RESEARCH LETTER

Diversity and cold adaptation of culturable endophytic fungi from bryophytes in the Fildes Region, King George Island, maritime Antarctica

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Abstract

Endophytic fungi associated with three bryophyte species in the Fildes Region, King George Island, maritime Antarctica, that is, the liverwort *Barbilophozia hatcheri*, the mosses *Chorisodontium aciphyllum* and *Sanionia uncinata*, were studied by culture-dependent method. A total of 128 endophytic fungi were isolated from 1329 tissue segments of 14 samples. The colonization rate of endophytic fungi in three bryophytes species were 12.3%, 12.1%, and 8.7%, respectively. These isolates were identified to 21 taxa, with 15 *Ascomycota*, 5 *Basidiomycota*, and 1 unidentified fungus, based on morphological characteristics and sequence analyses of ITS region and D1/D2 domain. The dominant fungal endophyte was *Hyaloscyphaceae* sp. in *B. hatcheri*, *Rhizoscyphus* sp. in *C. aciphyllum*, and one unidentified fungus in *S. uncinata*; and their relative frequencies were 33.3%, 32.1%, and 80.0%, respectively. Furthermore, different Shannon–Weiner diversity indices (0.91–1.99) for endophytic fungi and low endophytic fungal composition similarities (0.19–0.40) were found in three bryophyte species. Growth temperature tests indicated that 21 taxa belong to psychrophiles (9), psychrotrophs (11), and mesophile (1). The results herein demonstrate that the Antarctic bryophytes are an interesting source of fungal endophytes and the endophytic fungal composition is different among the bryophyte species, and suggest that these fungal endophytes are adapted to cold stress in Antarctica.

Introduction

Fungal endophytes are a diverse group of fungal species that inhabit living plants at some time during their life cycle without causing apparent symptoms of infection (Wilson, 1995). Most of them belong to the phylum *Ascomycota*, and others belong to *Basidiomycota* (Flor *et al.*, 2011; Koukol *et al.*, 2012; U'Ren *et al.*, 2012) and *Zygomycota* (Gazis & Chaverri, 2010). The host and habitat range of these fungi is also diverse; they have been isolated from many different land plants and from all terrestrial ecosystems ranging from the tropics to polar regions (Arnold & Lutzoni, 2007; Rosa *et al.*, 2009, 2010). These fungi have profound impacts on plant hosts in many ways: Some impact plant fitness (Rodriguez *et al.*, 2009), some affect plant disease resistance and susceptibility (Rodriguez *et al.*, 2009), and some decompose plant litter (Sun *et al.*, 2011). Despite their diversity and importance, the vast majority of fungal endophytes and their ecological significance have yet to be adequately characterized.

Most studies of fungal endophytes have focused on those species that live in vascular plants, but endophytes also live in nonvascular plants including bryophytes (i.e., mosses, liverworts, and hornworts), which are a functionally important in boreal forest and tundra ecosystems where they produce much of the biomass. A great phylogenetic diversity of endophytes were found in the tissues of mosses and liverworts in boreal, temperate and tropical forests (Davis *et al.*, 2003; Davis & Shaw, 2008; Kauserud *et al.*, 2008; U'Ren *et al.*, 2010). Further studies are needed to characterize the diversity, distribution, and ecological roles

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of endophytic fungi associated with bryophytes in different regions, including Antarctica.

The plant flora of Antarctica, which is geographically isolated from other landmasses and is the coldest continent, consists largely of bryophytes (*c.* 111 species of mosses, and 27 species of liverworts) (Bednarek-Ochyra *et al.*, 2000; Ochyra *et al.*, 2008) and includes only two native vascular plant species. As the dominant plant component in Antarctic terrestrial ecosystems, bryophytes are major primary producers and have important influences on the thermal and hydrologic cycles, and therefore likely play a significant role in regulating ecological processes. All these characteristics make Antarctica useful for studying the taxonomy, ecology, adaptation, and evolution of endophytic fungal communities associated with bryophytes. Only a few studies have reported on endophytic fungi in Antarctica, and these have been limited to a small number of bryophyte species in some scattered sites in the continental Antarctic zone (Azmi & Seppelt, 1998; McRae & Seppelt, 1999; Bradner *et al.*, 2000; Tosi *et al.*, 2002).

The abundance and diversity of organisms decrease from the maritime to the continental Antarctic zone (Ruisi *et al.*, 2007). To our knowledge, no reports on the endophytic fungal diversity associated with bryophytes in the maritime Antarctic zone are available. The aim of this study was to investigate the diversity, distribution, and cold adaptation of the endophytic fungi associated with three dominant bryophytes species (the liverwort *Barbilophozia hatcheri*, the mosses *Chorisodontium aciphyllum*, and *Sanionia uncinata*) in the Fildes Region (62°12′-62°13′S and 58°56′-58°57′W), which represents one of the largest ice-free areas in the maritime Antarctic zone.

Materials and methods

Study sites and sample collection

The study area is located in the Fildes Region (62°08′-62°14′S and 59°02′-58°51′W), consisting of the Fildes Peninsula, Ardley Island and adjacent islands, located in the southwestern part of King George Island, South Shetland Islands. The Fildes Region represents one of the largest ice-free areas in the maritime Antarctica and contains six permanent Antarctic stations, one of which is the Great Wall Station (China). Sampling was carried out near the Great Wall Station during China's 28th Antarctic expedition in December 2011. Fourteen liverwort and moss samples were collected from five sites in this area (Table 1).

Isolation of endophytic fungi

The entire plants were surface-sterilized by immersion in 75% ethanol for 1 min, in 2% sodium hypochlorite for 3 min, and in 75% ethanol for 0.5 min. After the samples were surface dried with sterile paper towels, they were cut into segments of 0.1–0.3 cm. Tissue segments were then evenly placed in each 9-cm-diameter Petri dishes containing potato dextrose agar (PDA, 1.5%), tetracycline (50 mg L^{-1}), and streptomycin sulfate (50 mg L^{-1}). Each sample was represented by 60–100 segments, and 20–30 segments were placed on each Petri dish. Petri dishes were sealed, incubated for 2 months at 4 °C, and examined periodically. When colonies developed, they were transferred to PDA slants. Subcultures were then incubated on PDA. All of the isolates were deposited in the China Pharmaceutical Culture Collection (CPCC), Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College.

DNA extraction, amplification, and sequencing

All isolates were initially sorted into morphospecies on the basis of phenotypic characteristics including colony aspect (texture and color), cell morphology (shape), and growth rate. After this preliminary screening, representative isolates of each morphospecies were selected for molecular identification. Because the nuclear internal transcribed spacers (ITS) region and D1/D2 domain of the large-subunit rRNA gene (LSU) are the default markers for study of fungi at the species level (Seifert, 2009), we used ITS and D1/D2 gene sequences for fungal identification.

Genomic DNA was extracted with a modified CTAB method (Cubero *et al.*, 1999). The ITS (ITS1-5.8 S-ITS2) region of the rRNA gene was amplified with the primers ITS1 and ITS4 as described by White *et al.* (1990). Amplification of the ITS region was performed as follows: 95 °C for 3 min, followed by 37 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 10 min. The D1/D2 domain was amplified with the primers NL1 and NL4 as described by Kurtzman & Robnett (1991). Amplification of the D1/D2 domain was performed as follows: 94 °C for 6 min, followed by 40 cycles of 94 °C for 60 s, 50 °C for 60 s, and 72 °C for 60 s; and a final extension at 72 °C for 5 min. PCR products were purified and sequenced with the same primers by Sangon Biotech Co., Ltd. (Beijing, China). The sequence data obtained in this study were deposited in GenBank under the accession numbers JX852321 to JX852420.

Molecular identification and phylogeny

Fungi were identified based on sequence similarity (i.e., assessment of the percentage of nucleotide identity with reference sequences) and the phylogenetic position. For

Table 1. Data for the 14 bryophyte samples in the Fildes Region of Antarctica

Sample code	Species	Sites (coordinates)	Sampling date
374	*Chorisodontium aciphyllum* (Hook. f. & Wils.) Broth.	Ardley Island (62°13′39.29″ S, 58°56′53.51″ W)	2011.12.21
375	*Sanionia uncinata* (Hedw.) Loeske	Ardley Island (62°13′39.29″ S, 58°56′53.51″ W)	2011.12.21
392	*Sanionia uncinata* (Hedw.) Loeske	Ardley Island (62°13′39.29″ S, 58°56′53.51″ W)	2011.12.21
417	*Chorisodontium aciphyllum* (Hook. f. & Wils.) Broth.	Ardley Island (62°13′39.29″ S,58°56′53.51″ W)	2011.12.21
426	*Barbilophozia hatcheri* (Evans) Loeske	Ardley Island (62°12′44.77″ S,58°56′27.47″ W, 34 m)	2011.12.21
451	*Chorisodontium aciphyllum* (Hook. f. & Wils.) Broth.	Ardley Island (62°12′44.77″S, 58°56′27.47″ W, 34 m)	2011.12.21
457	*Barbilophozia hatcheri* (Evans) Loeske	Ardley Island (62°12′44.77″ S,58°56′27.47″ W, 34 m)	2011.12.21
499	*Sanionia uncinata* (Hedw.) Loeske	Files Peninsula (62°12′52.83″S,58°57′42.00″W, 76 m)	2011.12.25
500	*Sanionia uncinata* (Hedw.) Loeske	Files Peninsula (62°12′52.83″S,58°57′42.00″W, 76 m)	2011.12.25
502	*Sanionia uncinata* (Hedw.) Loeske	Files Peninsula (62°12′52.83″S,58°57′42.00″W, 76 m)	2011.12.25
1-1	*Sanionia uncinata* (Hedw.) Loeske	Files Peninsula (62°13′1.61″ S,58°57′43.51″ W, 70 m)	2011.12.25
1-2	*Sanionia uncinata* (Hedw.) Loeske	Files Peninsula (62°12′57.05″S, 58°57′45.46″W, 13 m)	2011.12.25
1-3	*Sanionia uncinata* (Hedw.) Loeske	Files Peninsula (62°12′57.05″S, 58°57′45.46″W, 13 m)	2011.12.25
1-4	*Sanionia uncinata* (Hedw.) Loeske	Files Peninsula (62°12′57.05″S, 58°57′45.46″W, 13 m)	2011.12.25

sequence similarity determination, Megablast searches were performed in the GenBank public sequence databases to find the closest related species. Sequence data were also used to investigate phylogenetic relationships using Molecular Evolutionary Genetics Analysis (MEGA) software, version 5.0. The phylogenetic trees were constructed using the neighbor-joining (NJ) algorithm with bootstrap values calculated from 1000 replicate runs. The maximum composite likelihood model was used to estimate evolutionary distance.

Effect of temperature on growth

Representative isolates of each taxon were inoculated onto the PDA plates and kept at 4, 12, 20, 27, and 35 °C in the dark. Each combination of isolate and temperature was represented by three replicate plates. Plates were inspected for 1 month, and colony diameters were measured every 5 days. The mean diameter was obtained from the three replicates. The colony diameter growth rate was calculated as the mean diameter of the isolate divided by the incubation time in days.

Data analyses

Colonization rate (CR%) was calculated as the total number of tissue segments infected by fungi divided by the total number tissue segments incubated (Petrini *et al.*, 1982). Relative frequency (RF%) was calculated as the number of isolates of certain species divided by the total number of isolates. The endophytic fungal diversity was evaluated using the Shannon–Weiner diversity index which has two main components: evenness and the number of species (Shannon & Weiner, 1963). To evaluate the degree of community similarity of endophytic fungi between bryophyte species, Sorenson's similarity coefficient (*Cs*) was used and was calculated according to the formula $Cs = 2j/(a + b)$, where j is the number of endophytic fungal species coexisting in two bryophyte species, a is the total number of endophytic fungal species in one species, and b is the total number of endophytic fungal species in the other species (Sorensen, 1948).

Results

Colonization rate

In this study, we used a culture-dependent method to examine the endophytic fungi associated with three bryophytes species (*B. hatcheri*, *C. aciphyllum*, and *S. uncinata*) in the Fildes Region. From the 14 samples collected, a total of 128 isolates of fungal endophytes were obtained from 1329 tissue segments. The CR% of endophytic fungi was 12.3% for *B. hatcheri*, 12.1% for *C. aciphyllum*, and 8.7% for *S. uncinata* (Table 2).

Phylogenetic analyses

Fifty representative fungal isolates including all morphospecies were selected for sequence similarity analysis (Table 3) and phylogenetic reconstruction (Fig. 1), which were using the ITS rDNA sequences. The neighbor-joining tree indicated that most of culturable endophytic fungi belong to the phylum *Ascomycota* (41 isolates) and others belong to the phylum *Basidiomycota* (5 isolates) and 'unknown fungi' (4 isolates). The *Ascomycota* included four orders: *Helotiales* (37 isolates), *Microascales* (1 isolate), *Sordiariales* (1 isolate), and *Xylariales* (2 isolates). The *Basidiomycota* included the three orders: *Cystofilobasidiales* (1 isolate), *Platygloeales* (1 isolate), and *Sporidiobolales* (3 isolates). They were all supported by high bootstrap values (> 50%) in the phylogenetic tree.

Table 2. RF%, CR%, and Shannon–Weiner index (*H′*) of endophytic fungi from three bryophyte species

Taxon	No. of isolates with the indicated taxa (RF%)		
	Barbilophozia hatcheri	*Chorisodontium aciphyllum*	*Sanionia uncinata*
Ascomycota			
Annulohypoxylon sp.	1 (6.7%)		
Chaetomium sp.			1 (1.2%)
Hyphodiscus sp.	1 (6.7%)	8 (28.6%)	7 (8.2%)
Rhizoscyphus sp.	1 (6.7%)	9 (32.1%)	1 (1.2%)
Scopulariopsis sp.			1 (1.2%)
Thelebolus sp.			1 (1.2%)
Xenopolyscytalum sp.			1 (1.2%)
Dermateaceae sp.	2 (13.3%)		
Hyaloscyphaceae sp.	5 (33.3%)	2 (7.1%)	
Xylariaceae sp.	1 (6.7%)		
Helotiales sp. 1	2 (13.3%)		
Helotiales sp. 2	1 (6.7%)		
Helotiales sp. 3	1 (6.7%)		
Helotiales sp. 4			1 (1.2%)
Helotiales sp. 5		1 (3.6%)	
Basidiomycota			
Eocronartium sp.			1 (1.2%)
Mrakia sp.			1 (1.2%)
Rhodotorula sp. 1			1 (1.2%)
Rhodotorula sp. 2			1 (1.2%)
Sporidiobolales sp.		1 (3.6%)	
Unknown fungi			
Unidentified fungus		7 (25.0%)	68 (80.0%)
Total			
Number of tissue segments	122	232	975
Number of fungal isolates	15	28	85
Number of taxa	9	6	12
Colonization rate (%)	12.3	12.1	8.7
Shannon index of diversity (*H′*)	1.99	1.50	0.91

The endophytic fungi were identified to lower taxonomic levels by means of the phylogenetic analysis and similarity comparison. The 50 isolates were identified to 21 taxa: 11 to genus (i.e., *Annulohypoxylon* sp., *Chaetomium* sp., *Eocronartium* sp., *Hyphodiscus* sp., *Mrakia* sp., *Rhizoscyphus* sp., *Rhodotorula* sp.1, *Rhodotorula* sp.2, *Scopulariopsis* sp., *Thelebolus* sp., and *Xenopolyscytalum* sp.), 3 to family (i.e., *Dermateaceae* sp., *Hyloscyphaceae* sp., and *Xylariaceae* sp.), 6 to order (i.e., *Helotiales* sp. 1, *Helotiales* sp. 2, *Helotiales* sp. 3, *Helotiales* sp. 4, *Helotiales* sp. 5, and *Sporidiobolales* sp.), and 1 to 'unknown fungi' (unidentified fungus) (Table 3). For example, isolate I12F-02258 showed the highest similarity (99.8%) with *Rhizoscyphus ericae* (AY394907) and clustered with *R. ericae* in one group with high bootstrap values (99%) in the phylogenetic tree (Fig. 1); isolate I12F-02258 was therefore identified as *Rhizoscyphus* sp.

Fungal diversity

The fungal taxa isolated from *B. hatcheri*, *C. aciphyllum*, and *S. uncinata* were 9, 6, and 12, respectively (Table 2). Only two taxa coexisted in all three bryophyte species, and these were *Hyphodiscus* sp. and *Rhizoscyphus* sp. Six taxa were found only in the liverwort *B. hatcheri*. Two taxa were found only in the moss *C. aciphyllum*, and nine taxa were found only in the moss *S. uncinata* (Table 2).

The dominant fungal endophyte was *Hyaloscyphaceae* sp. in *B. hatcheri*, *Rhizoscyphus* sp. in *C. aciphyllum*, and one unidentified fungus in *S. uncinata*, and their relative frequencies were 33.3%, 32.1%, and 80.0%, respectively (Table 2). *Hyphodiscus* sp. was also a frequent taxon with relative frequencies of 6.7% in *B. hatcheri*, 28.6% in *C. aciphyllum*, and 8.2% in *S. uninata*. In contrast, several fungi were infrequent. For example, *Chaetomium* sp., *Eocronartium* sp., *Scopulariopsis* sp., *Thelebolus* sp., and *Xenopolyscytalum* sp. were isolated only one time (Table 2).

The Shannon–Weiner diversity index was used to evaluate and compare the diversity of the endophytic fungus community between bryophyte species. The indices were highest for *B. hatcheri*, lowest for *S. uncinata*, and intermediate for *C. aciphyllum* (Table 2).

The Sorenson's similarity coefficients for the endophytic fungi from the three bryophyte species were 0.19, 0.33, and 0.40, respectively. Similarity was highest between *B. hatcheri* and *C. aciphyllum*, intermediate between *C. aciphyllum* and *S. uncinata*, and lowest between *B. hatcheri* and *S. uncinata*. The results suggest that the distribution of the isolated fungal taxa on three bryophytes was obviously different.

Effect of temperature on growth rate *in vitro*

Based on their responses to temperature, the endophytic fungi could be divided into three groups according to Gounot (1986) and Robinson (2001). Nine fungal taxa were psychrophiles with an optimum temperature for growth of $\leq$ 15 °C and a maximum temperature for growth of $\leq$ 20 °C: *Eocronartium* sp., *Mrakia* sp., *Rhodotorula* sp.1, *Rhodotorula* sp.2, *Xylariaceae* sp., *Helotiales* sp. 1, *Helotiales* sp. 2, *Sporidiobolales* sp., and one unidentified fungus; eleven fungal taxa were psychrotrophs with an optimum temperature for growth of 15–20 °C and a maximum temperature for growth of > 20 °C: *Annulohypoxylon* sp., *Chaetomium* sp., Dermateaceae sp., *Hyphodiscus* sp., *Rhizoscyphus* sp., *Thelebolus* sp., *Xenopolyscytalum* sp., *Hyaloscyphaceae* sp., *Helotiales* sp. 3, *Helotiales* sp. 4, and *Helotiales* sp. 5; *Scopulariopsis* sp. was

Table 3. Molecular identification of endophytic fungi isolated from three bryophyte species

Host species	Representative isolate code	Gene type	GenBank accession number	Closest related species/GenBank accession number	Maximum identity (%)	Identification
B. hatcheri	I12F-02255	ITS	JX852325	Uncultured *Hyaloscyphaceae* (FJ475648)	476/513 (92.8%)	*Hyaloscyphaceae* sp.
		D1/D2	JX852375	Uncultured soil fungus (EU692229)	544/569 (95.6%)	
	I12F-02256	ITS	JX852326	Uncultured *Helotiales* (FM997939)	494/502 (98.4%)	*Helotiales* sp. 1
		D1/D2	JX852376	Uncultured fungus (GU174403)	575/587 (98.0%)	
	I12F-02258	ITS	JX852328	*Rhizoscyphus ericae* (AY394907)	496/502 (98.8%)	*Rhizoscyphus* sp.
		D1/D2	JX852378	Uncultured soil fungus (EU692281)	568/572 (99.3%)	
	I12F-02260	ITS	JX852330	*Annulohypoxylon* sp. (JQ327866)	517/523 (98.9%)	*Annulohypoxylon* sp.
		D1/D2	JX852380	*Xylariaceae* sp. (AB746916)	548/562 (97.5%)	
	I12F-02261	ITS	JX852331	*Xylariaceae* sp. (AY315402)	510/528 (96.6%)	*Xylariaceae* sp.
		D1/D2	JX852381	*Xylariaceae* sp. (JF773597)	547/559 (97.9%)	
	I12F-02269	ITS	JX852339	*Hyphodiscus* sp. (AB546944)	506/521 (97.1%)	*Hyphodiscus* sp.
		D1/D2	JX852389	*Hyphodiscus hymeniophilus* (GU727550)	566/574 (98.6%)	
	I12F-02296	ITS	JX852366	Uncultured soil fungus (GU083257)	520/529 (98.3%)	*Helotiales* sp. 2
		D1/D2	JX852416	Uncultured *Helotiales* (JF449495)	556/564 (98.6%)	
	I12F-02297	ITS	JX852367	*Mollisia minutella* (FR837920)	472/518 (91.1%)	*Dermateaceae* sp.
		D1/D2	JX852417	Uncultured fungus (EU292358)	543/558 (97.3%)	
	I12F-02299	ITS	JX852369	Uncultured fungus (HQ260283)	512/525 (97.5%)	*Helotiales* sp. 3
		D1/D2	JX852419	Uncultured *Helotiales* (EU046067)	562/567 (99.1%)	
C. aciphyllum	I12F-02251	ITS	JX852321	*Hyphodiscus* sp. (AB546944)	499/512 (97.5%)	*Hyphodiscus* sp.
		D1/D2	JX852371	*Hyphodiscus hymeniophilus* (GU727550)	573/578 (99.1%)	
	I12F-02263	ITS	JX852333	*Rhizoscyphus ericae* (AM887700)	511/518 (98.6%)	*Rhizoscyphus* sp.
		D1/D2	JX852383	Uncultured soil fungus (EU691216)	573/575 (99.7%)	
	I12F-02268	ITS	JX852336	Uncultured *Hyaloscyphaceae* (FJ475648)	476/513 (92.8%)	*Hyaloscyphaceae* sp.
		D1/D2	JX852386	Uncultured soil fungus (EU692229)	553/583 (94.8%)	
	I12F-02280	ITS	JX852350	Uncultured fungus (JN889728)	321/366 (87.7%)	*Sporidiobolales* sp.
		D1/D2	JX852400	*Basidiomycota* sp. (AB558448)	538/612 (87.9%)	
	I12F-02283	ITS	JX852353	Uncultured fungus (FJ237219)	806/857 (94.0%)	Unidentified fungus
		D1/D2	JX852403	Uncultured fungus (FJ456973)	339/415 (81.7%)	
	I12F-02289	ITS	JX852359	Fungal endophyte (HQ335303)	509/511 (99.6%)	*Helotiales* sp. 5
		D1/D2	JX852409	Uncultured fungus (EU292389)	560/571 (98.1%)	

Table 3. Continued

Host species	Representative isolate code	Gene type	GenBank accession number	Closest related species/GenBank accession number	Maximum identity (%)	Identification
S. uncinata	I12F-02253	ITS	JX852323	Uncultured fungus (FJ237219)	804/857 (93.8%)	Unidentified fungus
		D1/D2	JX852373	Uncultured fungus (FJ456973)	339/415 (81.7%)	
	I12F-02254	ITS	JX852324	*Hyphodiscus* sp. (AB546944)	504/517 (97.5%)	*Hyphodiscus* sp.
		D1/D2	JX852374	*Hyphodiscus hymeniophilus* (GU727550)	577/584 (98.8%)	
	I12F-02259	ITS	JX852329	*Mrakia psychrophilia* (EU224267)	597/598 (99.8%)	*Mrakia* sp.
		D1/D2	JX852379	*Mrakia* sp. (EU680778)	612/617 (99.2%)	
	I12F-02262	ITS	JX852332	Uncultured fungus (AB520435)	506/545 (92.8%)	*Eocronartium* sp.
		D1/D2	JX852382	*Eocronartium muscicola* (AY512844)	581/594 (97.8%)	
	I12F-02273	ITS	JX852343	Fungal endophyte (HQ335299)	497/499 (99.6%)	*Xenopolyscytalum* sp.
		D1/D2	JX852393	*Xenopolyscytalum* sp. (HE603984)	558/565 (98.8%)	
	I12F-02277	ITS	JX852347	*Rhodotorula* sp. (JF805370)	407/492 (82.7%)	*Rhodotorula* sp. 1
		D1/D2	JX852397	*Basidiomycota* sp. (JQ768846)	577/594 (97.1%)	
	I12F-02278	ITS	JX852348	*Rhodotorula* sp. (DQ870625)	543/609 (89.2%)	*Rhodotorula* sp. 2
		D1/D2	JX852398	*Rhodotorula* sp. (EU075184)	560/603 (92.9%)	
	I12F-02284	ITS	JX852354	Uncultured *Helotiales* (HQ212246)	523/528 (99.1%)	Helotiales sp. 4
		D1/D2	JX852404	Uncultured fungus (EU292389)	565/577 (97.9%)	
	I12F-02285	ITS	JX852355	*Rhizoscyphus ericae* (AM887700)	512/519 (98.7%)	*Rhizoscyphus* sp.
		D1/D2	JX852405	Uncultured soil fungus (EU691216)	565/578 (97.8%)	
	I12F-02287	ITS	JX852357	*Thelebolus microsporus* (GQ483644)	518/519 (99.8%)	*Thelebolus* sp.
		D1/D2	JX852407	*Thelebolus ellipsoideus* (FJ176895)	567/570 (99.5%)	
	I12F-02288	ITS	JX852358	*Chaetomium murorum* (HM365268)	504/532 (94.7%)	*Chaetomium* sp.
		D1/D2	JX852408	*Chaetomium globosum* (AB449688)	553/564 (98.0%)	
	I12F-02300	ITS	JX852370	*Scopulariopsis hibernica* (FJ946484)	583/592 (98.5%)	*Scopulariopsis* sp.
		D1/D2	JX852420	*Scopulariopsis* sp. (HQ676488)	527/538 (98.0%)	

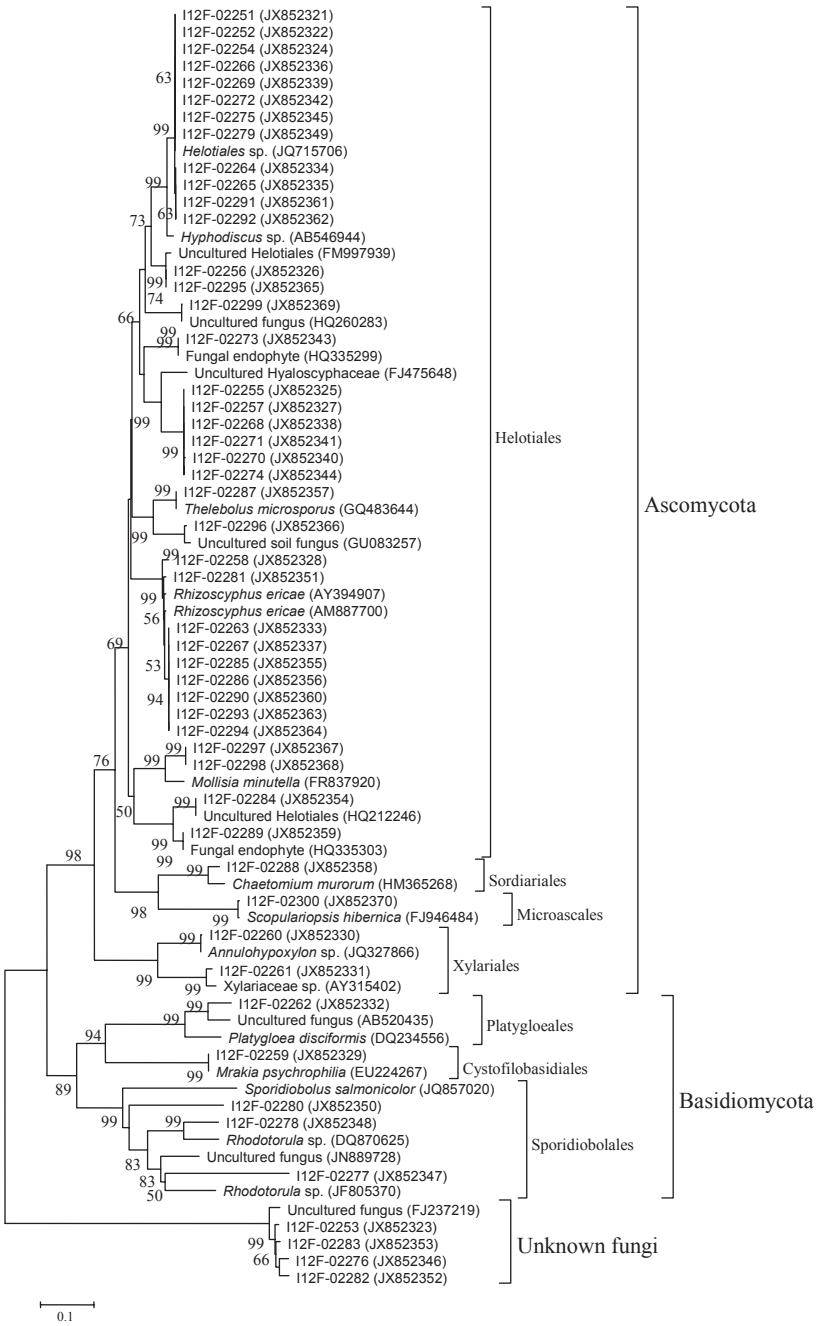

Fig. 1. Phylogenetic tree of the endophytic fungi isolated from three bryophytes and other fungal species based on the ITS (ITS1-5.8S-ITS2) region sequences of rDNA. The tree was constructed with the neighbor-joining method. Bootstrap support values are indicated for major nodes having values $\geq$ 50%.

mesophile with an optimum temperature above 20 °C (Table 4).

Discussion

Colonization rate

The CR% of the three bryophyte species by endophytic fungi were low (8.7–12.3%) in the present study. Davis & Shaw (2008) have revealed the CR% of endophytic fungi associated with bryophytes in tropical and temperate ecosystems: The CR% was 85.2% for 26 species in New Zealand, 96.6% for 18 species in North Carolina, 68.8% for 14 species in Germany, 60.9% for 22 species in Pacific Northwest. The above results show that the CR% of bryophytes by endophytic fungi is lower in the Antarctic ecosystem than in tropical and temperate ecosystems. These low CR% in this study suggest that a combination of abiotic conditions in Antarctica (i.e., dry, cold, oligotrophic, and UV radiation) may limit the survival of the endo-

phytic fungi in bryophytes. A similar trend was also found in vascular plant species. The CR% of the endophytic fungi in some vascular plant species in temperate environments ranged from 36.7% to 100% (Huang *et al.*, 2008; Gazis & Chaverri, 2010; Sun *et al.*, 2012), while the CR% of endophytic fungi was 34.8% for *Colobanthus quitensis* (Rosa *et al.*, 2010) and 9.4% for *Deschampsia antarctica* (Rosa *et al.*, 2009) in Antarctica.

Endophytic fungal composition

In the present study, 128 fungal isolates were identified, and these belonged to 20 fungal taxa in the *Ascomycota*, *Basidiomycota*, and 1 unknown fungus. The results indicate that the maritime Antarctic bryophytes are an interesting niche that harbors diverse fungal endophytes. Similar results were reported in previous studies of fungal endophytes in Antarctica. Azmi & Seppelt (1998) isolated fungi from the mosses *Bryum pseudotriquetrum*, *Ceratodon purpureus*, and *Grimmia antarctica* in the Windmill Islands region (66°17′S and 110° 32′E) and identified 21 fungal taxa. McRae & Seppelt (1999) studied the fungal diversity of the mosses *G. antarctici* and *B. pseudotriquetrum* in the Windmill Islands (66°17′S and 110°33′E) and identified 10 fungal taxa. Bradner *et al.* (2000) identified the microfungi isolated from the moss *Bryum argenteum* at Marble Point (77°24′S and 163°48′E) and found a new fungal isolate *Embellisia* species. Tosi *et al.* (2002) investigated the microfungi associated with eight moss species (*B. pseudotriquetrum*, *Syntrichia princeps*, *Schistidium antarctici*, *B. argenteum*, *Sarconeurum glaciale*, *C. purpureus*, *Hennediella heimii*, and *Campylopus pyriformis*) in Victoria Land (74–76°S and 162–165°E) and identified 28 fungal taxa belonged to 18 genera. The above results concerning fungal endophytes of bryophytes do not support the hypothesis that the diversity of organisms decreases from the maritime to the continental Antarctic zone (Ruisi *et al.*, 2007).

Of the 21 fungal taxa in the present study, only two were previously reported as endophytes associated with mosses in Antarctica: *Thelebolus* and *Chaetomium* species were isolated from the mosses in the continental Antarctic zone (Azmi & Seppelt, 1998; McRae & Seppelt, 1999; Tosi *et al.*, 2002). Endophytic *Hyphodiscus* and *Xylariaceae* have been documented in bryophytes in other ecosystems (Davis *et al.*, 2003; Davis & Shaw, 2008; Kauserud *et al.*, 2008). Additionally, two fungal taxa were reported to be associated with mosses in the previous studies, but they are not known to be endophytic; for example, *Eocronartium muscicola* is a parasitic bryophilous basidiomycete and has been found on at least 21 moss species (Boehm & McLaughlin, 1988, 1989), and *R. ericae* is an ericoid mycorrhizal fungus living in the tissues of the liverwort *Cephaloziella varians* in the maritime and sub-Antarctic (Upson *et al.*, 2007; Newsham, 2010). Some fungal species were isolated from nonbryophyte samples in Antarctica, for example *Scopulariopsis* species (Bialasiewicz & Czarnecki, 1999), and the yeast *Mrakia* and *Rhodotorula* species (Connell *et al.*, 2008; Loque *et al.*, 2010). Species of *Annulohypoxylon*, *Dermateaceae*, *Helotiales*, and *Hyaloscyphaceae* were also reported to be endophytes of vascular plants in other ecosystems (Zhang *et al.*, 2009; Gazis & Chaverri, 2010; Kernaghan & Patriquin, 2011). In the present study, *Xenopolyscytalum* species and one unidentified fungus (represented by isolates I12F-02253, -02276, -02282, and -02283) were first reported as fungal endophytes of plants. This unidentified fungus showed very low levels of sequence similarity with fungal species in the NCBI, suggesting that this taxon may be indigenous to Antarctica, and more taxonomic studies are necessary to determine its correct identification.

Values of the Shannon–Weiner diversity index, which was used to evaluate and compare the fungal diversity between the three bryophyte species, ranged from 0.91 to 1.99 in this study. Comparable Shannon–Weiner diversity indices (1.44 ± 0.3) were reported for endophytic fungi in the native Antarctic vascular plant species *C. quitensis* (Rosa *et al.*, 2010). In Antarctica, several abiotic stresses, such as cold and UV radiation, may lead to such low diverse endophytic fungal community that only a few stress-adapted fungal groups could survive in this environment.

The Sorenson's similarity coefficients for the endophytic fungi from the three plant species ranged from 0.18 to 0.40 in this study. These low Sorenson's similarity coefficients indicate that the fungi have different distributions and perhaps host specificity. This contrast to the findings by Davis & Shaw (2008) who reported that endophytic fungi of liverworts are restricted by geography but not by host.

Effect of temperature on growth *in vitro*

The results of growth temperature tests suggest that most of the endophytic fungi from bryophytes in the Fildes Region possess a remarkable ability to grow when temperatures are low. Tosi *et al.* (2002) found that most fungi isolated from mosses in Victoria Land could grow $\leq$ 5 °C and had their optimum between 10 and 24 °C, indicating that most endophytic fungi isolated from mosses in continental Antarctica are also psychrophilic and psychrotrophic. These psychrophilic and psychrotrophic endophytic fungi in bryophytes may rely on possible physiological (e.g., the accumulation of trehalose, the alteration of membrane lipid composition, antifreeze proteins, and cold-active enzymes) and morphological adaptations (e.g., the predominance of sterile fungi) to

Table 4. *In vitro* growth rate (mm day^{-1}) of endophytic fungi isolated from three bryophytes as affected by temperatures.

Taxon	Isolates tested	Temperature (°C)				
		4	12	20	27	35
Annulohypoxylon sp.	I12F-02260	0.97	1.27	1.34	-	-
Chaetomium sp.	I12F-02288	2.37	2.55	3.64	0.53	-
Eocronartium sp.	I12F-02262	1.76	1.53	1.22	-	-
Hyphodiscus sp.	I12F-02251	0.74	1.13	1.10	-	-
	I12F-02265	1.06	1.19	1.22	-	-
	I12F-02272	0.93	1.02	1.07	-	-
	I12F-02275	0.89	0.92	1.02	-	-
Mrakia sp.	I12F-02259	1.39	0.84	-	-	-
Rhizoscyphus sp.	I12F-02263	1.00	1.04	1.43	0.15	-
	I12F-02281	0.76	0.94	1.04	0.30	-
	I12F-02258	1.11	1.38	1.47	0.41	-
Rhodotorula sp. 1	I12F-02277	0.82	1.02	0.97	-	-
Rhodotorula sp. 2	I12F-02278	0.38	0.77	0.71	-	-
Scopulariopsis sp.	I12F-02300	0.50	0.66	1.17	1.36	-
Thelebolus sp.	I12F-02287	2.65	2.80	3.97	-	-
Xenopolyscytalum sp.	I12F-02273	0.93	0.90	1.27	-	-
Dermateaceae sp.	I12F-02297	0.28	0.30	0.87	-	-
Hyaloscyphaceae sp.	I12F-02257	0.76	1.05	1.37	0.56	-
Xylariaceae sp.	I12F-02261	1.43	1.44	1.34	-	-
Helotiales sp. 1	I12F-02256	0.75	0.94	0.85	-	-
Helotiales sp. 2	I12F-02296	0.45	0.52	0.32	-	-
Helotiales sp. 3	I12F-02299	0.19	0.36	0.48	-	-
Helotiales sp. 4	I12F-02284	0.96	1.52	2.39	-	-
Helotiales sp. 5	I12F-02289	1.11	1.20	2.13	-	-
Sporidiobolales sp.	I12F-02280	0.83	0.53	0.14	-	-
Unidentified fungus	I12F-02253	0.33	0.41	0.35	-	-

Values are means of three replicate plates containing PDA.

grow at low temperature. These physiological and morphological mechanisms have been reported in Antarctic fungi from other environmental samples (Robinson, 2001; Ruisi *et al.*, 2007). Additionally, life history of inhabit plant's tissue may help fungal endophytes coping with cold stress in the environments.

The effect of the endophytic fungi on Antarctic bryophytes remains unknown. Although endophytes may improve the tolerance of their hosts to abiotic stress (Khan *et al.*, 2012), further study is needed to determine whether the fungal endophytes could increase the tolerance of their bryophyte hosts to stress conditions in the Antarctica. It will be more interesting to study the physiology, evolution, and ecological role of these fungal endophytes in the future.

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