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Research Article

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

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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, GA_1 (0.465 ng/mL), GA_3 (1.808 ng/mL) along with other physiologically inactive GA_9 (0.054 ng/mL) and GA_{24} (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

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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°4′57.48″, E 126°23′30.39″	Halophytic
2	*Limonium teragonum*	Lt	N 35°3′54.22″, E 126°21′59.84″	Halophytic
3	*Suaeda australis*	Sa	N 35°3′52.69″, E 126°22′0.29″	Halophytic
4	*Pharagmites australis*	Pa	N 35°3′52.23″, E 126°21′59.93″	Halophytic
5	*Suaeda glauca Bunge*	Su	N 35°4′4.56″, E 126°22′1.47″	Halophytic

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 collected in separate sterile plastic bags, labeled, transported to the laboratory, and stored at 4°C until processed. The local sites, scientific names, and codes of the 5 plant species from which samples were taken are listed in Table 1.

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 [8, 9].

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 flasks 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 GTA 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.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 (*Dmn*) 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 pellet and the supernatant were 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

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

Diversity indices	Formula
Shannon diversity index (H')	$H' = -\sum_{i=1}^{R} pi \cdot \ln pi$
Simpson's index of diversity ($1 - D$)	$D = \sum_{i=1}^{R} \frac{ni(ni-1)}{N(N-1)}$
Menhinick's index (*Dmn*)	$Dmn = \frac{S}{\sqrt{N}}$
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_{18} 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 [$^2H_2$] 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

Table 3. Identification of endophytic fungal isolates from the roots of coastal plants

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 longipes* (HG423537)	99	KP017771
Sm-1-2-3-1	*Fusarium longipes* (HG423537)	99	KP017772
Sm-1-4-4	*Pestalotiopsis clavispora* isolate Ara-1 (JQ008944)	100	KP017773
Sm-1-5-3	*Fusarium incarnatum* strain FI-00602 (KJ572780)	99	KP017774
Sm-1-5-4	*Gibberella fujikuroi* (KJ677254)	99	KP017775
Sm-1-6-4	*Fusarium incarnatum* strain FI-00602 (KJ572780)	99	KP017776
Sm-1-9-2	*Fusarium incarnatum* strain FI-00602 (KJ572780)	99	KP017777
Sm-1-9-3	*Penicillium oxalicum* strain SY20-5 (KJ619622)	100	KP017778
Sm-1-9-4	*Penicillium expansum* isolate VG100 (KC894714)	100	KP017779
Sm-2-1-1	*Fusarium oxysporum* strain C-2 (KJ623246)	99	KP017780
Sm-2-3-3	*Penicillium expansum* isolate VG100 (KC894714)	99	KP017781
Sm-2-3-4	*Fusarium longipes* (HG423537)	99	KP017782
Sm-2-4-1	*Fusarium oxysporum* strain C-2 (KJ623246)	99	KP017783
Sm-2-5-1	*Bionectria pseudochroleuca* (KJ499909)	99	KP017784
Sm-2-5-1-1	*Paraphaeosphaeria sporulosa* strain CBS (JX496066)	100	KP017785
Sm-2-5-2	*Talaromyces marneffei* strain LCC29 (KF990145)	99	KP017786
Sm-2-6-1	*Fusarium oxysporum* strain HPA2 (KJ677253)	99	KP017787
Sm-2-7-3-1	*Fusarium caeruleum* (KJ680136)	99	KP017788
Sm-2-7-3-2	*Fusarium caeruleum* (KJ680136)	99	KP017789
Sm-2-8-2	*Bionectria pseudochroleuca* (KJ499909)	99	KP017790
Sm-2-9-1	*Fusarium oxysporum* strain HPA2 (KJ677253)	100	KP017791
Sm-2-10-1	*Penicillium brasilianum* strain 028M (KJ458973)	99	KP017792
Sm-2-10-2	*Fusarium longipes* (HG423537)	98	KP017793
Sm-3-1-1	*Alternaria alternata* isolate SDAU (KJ682318)	100	KP017794
Sm-3-1-2	*Fusarium oxysporum* (KJ653447)	99	KP017795
Sm-3-1-3	*Alternaria alternata* strain HMA1D (KJ677246)	100	KP017796
Sm-3-1-4	*Fusarium oxysporum* (KJ653447)	99	KP017797
Sm-3-1-5	*Fusarium oxysporum* strain HPA2 (KJ677253)	99	KP017798
Sm-3-2-2	*Fusarium incarnatum* strain FI-00602 (KJ572780)	100	KP017799
Sm-3-2-4	*Fusarium oxysporum* (KJ653447)	99	KP017800
Sm-3-3-1	*Fusarium oxysporum* (KJ653447)	99	KP017801
Sm-3-3-3	*Rhinocladiella similis* strain 152wat (KF811431)	100	KP017802
Sm-3-3-6	*Pestalotiopsis* sp. GRPS-3 (KF564287)	100	KP017803
Sm-3-4-3-1	*Monochaetia karstenii* (KC537806)	99	KP017804
Sm-3-4-4	*Talaromyces albobiverticillius* strain CBS (KF114736)	100	KP017805

Table 3. Continued

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 (KF990145)	99	KP017817
Sm-3-8-3	*Pestalotiopsis clavispora* strain P44 (JX045813)	100	KP017818
Sm-3-8-4	*Talaromyces marneffei* strain LCC29 (KF990145)	100	KP017819
Sm-3-9-6	*Aspergillus brasiliensis* (KJ677257)	100	KP017820
Sm-3-10-1	*Penicillium oxalicum* strain TMPS3 (DQ986355)	99	KP017821
Sm-3-10-2	*Talaromyces pinophilus* isolate OK3SP103P (KF871458)	99	KP017822
Lt-1-1-1	*Ophiosphaerella 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	*Lewia* 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 (KF999031)	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 SCLB5 (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	*Rhinocladiella similis* strain 152wat (KF811431)	100	KP017842
Lt-3-9-1	*Stemphylium solani* strain CEF-772 (KF999031)	100	KP017843
Lt-3-9-1-1	*Penicillium* sp. CMV-2013f strain CV26 (JX140791)	100	KP017844
Sa-1-1-2	*Stemphylium solani* isolate A2SX2410511 (KC172065)	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-1-3-2	*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 jeanselmei* (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

Table 3. Continued

Fungal isolates	Closest relative based on sequence homology	Similarity (%)	Accession No.
Sa-3-1-1	*Fusarium merismoides* strain LCC24 (KF990140)	99	KP017864
Sa-3-2-1	*Macrophoma* sp. TXc4-6 (HQ262514)	100	KP017865
Sa-3-2-2	*Pleospora bjoerlingii* (JX045842)	100	KP017866
Sa-3-2-3	*Macrophoma* sp. TXc4-6 (HQ262514)	100	KP017867
Sa-3-3-2	*Macrophoma* sp. TXc4-6 (HQ262514)	100	KP017868
Sa-3-5-1	*Trichoderma* sp. T86 BD-2013 (KC555170)	99	KP017869
Sa-3-5-3	*Pestalotiopsis* sp. GRPS-3 (KF564287)	100	KP017870
Sa-3-6-1	*Macrophoma* sp. TXc4-6 (HQ262514)	100	KP017871
Sa-3-6-2	*Alternaria* sp. DX-FOF7 (KF558883)	100	KP017872
Sa-3-10-2	*Metarhizium pingshaense* strain CQM132 (JF827149)	100	KP017873
Sa-3-10-3	*Macrophoma* sp. TXc4-6 (HQ262514)	100	KP017874
Pa-1-1-1	*Lewia* sp. OUCMBI101191 (HQ914885)	100	KP017875
Pa-1-1-2	*Penicillium* sp. CCF3828 (FJ430753)	99	KP017876
Pa-1-7-1	*Trichocladium asperum* strain H2F1 (KJ589597)	100	KP017877
Pa-1-10-1-1	*Purpureocillium lilacinum* strain E303 (KJ540087)	100	KP017878
Pa-2-1-1	*Aspergillus lentulus* (HE578064)	100	KP017879
Pa-2-2-1	*Lecanicillium fungicola* strain 4645 (JX500428)	100	KP017880
Pa-2-2-3	*Macrophoma* sp. TXc4-6 (HQ262514)	100	KP017881
Pa-2-2-4	*Meira* sp. JCM 18504 (AB778892)	100	KP017882
Pa-2-3-1	*Penicillium simplicissimum* (KF906546)	100	KP017883
Pa-2-3-2	*Mortierella elongata* (AB542099)	100	KP017884
Pa-2-3-3	*Exophiala oligosperma* (AB480204)	100	KP017885
Pa-2-4-1	*Penicillium simplicissimum* (KF906546)	100	KP017886
Pa-2-7-2	*Alternaria alternata* strain HMA1D (KJ677246)	100	KP017887
Pa-2-8-2	*Myceliophthora sepedonium* (JN031013)	99	KP017888
Pa-2-10-2	*Macrophoma* sp. TXc4-6 (HQ262514)	100	KP017889
Pa-3-1-1	*Pleospora bjoerlingii* (JX045842)	100	KP017890
Pa-3-1-2	*Exophiala oligosperma* (AB777520)	100	KP017891
Pa-3-3-3	*Alternaria* sp. DX-FOF7 (KF558883)	100	KP017892
Pa-3-5-2	*Exophiala oligosperma* (AB480204)	100	KP017893
Pa-3-7-2	*Lewia* sp. OUCMBI101191 (HQ914885)	100	KP017894
Pa-3-9-1	*Penicillium simplicissimum* (KF906546)	100	KP017895
Pa-3-10-1	*Alternaria alternata* isolate SDAU (KJ682318)	100	KP017896
Pa-3-10-3	*Cladosporium cladosporioides* isolate ZJ18 (KJ572146)	100	KP017897
Su-1-1-1	*Pleospora bjoerlingii* (JX045842)	100	KP017898
Su-1-4-2	*Penicillium sumatrense* strain CV503 (JX140883)	100	KP017899
Su-1-4-3	*Gibellulopsis nigrescens* isolate Cvn-HNh (KC156644)	100	KP017900
Su-1-5-1	*Fusarium oxysporum* strain HPA2 (KJ677253)	100	KP017901
Su-1-5-3	*Penicillium simplicissimum* (KF906546)	100	KP017902
Su-1-6-1	*Trichoderma aureoviride* strain SL (KJ610807)	99	KP017903
Su-1-8-2	*Exophiala oligosperma* (KJ652931)	99	KP017904
Su-1-8-3	*Pleospora bjoerlingii* (JX045842)	100	KP017905
Su-1-9-3	*Pleospora bjoerlingii* (JX045842)	100	KP017906
Su-1-10-2	*Penicillium sumatrense* strain CV503 (JX140883)	100	KP017907
Su-1-10-3	*Penicillium* aff. *janthinellum* P49 (JN246047)	100	KP017908
Su-2-2-2	*Aspergillus terreus* isolate D34 (KF971363)	100	KP017909
Su-2-4-1	*Fusarium oxysporum* strain HPA2 (KJ677253)	100	KP017910
Su-2-4-4	*Aspergillus brasiliensis* (KJ677257)	100	KP017911
Su-2-6-1	*Fusarium oxysporum* strain P28 (JX045794)	100	KP017912
Su-2-10-3	*Pestalotiopsis vismiae* isolate LH04Pv (JX305714)	99	KP017913
Su-3-2-2	*Colletotrichum acutatum* strain 11E031 (KF717039)	100	KP017914
Su-3-3-2	*Penicillium* sp. FZ99 (KF848940)	100	KP017915
Su-3-3-3	*Pleospora bjoerlingii* (JX045842)	100	KP017916
Su-3-4-1	*Cladosporium cladosporioides* strain GKF2 (KJ589558)	100	KP017917
Su-3-4-2	*Penicillium* sp. FZ99 (KF848940)	100	KP017918
Su-3-4-3	*Talaromyces pinophilus* isolate SCLB5 (KF913534)	100	KP017919
Su-3-4-5	*Alternaria alternata* strain HMA3B (KJ677249)	100	KP017920
Su-3-5-1	*Fusarium* sp. X3-1 XZ-2010 (HM214466)	100	KP017921

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 (KF564287)	100	KP017929

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

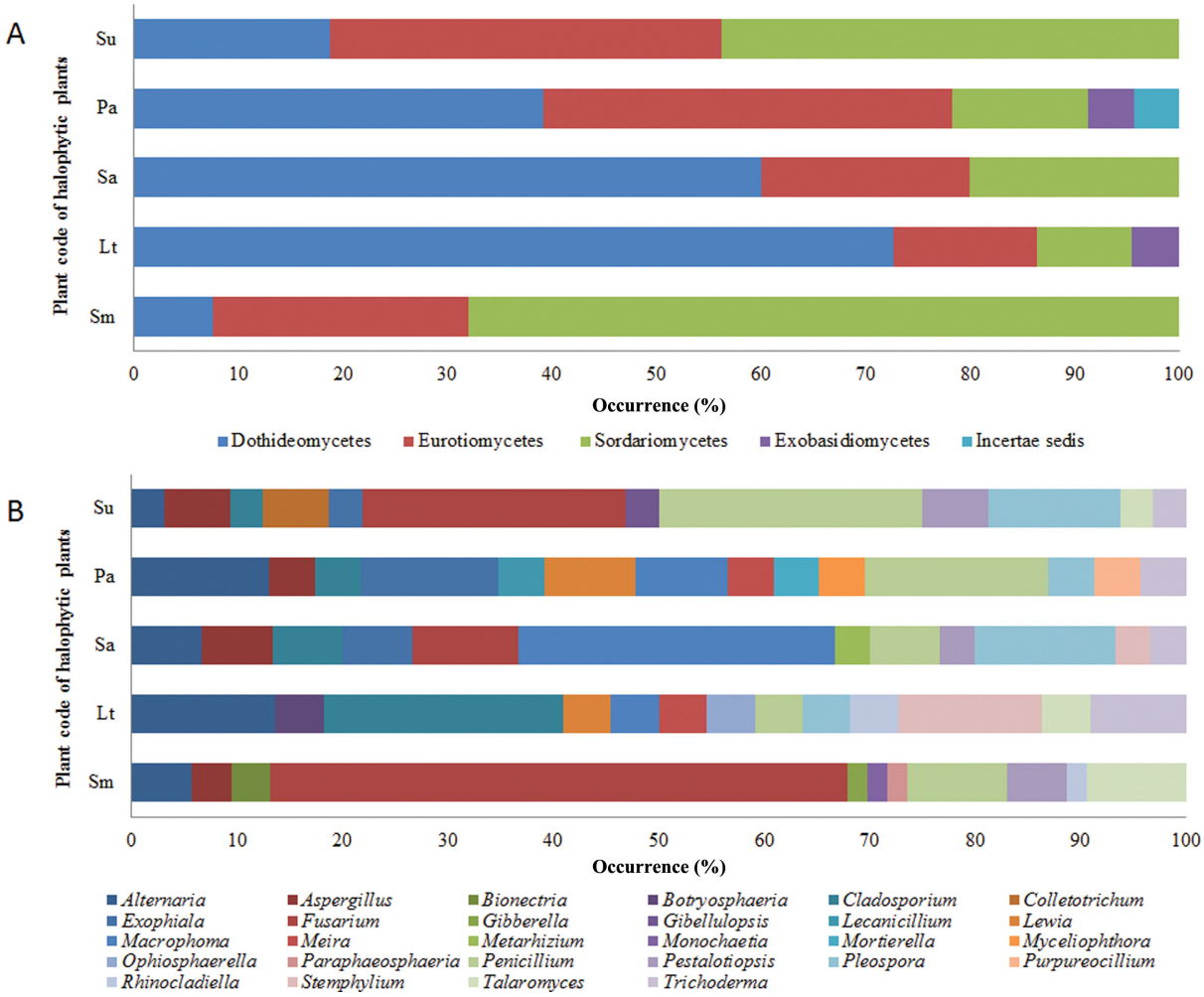

Fig. 1. Distribution of fungal isolates in different plant samples at the class (A) and genus (B) levels. Sm, *Suaeda maritima*; Lt, *Limonium tetragonum*; Sa, *Suaeda australis*; Pa, *Phragmites australis*; Su, *Suaeda glauca* Bunge.

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.

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*, *Ophiosphaerella*, *Paraphaeosphaeria*, *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 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 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	2
Bionectria	2	-	-	-	-
Botryosphaeria	-	1	-	-	-
Cladosporium	-	5	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	-
Ophiosphaerella	-	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
S	11	13	12	14	12
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 (*Dmn*)	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 (α)	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.

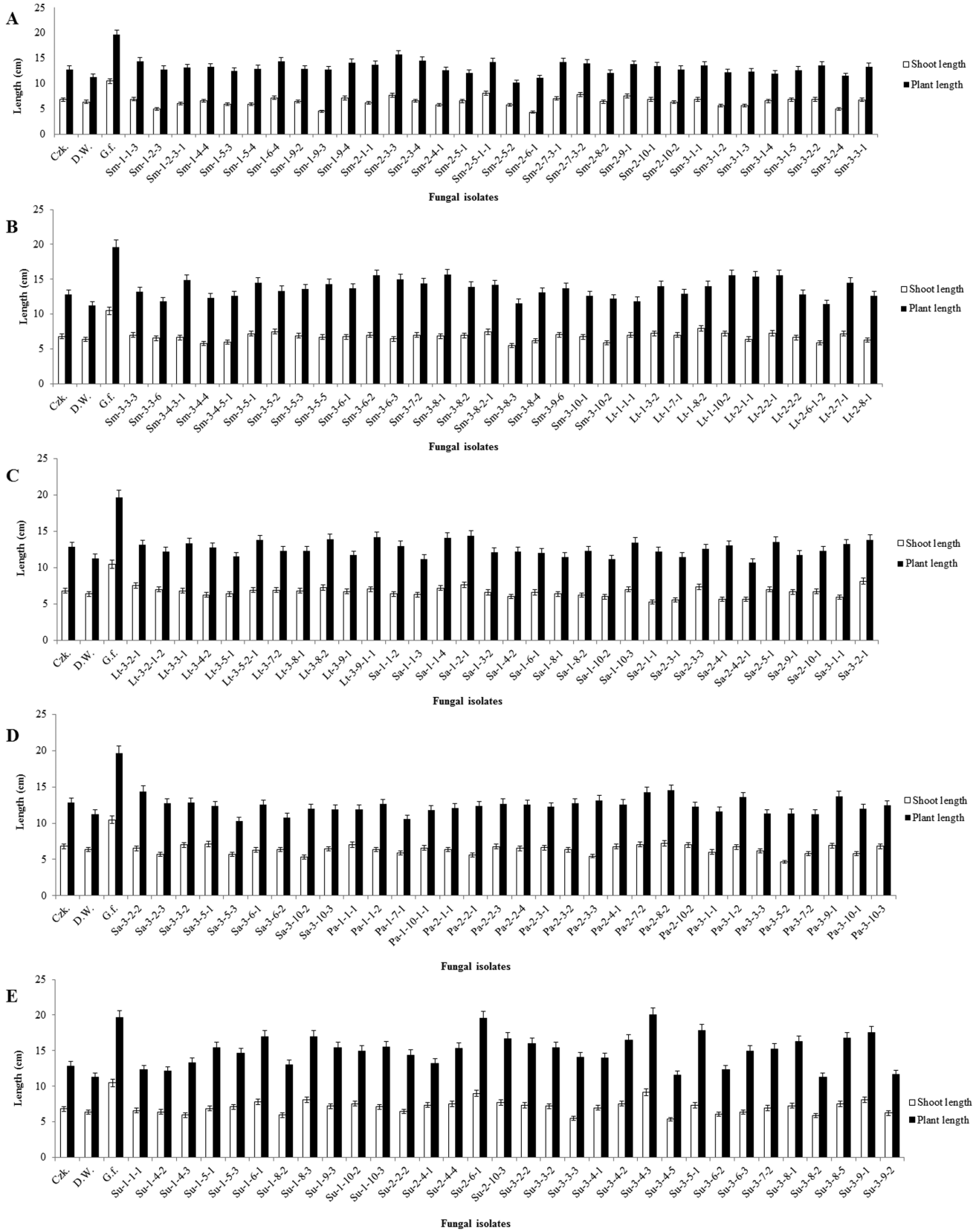

Fig. 2. Screening for plant growth promoting of Waito-C rice seedlings with fungal culture filtrates of fungal endophytes isolated from halophytes (A~E). Ten microliters of lyophilized culture filtrates was treated to Waito-C rice seedlings. The shoot length and plant length of the Waito-C rice seedlings 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 fukikuroi*.

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 culturable 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 GA_1 (0.465 ng/mL), GA_3 (1.808 ng/mL), other inactive GA_9 (0.054 ng/mL) and GA_{24} (0.044 ng/mL) (Fig. 3). It was confirmed that Su-3-4-3 produced as much GA_1, GA_3, GA_9 and GA_{24} as *G. fujikuroi*.

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 *Penicillium*. The group of endophytic fungi isolated from *Phragmites australis* was the

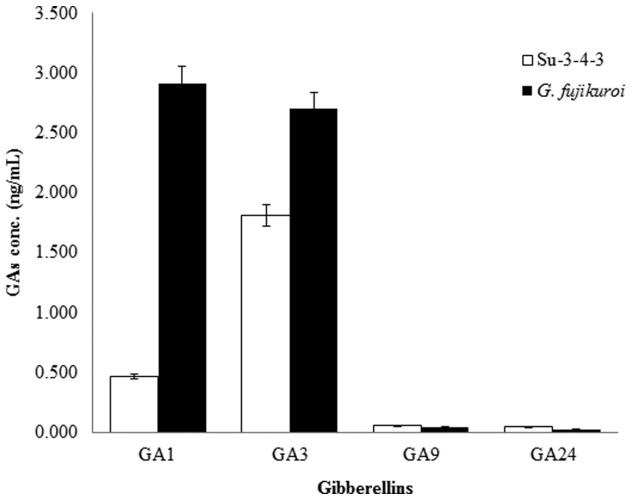

Fig. 3. Gibberellins (GAs) content of fungal culture filtrates of the Su-3-4-3 strain and wild type *Gibberella fujikuroi*. Gas chromatograph/mass spectrometer selected ion monitoring analysis of culture filtrate extracts from the Su-3-4-3 fungal strain detected two bioactive GAs. Su-3-4-3 showed the presence of bioactivity of GA_1, GA_3, and other inactive GA. The standard deviation from means was calculated using Microsoft Excel.

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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