Exserohilum                  |    | 1  |    |    |    |    |    |    |    |    |    |    |
| Fusarium                     |    | 1  | 1  | 1  |    |    |    |    |    |    | 1  |    |
| Geomyces                     |    |    |    |    | 1  |    |    |    |    |    |    |    |
| Lecanicillium                |    | 1  |    |    |    |    |    |    |    |    |    |    |
| Leptodontidium               | 2  |    |    |    |    |    |    |    |    |    |    |    |
| Leptosphaeria                | 1  | 1  |    |    |    |    |    |    |    |    |    |    |
| Merimbla                     |    |    |    |    |    |    |    |    |    |    |    | 1  |
| Meyerozyma                   | 1  | 1  | 1  |    |    |    |    |    |    |    |    |    |
| Neosartorya                  |    |    |    |    |    |    |    |    |    |    |    | 1  |
| Oidiodendron                 |    | 1  |    |    |    |    |    |    |    |    |    |    |
| Paecilomyces                 |    |    |    |    |    |    |    |    |    |    |    | 1  |
| Paraphoma                    |    |    |    |    |    |    |    |    |    |    | 4  |    |
| Penicillium                  | 15 | 3  | 15 | 15 | 10 | 10 | 2  | 1  |    |    |    |    |
| Phialophora                  |    | 1  | 1  |    |    |    |    |    |    |    |    |    |
| Phoma                        |    | 1  |    |    |    |    |    |    |    |    |    |    |
| Phomopsis                    |    |    |    |    |    |    |    |    |    |    |    | 1  |
| Pseudocercosporella          |    | 1  |    |    |    |    |    |    |    |    |    |    |
| Ramichloridium               |    |    |    |    |    |    |    |    |    |    |    | 1  |
| Talaromyces                  |    |    |    |    |    |    |    |    |    |    | 1  |    |
| Trichoderma                  |    |    |    |    |    |    |    |    |    |    |    | 1  |
| Zalerion                     |    |    |    |    |    |    |    |    |    |    |    |    |
| Generically unclassified     |    | 1  | 1  | 1  |    |    |    |    |    |    |    | 1  |

**As**, *Aster sphathulifolius* Maxim.; **Cd**, *Cynodon dactylon* (L.) Pers.; **Cf**, *Cerastium fischerianum* Ser.; **Cj**, *Cnidium japonicum* Miq.; **Lm**, *Lysimachia mauritiana* Lamark; **Pa**, *Phragmites australis*; **Pc**, *Plantago camtschatica* Cham.; **Pt**, *Pinus thunbergii* Parlatore; **Sk**, *Sedum kamtschaticum* Fisch. & Mey.; **Sm**, *Spergularia marina* (L.) Griseb.; **So**, *Sedum oryzifolium* Makino; **Sv**, *Setaria viridis* var. pachystachys Makino & Nemoto.
during the early stage of plant growth. Thus, these 2 species likely help their plant host to absorb nutrients, and they also promote host's growth.

Two of the identified species are known to improve the resilience of plant-host defense systems against pathogens. *P. simplicissimum* has been reported to activate multiple host defense signals [18] and *P. restrictum* exerts antagonistic action on pathogenic fungi [19]. Because plants are exposed to a large number of pathogenic microorganisms in the soil or air, it is important for plants to possess such defense systems.

In summary, a total of 194 fungal strains were isolated from 12 plants inhabiting the East Coast and were classified into 1 phylum, 5 classes, 11 orders, 16 families, and 31 genera. *Penicillium* (class Eurotiomycetes) was the most dominant genus followed by *Aspergillus*. The group of fungi isolated from *Phragmites australis* was the most diverse according to diversity analysis. Species helping plant growth and survival such as *P. citrinum*, *P. funiculosum*, *P. janthinellum*, *P. restrictum*, and *P. simplicissimum* were also identified. This study provides basic data on the symbiosis of coastal plants and fungi.

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