Diversity and Plant Growth Promoting Capacity of Endophytic Fungi Associated with Halophytic Plants from the West Coast of Korea

Irina Khalmuratova†, Hyun Kim†, Yoon-Jong Nam†, Yoosun Oh†, Min-Ji Jeong†, Hye-Rim Choi†, Young-Hyun You†, Yeon-Sik Choo2, In-Jung Lee3, Jae-Ho Shin3, Hyeokjun Yoon4, and Jong-Guk Kim4,*

1School of Life Sciences and Biotechnology, Institute for Microorganisms, Kyungpook National University, Daegu 41566, Korea
2Department of Biology, College of National Sciences, Kyungpook National University, Daegu 41566, Korea
3School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea
4Biological and Genetic Resources Assessment Division, National Institute of Biological Resources, Incheon 22689, Korea

Abstract Five halophytic plant species, Suaeda maritima, Limonium tetragonum, Suaeda australis, Phragmites australis, and Suaeda glauca Bunge, which are native to the Muan salt marsh of South Korea, were examined for fungal endophytes by sequencing the internal transcribed spacer (ITS) region containing ITS1, 5.8S rRNA, and ITS2. In total, 160 endophytic fungal strains were isolated and identified from the roots of the 5 plant species. Taxonomically, all 160 strains belonged to the phyla Ascomycota, Basidiomycota, and Zygomycota. The most dominant genus was Fusarium, followed by the genera Penicillium and Alternaria. Subsequently, using 5 statistical methods, the diversity indices of the endophytes were determined at genus level. Among these halophytic plants, P. australis was found to host the greatest diversity of endophytic fungi. Culture filtrates of endophytic fungi were treated to Waito-C rice seedlings for plant growth-promoting effects. The fungal strain Su-3-4-3 isolated from S. glauca Bunge provide the maximum plant length (20.1 cm) in comparison with wild-type Gibberella fujikuroi (19.6 cm). Consequently, chromatographic analysis of the culture filtrate of Su-3-4-3 showed the presence of physiologically active gibberellins, GA1 (0.465 ng/mL), GA3 (1.808 ng/mL) along with other physiologically inactive GA9 (0.054 ng/mL) and GA24 (0.044 ng/mL). The fungal isolate Su-3-4-3 was identified as Talaromyces pinophilus.

Keywords Fungal endophytes, Genetic diversity, Gibberellin, Halophytic plants, Plant growth promotion, Salt marsh

Marshes are transitional areas between terrestrial and aquatic ecosystems and are dominated by various living species that provide numerous ecological services such as coastal protection, carbon sequestration, and buffering of coastal waters from terrestrial pollutants, which helps to improve water quality. Coastal salt marshes also reduce storm damage by absorbing high wind and wave energy [1]. Salt marshes contain highly diverse hydrophytes, salt-tolerant plants, and microorganisms [2]. Soil microbes are directly connected to the productivity and diversity of plants [3]. Symbiosis between plants and microbes is important for the settlement of coastal plants.

Endophytes are microorganisms (fungi, actinomycetes, and other bacteria) that live within host plant tissues without causing any detectable symptoms of disease to the host. Endophytic microorganisms have been isolated from nearly all plant families, including species growing in many different climatic regions. Fungal endophytes live in symbiotic association with all plants in natural ecosystems, play an important role in the resistance of plants to various diseases and abiotic and biotic stresses, and also promote plant growth [4, 5]. Such symbiotic fungal endophytes produce a number of important plant hormones including gibberellins (GAs), indole acetic acid, and abscisic acid [6, 7]. The purpose of the present study was to investigate the distribution of fungal endophytes in the roots of halophytic plants and analyze their diversity. Additionally, isolated strains were screened on Waito-C rice seedlings to investigate...
their plant growth promoting activity. The fungal culture filtrates were subjected to chromatographic techniques to isolate and detect secondary metabolites.

**MATERIALS AND METHODS**

**Collection of plant materials.** For the isolation of fungal endophytes, plant samples were collected from a salt marsh located in Muan County in South Korea. Healthy and fresh roots of the plants *Suaeda maritima*, *Limonium tetragonum*, *Suaeda australis*, *Phragmites australis*, and *Suaeda glauca* Bunge were washed with tap water to remove sand particles and treated with Tween 80 solution (200 µL in 100 mL distilled water) for 10 min. Samples were surface sterilized twice with 1% (w/v) perchloric acid solution for 10 min, followed by washing with distilled water. The sterilized roots were cut into 3~4 cm pieces, cultivated on Hagem minimal media containing streptomycin, and incubated at 25°C. After the emergence of fungi from inside the root pieces, the fungi were then transferred onto potato dextrose agar. The isolated pure cultures of root fungi were stored on potato dextrose agar plates and slants.

**Isolation of endophytic fungi from roots.** Root samples of the halophytes were washed with tap water to remove sand particles and treated with Tween 80 solution (200 µL in 100 mL distilled water) for 10 min. Samples were surface sterilized twice with 1% (w/v) perchloric acid solution for 10 min, followed by washing with distilled water. The sterilized roots were cut into 3~4 cm pieces, cultured on Hagem minimal media containing streptomycin, and incubated at 25°C. After the emergence of fungi from inside the root pieces, the fungi were then transferred onto potato dextrose agar. The isolated pure cultures of root fungi were stored on potato dextrose agar plates and slants.

**DNA extraction, PCR, and identification.** The fungal strains were subcultured and incubated in potato dextrose broth for 6~8 days. For DNA extraction, mycelia of fungi were transferred into 100 mL Erlenmeyer's flask containing 50 mL potato dextrose broth medium in a shaking incubator for 7~9 days at 28 ± 2°C and 110 rpm. The lyophilized samples were used for identification. Fungal genomic DNA was isolated using a DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. PCR was performed using the primers ITS1 (5’-TCC GTG GGT GAA CCT GCG G-3’) and ITS4 (5’-TCC TCC GCT TAT TGA TAT GC-3’). The following PCR thermal cycle parameters were used: 95°C for 2 min, 35 cycles of 30 sec at 94°C, 40 sec at 55°C, and 35 sec at 72°C, and a final extension step at 72°C for 7 min. The amplified products were observed by agarose gel electrophoresis with ethidium bromide staining. The resulting products were purified using a PCR Purification Kit (Qiagen) and then sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 310 DNA Sequencer (Applied Biosystems). Sequences were identified using the Basic Local Alignment Search Tool search program (http://www.ncbi.nlm.nih.gov/BLAST/) of the National Center for Biotechnology Information (Bethesda, MD, USA).

**Statistical analysis of fungi.** The richness and diversity of fungi were analyzed at the genus level in the plant samples. Fungal genus diversity was evaluated using the Simpson's, Fisher's alpha (α), and Shannon (H) indices of diversity [10-12]. Menhinick's (Dmm) and Margalef’s (Dmg) indices were used to determine the richness of each genus among the fungal community [13, 14]. The formulas used for calculating diversity indices are listed in Table 2.

**Bioassay on Waito-C rice seedlings.** The filtered culture media of the isolated fungal strains were screened on Waito-C rice seedlings for their plant growth promoting activity. The fungal isolates were grown on a shaking incubator for 7 days at 25°C and 180 rpm on Czapek broth medium. Forty-five milliliters of culture fluid was harvested and the supernatant was stored at −70°C and then lyophilized. The lyophilized supernatants were mixed with 1 mL of autoclaved distilled water. The Waito-C rice seeds were surface sterilized in spotac solution for 1 day and their germination was used as the output of the bioassay.

**Table 1. Geographic coordinates and scientific names of the native plants in the Muan salt marsh**

| No. | Scientific name         | Code | Site of collection | Habitat    |
|-----|-------------------------|------|--------------------|------------|
| 1   | *Suaeda maritima*       | Sm   | N 35°45‘37.48”, E 126°23‘30.39” | Halophytic |
| 2   | *Limonium tetragonum*   | Lt   | N 35°35‘42.22”, E 126°21‘59.84” | Halophytic |
| 3   | *Suaeda australis*      | Sa   | N 35°35‘22.69”, E 126°22‘20.29” | Halophytic |
| 4   | *Phragmites australis*  | Pa   | N 35°35‘22.23”, E 126°21‘59.93” | Halophytic |
| 5   | *Suaeda glauca* Bunge   | Su   | N 35°44‘56”, E 126°22‘14.7” | Halophytic |

**Table 2. Formulas of the diversity indices used in this study**

| Diversity indices | Formula |
|------------------|---------|
| Shannon diversity index (H) | \( H' = - \sum_{i=1}^{S} p_i \cdot \ln p_i \) |
| Simpson’s index of diversity (1 − D) | \( D = \frac{\sum_{i=1}^{S} n_i(n_i - 1)}{N(N - 1)} \) |
| Menhinick’s index (Dmm) | \( Dmn = \frac{S}{\sqrt{N}} \) |
| Margalef’s index (Dmg) | \( Dmg = \frac{(S - 1)}{\ln(N)} \) |
| Fisher’s alpha index (α) | \( S = \alpha \cdot \ln \left( 1 + \frac{N}{\alpha} \right) \) |

ni, number of clones in the ith OUT; N, total number of individuals in each sample; pi, ni over N; S, number of different genera in a sample.
treated with growth inhibitor uniconazol (20 ppm). After treatment Waito-C rice seeds were washed clearly and soaked in autoclaved distilled water until sprouts emerged. These Waito-C rice seedlings were transplanted in glass tubes containing 0.6% water-agar medium and grown in a growth chamber. When Waito-C rice seedlings had reached the two leaf stage, the meristems were treated with 10 µL of supernatant solution of each fungal culture filtrates. One week after treatment, the shoot and plant length was observed and compared with culture filtrate of *Gibberella fujikuroi*, lyophilized Czapek broth medium, and distilled water. Culture filtrates of *G. fujikuroi*, Czapek broth medium and distilled water were applied as controls to determine the shoot and plant length of Waito-C rice seedlings.

**Quantification of endogenous GAs.** The culture filtrates of fungal isolate was analyzed for the presence of GAs by gas chromatography/mass spectrometry (GC/MS). For these experiments, fungal strains were cultured in 250 mL Czapek broth medium for 7 days at 25°C in a shaking incubator at 180 rpm. The extracted GAs was analyzed with reverse-phase C<sub>n</sub> high-performance liquid chromatography (HPLC). The fractions were collected and were prepared for GC/MS with a selected ion monitoring (SIM). After the GC/MS data were analyzed, the three major ions of the supplemented [2H₂] GAs internal standards and the fungal GAs were monitored. The retention time was determined using hydrocarbon standards to calculate the Kovats Retention Index value, while the GAs quantification was based on peak area rations of nondeuterated (extracted) GAs to deuterated GAs.

**RESULTS AND DISCUSSION**

**Endophyte identification.** The endophytic fungal strains were identified by obtaining the nucleotide sequences of

| Fungal isolates | Closest relative based on sequence homology | Similarity (%) | Accession No. |
|-----------------|-------------------------------------------|----------------|---------------|
| Sm-1-1-3        | *Fusarium andiyazi* strain CBS 134430 (KC954400) | 100 | KP017770 |
| Sm-1-2-3        | *Fusarium luteum* (HG423537) | 99 | KP017771 |
| Sm-1-4-1        | *Pestalotiopsis clavispora* isolate Ara-1 (JQ008944) | 100 | KP017773 |
| Sm-1-5-3        | *Fusarium incarnatum* strain FI-00602 (KJ572780) | 99 | KP017774 |
| Sm-1-6-4        | *Fusarium incarnatum* strain FI-00602 (KJ572780) | 99 | KP017775 |
| Sm-1-9-2        | *Fusarium incarnatum* strain FI-00602 (KJ572780) | 99 | KP017776 |
| Sm-1-9-3        | *Penicillium expansum* strain SY20-5 (KJ619622) | 100 | KP017777 |
| Sm-1-9-4        | *Penicillium expansum* strain VG100 (KJ619622) | 100 | KP017778 |
| Sm-2-1-1        | *Fusarium oxysporum* strain C-2 (KJ623246) | 99 | KP017779 |
| Sm-2-3-3        | *Penicillium expansum* strain VG100 (KJ619622) | 99 | KP017780 |
| Sm-2-3-4        | *Fusarium luteum* (HG423537) | 99 | KP017781 |
| Sm-2-4-1        | *Fusarium oxysporum* strain C-2 (KJ623246) | 99 | KP017782 |
| Sm-2-5-1        | *Bionectria pseudochroleuca* (KJ499909) | 99 | KP017783 |
| Sm-2-5-1-1      | *Paraphaeosphaeria sporulosa* strain CBS (JX496066) | 100 | KP017784 |
| Sm-2-6-1        | *Talaromyces marneffei* strain LCC29 (KF990145) | 99 | KP017785 |
| Sm-2-7-3-1      | *Fusarium caeruleum* (KJ680136) | 99 | KP017786 |
| Sm-2-7-3-2      | *Fusarium caeruleum* (KJ680136) | 99 | KP017787 |
| Sm-2-8-2        | *Bionectria pseudochroleuca* (KJ499909) | 99 | KP017788 |
| Sm-2-9-1        | *Fusarium oxysporum* strain HPA2 (KJ677253) | 100 | KP017789 |
| Sm-2-10-1       | *Penicillium brasiliense* strain 028M (KJ458973) | 99 | KP017790 |
| Sm-2-10-2       | *Fusarium longipes* (HG423537) | 98 | KP017791 |
| Sm-3-1-1        | *Alternaria alternata* isolate SDAU (KJ682318) | 100 | KP017792 |
| Sm-3-1-2        | *Fusarium oxysporum* (KJ653447) | 99 | KP017793 |
| Sm-3-1-3        | *Alternaria alternata* strain HMA1D (KJ677246) | 100 | KP017794 |
| Sm-3-1-4        | *Fusarium oxysporum* (KJ653447) | 99 | KP017795 |
| Sm-3-1-5        | *Fusarium oxysporum* strain HPA2 (KJ677253) | 99 | KP017796 |
| Sm-3-2-2        | *Fusarium incarnatum* strain FI-00602 (KJ572780) | 100 | KP017797 |
| Sm-3-2-4        | *Fusarium oxysporum* (KJ653447) | 99 | KP017798 |
| Sm-3-3-1        | *Fusarium oxysporum* (KJ653447) | 99 | KP017799 |
| Sm-3-3-3        | *Rhinocladiella similis* strain 152wat (KF811431) | 100 | KP017800 |
| Sm-3-3-6        | *Pestalotiopsis sp.* GRPS-3 (KF564287) | 100 | KP017801 |
| Sm-3-4-3-1      | *Monochaetia karstenii* (KC537806) | 99 | KP017802 |
| Sm-3-4-4        | *Talaromyces albobicervicillus* strain CBS (KF114736) | 100 | KP017803 |
| Fungal isolates | Closest relative based on sequence homology | Similarity (%) | Accession No. |
|----------------|------------------------------------------|----------------|---------------|
| Sm-3-4-5-1     | Fusarium armeniacum (KC477845)           | 99             | KP017806      |
| Sm-3-5-1       | Fusarium oxysporum strain C-2 (KJ623246) | 100            | KP017807      |
| Sm-3-5-2       | Fusarium incarnatum strain FI-00602 (KJ572780) | 100           | KP017808      |
| Sm-3-5-3       | Fusarium anthophilum (KJ598869)          | 100            | KP017809      |
| Sm-3-5-5       | Fusarium longipes (HG423537)             | 99             | KP017810      |
| Sm-3-6-1       | Aspergillus brasiliensis (KJ677257)      | 100            | KP017811      |
| Sm-3-6-2       | Fusarium oxysporum (KJ653447)            | 100            | KP017812      |
| Sm-3-6-3       | Fusarium oxysporum (KJ653447)            | 100            | KP017813      |
| Sm-3-7-2       | Alternaria alternata strain HMA3B (KJ677249) | 100           | KP017814      |
| Sm-3-8-1       | Fusarium oxysporum strain C-2 (KJ623246) | 100           | KP017815      |
| Sm-3-8-2       | Fusarium longipes (HG423537)             | 99             | KP017816      |
| Sm-3-8-2-1     | Talaromyces marneffei strain LCC29 (KJ990145) | 99           | KP017817      |
| Sm-3-8-3       | Pestalotiopsis clavispora strain P44 (JX045813) | 100       | KP017818      |
| Sm-3-8-4       | Talaromyces marneffei strain LCC29 (KJ990145) | 100       | KP017819      |
| Sm-3-9-6       | Aspergillus brasiliensis (KJ677257)      | 100            | KP017820      |
| Sm-3-10-1      | Penicillium oxalicum strain TMP53 (DQ986355) | 99           | KP017821      |
| Sm-3-10-2      | Talaromyces pinophilus isolate OK3SP103P (KF871458) | 99     | KP017822      |
| Lt-1-1-1       | Ophiostepella agrostis isolate ZJ5 (KJ572127) | 99           | KP017823      |
| Lt-1-3-2       | Trichoderma harzianum strain ML16-1 (KJ619615) | 100       | KP017824      |
| Lt-1-7-1       | Alternaria alternata strain HMA1D (KJ677246) | 100       | KP017825      |
| Lt-1-8-2       | Alternaria tenuissima (GQ503332)         | 100            | KP017826      |
| Lt-1-10-2      | Botryosphaeria sp. XSH25 (KJ572244)      | 0.98           | KP017827      |
| Lt-2-1-1       | Alternaria alternata strain HMA1D (KJ677246) | 100       | KP017828      |
| Lt-2-2-1       | Cladosporium oxysporum strain B2F2 (KJ589590) | 100     | KP017829      |
| Lt-2-2-2       | Pleospora bjoerlingii (JX045842)         | 100            | KP017830      |
| Lt-2-6-1-2     | Trichoderma harzianum strain ML16-1 (KJ619615) | 100       | KP017831      |
| Lt-2-7-1       | Lewisia sp. OUCMBI101191 (HQ914885)      | 99             | KP017832      |
| Lt-2-8-1       | Cladosporium cladosporioides strain GKF2 (KJ589558) | 100     | KP017833      |
| Lt-3-2-1       | Cladosporium oxysporum strain B2F2 (KJ589590) | 100     | KP017834      |
| Lt-3-2-1-2     | Cladosporium oxysporum strain B2F2 (KJ589590) | 100     | KP017835      |
| Lt-3-3-1       | Stemphylium solani strain CEF-772 (KP999031) | 100     | KP017836      |
| Lt-3-4-2       | Meira sp. JCM 18504 (AB778892)           | 100            | KP017837      |
| Lt-3-5-1       | Macrophoma sp. TXc4-6 (HQ262514)         | 100            | KP017838      |
| Lt-3-5-2-1     | Talaromyces pinophilus isolate SCLBS (KF913534) | 100   | KP017839      |
| Lt-3-7-2       | Stemphylium solani strain PB2 (KC796609)  | 100            | KP017840      |
| Lt-3-8-1       | Cladosporium oxysporum strain B2F2 (KJ589590) | 100     | KP017841      |
| Lt-3-8-2       | Rhinocladia similis strain 152wat (KF811431) | 100    | KP017842      |
| Lt-3-9-1       | Stemphylium solani strain CEF-772 (KP999031) | 100     | KP017843      |
| Lt-3-9-1-1     | Penicillium sp. CMV-2013f strain CV26 (JX140791) | 100  | KP017844      |
| Sa-1-1-2       | Stemphylium solani isolate ASX241051 (KC72065) | 100     | KP017845      |
| Sa-1-1-3       | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100   | KP017846      |
| Sa-1-1-4       | Alternaria alternata strain HMA1D (KJ677246) | 100   | KP017847      |
| Sa-1-2-1       | Macrophoma sp. TXc4-6 (HQ262514)         | 99             | KP017848      |
| Sa-1-2-2       | Macrophoma sp. TXc4-6 (HQ262514)         | 100            | KP017849      |
| Sa-1-4-2       | Pleospora bjoerlingii (JX045842)         | 100            | KP017850      |
| Sa-1-6-1       | Fusarium longipes (HG423537)             | 99             | KP017851      |
| Sa-1-8-1       | Penicillium expansum isolate VG100 (KC894714) | 100   | KP017852      |
| Sa-1-8-2       | Pleospora bjoerlingii (JX045842)         | 99             | KP017853      |
| Sa-1-10-2      | Fusarium oxysporum strain C-2 (KJ623246) | 100            | KP017854      |
| Sa-1-10-3      | Aspergillus ustus strain EDT12-21 (JX076971) | 100   | KP017855      |
| Sa-2-1-1       | Exophiala jeanselmi (AB531492)           | 100            | KP017856      |
| Sa-2-3-1       | Cladosporium cladosporioides strain GKF2 (KJ589558) | 100  | KP017857      |
| Sa-2-3-3       | Macrophoma sp. TXc4-6 (HQ262514)         | 100            | KP017858      |
| Sa-2-4-1       | Exophiala oligosperma (AB777520)         | 100            | KP017859      |
| Sa-2-4-2-1     | Pleospora bjoerlingii (JX045842)         | 99             | KP017860      |
| Sa-2-5-1       | Penicillium sp. KJ-2012 strain GZU (JQ965022) | 100  | KP017861      |
| Sa-2-9-1       | Macrophoma sp. TXc4-6 (HQ262514)         | 100            | KP017862      |
| Sa-2-10-1      | Aspergillus brasiliensis (KJ677257)      | 100            | KP017863      |
| Fungal isolates | Closest relative based on sequence homology | Similarity (%) | Accession No. |
|-----------------|--------------------------------------------|----------------|---------------|
| Su-3-1-1        | Fusarium oxysporum strain HPA2 (KJ677257)  | 99             | KP017919      |
| Su-3-1-2        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-1-3        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-1-4        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-1-5        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-1-6        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-1-7        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-1-8        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-1-9        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-1-10       | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-2-1        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-2-2        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-2-3        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-2-4        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-2-5        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-2-6        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-2-7        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-2-8        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-2-9        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-2-10       | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-3-1        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-3-2        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-3-3        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-3-4        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-3-5        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-3-6        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-3-7        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-3-8        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-3-9        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-3-10       | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-4-1        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-4-2        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-4-3        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-4-4        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-4-5        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-4-6        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-4-7        | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
| Su-3-4-8        | Alternaria alternative strain SDAU (KJ682318) | 100            | KP017919      |
| Su-3-4-9        | Cladosporium cladosporioides isolate ZJ18 (KJ572146) | 100            | KP017940      |
| Su-3-4-10       | Penicillium simplicissimum (KF906546)     | 100            | KP017940      |
the region of ITS1 5.8S ITS2, and the sequences were registered in the GenBank database of the National Center for Biotechnology Information (accession Nos. KP017770~KP017929) (Table 3). In total, 160 strains of endophytic fungi were isolated from the roots of 5 halophytic plants belonging to 5 species that were growing naturally in the Muan salt marsh. The identified fungi were classified into 28 genera and 48 species.

The identified strains were categorized into the phyla Ascomycota (157 strains), Basidiomycota (2 strains), and Zygomycota (1 strain). The class Sordariomycetes (61 strains) accounted for the highest number of strains followed by the classes Dothideomycetes (53 strains), Eurotiomycetes (43 strains), Exobasidiomycetes (2 strains), and incertae sedis (1 strain). At the genus level, Fusarium (40 strains) accounted for the highest proportion followed by Penicillium (20 strains) and Alternaria (12 strains).

Taxonomic placement of fungi in each plant sample

Table 3. Continued

| Fungal isolates | Closest relative based on sequence homology | Similarity (%) | Accession No. |
|-----------------|-------------------------------------------|---------------|--------------|
| Su-3-6-2        | *Penicillium oxalicum* strain SY20-5 (KJ619622) | 100           | KP017922     |
| Su-3-6-3        | *Fusarium oxysporum* strain HPA2 (KJ677253)   | 100           | KP017923     |
| Su-3-7-2        | *Colletotrichum gloeosporioides* strain CG60 (KJ632430) | 100           | KP017924     |
| Su-3-8-1        | *Fusarium incarnatum* strain LS 03 (KJ721990)  | 100           | KP017925     |
| Su-3-8-2        | *Penicillium sumatrense* strain CV503 (JX140883) | 100           | KP017926     |
| Su-3-8-5        | *Fusarium oxysporum* strain C-2 (KJ623246)     | 100           | KP017927     |
| Su-3-9-1        | *Fusarium oxysporum* strain C-2 (KJ623246)     | 100           | KP017928     |
| Su-3-9-2        | *Pestalotiopsis* sp. GRPS-3 (KF6564287)        | 100           | KP017929     |

Fig. 1. Distribution of fungal isolates in different plant samples at the class (A) and genus (B) levels. Sm, *Suaeda maritima*; Lt, *Limonium tetragonum*; Sa, *Suaeda australis*; Pa, *Phragmites australis*; Su, *Suaeda glauca* Bunge.
was performed at the class and genus levels (Fig. 1). Sordariomycetes accounted for the highest percentage of strains at the class level, except for the plants L. tetragonum, S. australis, and P. australis. Dothideomycetes accounted for the highest percentage in samples from the plants L. tetragonum, S. australis, and P. australis. At the genus level, Fusarium (25%) was the most prominent genus, while in samples from the plants S. maritima and S. glauca Bunge, Penicillium (12.5%) was the second most dominant genus among all fungal isolates and was represented in every plant sample tested.

In the present study, the majority of the isolated fungal endophytes belonged to the phylum Ascomycota and a few of them belonged to Basidiomycota and Zygomycota. The genera of the endophytic fungi isolated from the tested halophytic plants were Alternaria, Aspergillus, Bionectria, Botryosphaeria, Cladosporium, Colletotrichum, Exophiala, Fusarium, Gibberella, Gibellulopsis, Lecanicillium, Lewia, Macrophoma, Meira, Metarhizium, Monochaetia, Mortierella, Myceliophthora, Ophiobolus, Ophiostoma, Penicillium, Pestalotiopsis, Pleospora, Purpureocillium, Rhinocladiella, Stemphylium, Talaromyces, and Trichoderma. The most dominant genus was Fusarium (25%), followed by Penicillium (12.5%) and Alternaria (7.5%).

Previously, molecular methods have been successfully used for the identification of the strains comprising endophytic fungal communities [15, 16]. In this study, we followed a similar molecular strategy to those previously reported in order to identify these endophytic fungi by means of sequencing internal transcribed spacer rRNA genes and employing a phylogenetic classification system.

Previous studies have reported that these endophytes could play a role in plant development. Fusarium oxysporum, which was isolated from all Suaeda species, reportedly produces GAs and indole acetic acids that stimulate plant growth and development and may reduce the hazardous effect of salinity on the host plant [17]. GAs are known to influence stem elongation, seed germination, pollen maturation, leaf expansion, and the induction of flowering [18], while indole acetic acid modulates cell division and enlargement, tissue differentiation, and responses to gravity and light [19]. Endophytic fungi of the species Alternaria alternata were isolated from all plant samples, and they were previously isolated from the leaves of Solanum nigrum, where they were shown to produce indole acetic acid [20]. Members of the genus Penicillium were represented in all of the studied plant samples. Previous studies have revealed that some species of Penicillium can promote plant growth by several different mechanisms, such as the production of plant growth promoting secondary metabolites (auxin and GAs), antagonism to plant pathogens, and solubilization of

### Table 4. Endophytic fungi (160 strains) isolated from the 5 coastal plants

| Scientific name of plant sample | Abbreviated plant name | Taxon of fungal strains | No. of isolates |
|---------------------------------|------------------------|-------------------------|----------------|
| Suaeda maritima                  | Sm                     | 11 genera, 21 species   | 53             |
| Limonium tetragonum             | Lt                     | 13 genera, 10 species   | 22             |
| Suaeda australis                | Sa                     | 12 genera, 13 species   | 30             |
| Phragmites australis            | Pa                     | 14 genera, 11 species   | 23             |
| Suaeda glauca Bunge             | Su                     | 12 genera, 8 species    | 32             |

Sm, Suaeda maritima; Lt, Limonium tetragonum; Sa, Suaeda australis; Pa, Phragmites australis; Su, Suaeda glauca Bunge.

### Table 5. Diversity indices and distribution of endophytic fungi isolated from native plants in the Muan salt marsh

| Fungal taxon | Sm | Lt | Sa | Pa | Su |
|--------------|----|----|----|----|----|
| Alternaria   | 3  | 3  | 2  | 3  | 1  |
| Aspergillus  | 2  | 2  | 1  | 3  | 2  |
| Bionectria   | 2  | -  | -  | -  | -  |
| Botryosphaeria | - | 1  | -  | -  | -  |
| Cladosporium | -  | -  | 2  | 1  | 1  |
| Colletotrichum | - | -  | -  | -  | 2  |
| Exophiala    | -  | -  | 2  | 3  | 1  |
| Fusarium     | 29 | -  | 3  | -  | 8  |
| Gibberella   | 1  | -  | -  | -  | -  |
| Gibellulopsis | - | -  | -  | -  | 1  |
| Lecanicillium | - | -  | -  | -  | 1  |
| Lewia        | -  | 1  | -  | 2  | -  |
| Macrophoma   | -  | 1  | 9  | 2  | -  |
| Meira        | -  | 1  | -  | -  | 1  |
| Metarhizium  | -  | 1  | -  | -  | -  |
| Monochaetia  | 1  | -  | -  | -  | -  |
| Mortierella  | -  | -  | 1  | -  | -  |
| Myceliophthora | - | -  | -  | -  | 1  |
| Ophiobolus   | -  | -  | -  | 1  | -  |
| Paraphaeosphaeria | - | -  | -  | -  | 1  |
| Penicillium  | 5  | 1  | 2  | 4  | 8  |
| Pestalotiopsis | 3 | -  | 1  | -  | 2  |
| Pleospora    | -  | 1  | 4  | 1  | 4  |
| Purpureocillium | - | -  | -  | 1  | -  |
| Rhinocladiella | - | 1  | -  | 1  | -  |
| Stemphylium  | -  | -  | 3  | 1  | -  |
| Talaromyces  | 5  | -  | 1  | -  | 1  |
| Trichoderma  | -  | 2  | 1  | 1  | 1  |
| N            | 53 | 22 | 30 | 23 | 32 |
| Shannon diversity index \((H')\) | 1.24 | 1.61 | 1.67 | 1.95 | 1.56 |
| Simpson’s index of diversity \((1 – D)\) | 0.69 | 0.93 | 0.89 | 0.94 | 0.87 |
| Menhinick’s index \((Dnn)\) | 1.51 | 2.77 | 2.19 | 2.92 | 2.12 |
| Margalef’s index \((Dmg)\) | 2.52 | 3.88 | 3.23 | 4.15 | 3.17 |
| Fisher’s diversity \((e)\) | 4.22 | 13.35 | 7.41 | 15.18 | 6.97 |

Sm, Suaeda maritima; Lt, Limonium tetragonum; Sa, Suaeda australis; Pa, Phragmites australis; Su, Suaeda glauca Bunge; N, total number of individuals in each sample; S, number of different genera in a sample.
Fig. 2. Screening for plant growth promoting of Waito-C rice seedlings with fungal culture filtrates of fungal endophytes isolated from halophytes (A–E). Ten microliters of lyophilized culture filtrates was treated to Waito-C rice seedlings. The shoot length and plant length of the Waito-C rice seedlings were measured after 7 days of treatment. The standard deviation from means was calculated using Microsoft Excel. Czk, Czapek media; D.W., distilled water; G.f., Gibberella fujikuroi.
minerals [21-25].

**Diversity of endophytic fungi at the genus level in the sampled plant species.** The identified endophytic fungal strains were characterized into 11 genera and 21 species from *S. maritima*, 13 genera and 10 species from *L. tetragonum*, 12 genera and 13 species from *S. australis*, 14 genera and 11 species from *P. australis*, and 12 genera and 18 species from *S. glauca* Bunge (Table 4).

Generic richness and diversity were calculated based on counting of fungal genera by plant samples (Table 5). The results showed that *P. australis* had the highest score in Margalef’s (4.15) and Menhinick’s (2.92) indices of richness and in Fisher’s α (15.18), Shannon’s (1.95) and Simpson’s (0.94) indices of diversity. The Shannon diversity index (*H’*) ranged from 1.24~1.95 (this index is usually between 1.5~3.5; 3.5 representing the highest diversity and 1.5 the lowest). Based on this result, the Shannon index is less sensitive to evenness, compared with other diversity indices [12]. The plant *P. australis* had the highest diversity indices, showing that the endophytic fungal community isolated from this plant had the most diversity among those from our plant specimens. *P. australis* is often found to form homogenous belts in temperate zone freshwater lakes, and it is ecologically important because it filters pollutants, stabilizes shores, and houses rich wildlife. *P. australis* is adapted to its aquatic environment, most importantly, in its ability to form aerenchyma to supply the underground plant parts with oxygen, allowing the plant to survive in anoxic and waterlogged sediments [26, 27]. Thus, this creation of an oxygen rich environment may facilitate the establishment of an especially rich and diverse endophytic community in its roots. Environmental conditions may also play an important role in the assemblages and diversity of endophytic fungi.

**Bioassay of culture filtrates for plant growth promotion Waito-C rice seedlings.** The bioassay on Waito-C rice seedlings was carried out to check plant growth promotion capacity of fungal culture filtrates. All fungi were checked, of which Su-3-4-3 fungal strain indicated 20.1 cm of plant length and 9.2 cm of shoot length and was found as growth promoter. The fungal isolate Su-3-4-3 significantly promoted whole plant length as compared with *Gibberella fujikuroi* (Fig. 2).

The use of Waito-C rice seedlings is profitable as they can easily grow under controlled and sterilized conditions, hydroponically, using autoclaved water-agar media. Since this media is free of any nutrient, the sole effect of culture filtrate can easily be evaluated. Waito-C rice is a known dwarf rice mutant with reduced GA biosynthesis. Treatment of its seeds with uniconazol, as a GA biosynthesis retardant, further suppresses the endogenous GAs production by blocking its biosynthesis pathway in the plant. Shoot elongation of these seedlings can thus efficiently be related to activity of plant growth promoting secondary metabolites from fungal culture filtrates applied [28, 29]. Similarly, it has been reported the biotechnological application of *Piriformospora indica*, a cultivable mycelium possessing growth promoting effects in a vast range of plant hosts. The Su-3-4-3 fungal strain, which has strain plant growth promoting effects, was analyzed using Waito-C rice seedlings.

**Analysis of culture filtrates of Su-3-4-3 for the presence of GAs.** GA, the plant hormone produced by fungal endophytes isolated from salt tolerant plants, was analyzed with HPLC and GC/MS. Therefore, a variety of GAs were confirmed from the culture filtrate of the Su-3-4-3 fungal strain; the result of the GC/MS SIM analysis showed that Su-3-4-3 produced GA1 (0.465 ng/mL), GA3 (1.808 ng/mL), other inactive GA9 (0.054 ng/mL) and GA24 (0.044 ng/mL) (Fig. 3). It was confirmed that Su-3-4-3 produced as much GA1, GA3, and other inactive GA.

The GC/MS with SIM technique has the ability to analyze highly complex mixtures and to detect compounds of different classes [30], and so was used for culture filtrate analysis of the Sm-3-7-5 fungal strain. GC/MS SIM is useful to investigate a number of compounds and is often used in plant experimentation [31, 32]. By reason of its reliability, GC/MS SIM was used in quantitative analysis of various plant hormones.

In summary, a total of 160 fungal strains were isolated from 5 plants inhabiting the Muan salt marsh and were classified into 3 phyla, 5 classes, 10 orders, 18 families, and 28 genera. *Fusarium* (class Sordariomycetes) was the most dominant genus followed by *Piriformospora indica*. The group of endophytic fungi isolated from *Phragmites australis* was the
most diverse according to the diversity analysis. Plant growth promotion activity of Waito-C rice seedlings was confirmed by culture filtrate of _Talaromyces pinophilus_ Su-3-4-3. Our recent study reports the information on the capacity of _Talaromyces pinophilus_ Su-3-4-3 producing GAs. Therefore, the present study was performed to provide basic data on the symbiosis of halophytic plants and fungi. Understanding such endophytic interactions may significantly improve the quality and productivity of agricultural crops.

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