Journal of Clinical and Medical Images

Research Article

ISSN: 2640-9615 | Volume 6

Studies on Endolichenic fungi in Myelochroa (Lichenized fungi, Ascomycota) in Jeju Island, Korea

Changtian L[1], FenghuaT[1], Jae-Seoun HUR[2], Yu LI[1] and Ahmadi H[3*]

[1]Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China

[2]Korean Lichen Research Institute, Sunchon National University, Korea

[3]Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China

***Corresponding author:**

Hayatullah Ahmadi,
Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, Jilin 130118, China, E-mail: Hayatullahahmadi114@gmail.com

Received: 28 Jul 2022
Accepted: 08 Aug 2022
Published: 13 Aug 2022
J Short Name: JCMI

Citation:

Ahmadi H, Studies on Endolichenic fungi in Myelochroa (Lichenized fungi, Ascomycota) in Jeju Island, Korea. J Clin Med Img. 2022; V6(15): 1-9

Keywords:

Host; Multivariate analysisl Phylogeny; Site factors; Taxonomy

1. Abstract

In this study, the endolichenic fungi were isolated from lichen of Myelochroa spp. in Jeju Island, Korea. The taxonomic placement of the isolated endolichenic fungi was studied using a combination of morphological and phylogenetic approaches. Morphological differences among the fungal isolates indicated that diverse distinct morphotypes might be present within the Myelochroa. Fifty-six fungal isolates were selected for further molecular phylogenetic analysis using nuclear ribosomal DNA sequences, including both the internal transcribed spacers and the 5.8S gene region. The 23 endophytes were identified to various taxonomic levels, and some to the species level based on morphological description and comparison. Taken together, our results suggested that Xylaria and Hansfordia were the dominant fungal endophytes in Myelochroa, and some of these endolichenic fungi exhibited host specificity. The importance of host-endolichenic fungal interactions was supported by data showing that the overlap of endolichenic fungi in Myelochroa species by pairwise comparisons was low (0–20%). Multivariate analysis of the data obtained from direct observations confirmed the finding that endolichenic fungi assemblages were strongly affected by host and site factors.

2. Introduction

Lichens are a stable and self-supporting symbiosis between fungi (the mycobionts) and algal partners. Approximately 19,387 lichen taxa have been described worldwide [25]. A group of non-obligate micro-fungi also resides inside theinternal tissues of the lichen thalli without causing any harm tothem, which are called as Endolichenic Fungi (ELF). ELF always/often occur asymptomatically within thalli, which live within the lichen thallus in a similar fashion as endophytes that live between cells in plant tissues. Previous studies of endophyte diversity suggested that all plant species surveyed to date harbor one or more endophytic symbionts in their photosynthetic tissues [12, 13, 29]. Like endophytic fungi, endolichenic fungi might be abundant in different species of lichens. Mostly, these ELF species belongs to subphylum Pezizomycotina, and multilocus phylogenetic analysis related to seven orders, such as Hypocreales, Xylariales, and Ploesporales [32]. The diversity and prevalence of endolichenic fungi have been studied extensively [31], and a few reports have been published on isolation and identification of endolichenic fungi from lichens [11, 16]. Furthermore, endolichenic fungi could become the valuable sources of new bioactive natural products [28]. Thus, it is very important to evaluate diversity of endolichenic fungi for future development and utilization. Most studies conducted to date have indicated that host relationships above the species level affected endophyte assemblages, however, in the present study, we analyzed the diversity of endolichenic fungi within Myelochroa. Meanwhile, effects of different lichen species collected on Jeju Island., substrata (tree species) and localities on diversity of endolichenic fungi were investigated herein.

3. Methods

3.1. Study sites and sample collection

The lichens, including Myelochroa coreana, M. aurulenta, M. rhytidodes and M. irrugans (Nyl.) (Table 1), were sampled from an altitude of 250–1100 m in Jeju Island. The foliose lichen species, Myelochroa spp., were collected in each of three previously demarcated localities (Worum, Mt. Halla, and Temple Guanum). More than 30 lichen thalli of each species at each locality were collected. Lichen samples were packed with sterilized polythene bags and then transported to the laboratory for fungal isolation.

Table 1: Myelochroa speceis collected from Jeju Island, Korea for this study.

Collection No.	Species	Location and host tree	Altitude (m)
08082401-08082430	*M. coreana*	Worum, *Pinus thunbergii*	717
08082501-08082530	*M. aurulenta*	Worum, *Quercus gilva*	717
08082601-08082630	*M. rhytidodes*	Worum, *Quercus gilva*	717
08082701-08082730	*M. rhytidodes*	Worum, *Quercus gilva*	717
08082801-08082830	*M. rhytidodes*	Worum, *Quercus gilva*	717
08082901-08082930	*M. irrugans*	Mt. Halla, *Carpinus laxiflora*	1100
08083001-08083030	*M. coreana*	Guanum Temple, *Prunus serrulata*	283
08083101-08083130	*M. coreana*	Guanum Temple, *Prunus serrulata*	283

3.2. Isolation of endolichenic fungi

Fungal isolations were conducted according to the surface sterilization method [19]. In detail, healthy looking parts of each lichen talli were cut into small fragments (ca. 1 cm2). More than 12 pieces of each lichen species at each locality were prepared from 30 different lichen samples for isolation. The fragments were washed in tap water for 2 h and then sterilized by consecutive immersion for 10 s in 95% ethanol, 3 min in 0.5% sodium hypochloride and 30 s in 75% ethanol. Finally, each fragment was gently rinsed with sterilized distilled water. The rinsed thalli were dried with sterile filter papers and then plated on PDA with 0.01% streptomycin and incubated at 25 °C. Fungal isolates growing from each lichen fragment were purified on 2% MEA and then deposited in the Korea Lichen & Allied Bioresources Center (KOLABIC), the Korea Lichen Research Institute (KoLRI), Sunchon National University.

3.3. Morphological observation

Colonies were cultured on spore-inducing media (V8, MEA, CMA, SNA) at 25 °C until fruiting structures formed. Once fruiting bodies or conidia were observed, the samples were used for morphologic identification. All isolates were sorted into morphotypes based on morphological characteristics such as color of the colony and medium, growth rate, surface texture, margin characters, aerial mycelium, and spore production. Representative isolates of each group were selected for further identification using DNA sequence analysis.

3.4. DNA extraction, PCR amplification and sequence analyses

Approximately 10 mg of fungal mycelia were scraped with a sterile nipper from fresh cultures growing on PDA plates at 25 ˚C for 5–15 d. Fungal genomic DNA was prepared using a NucleoSpin® DNA extraction kit (Macherey Nagel, Düren, Germany). The amplification profile was according to the follow stages: initial denaturation at 95 °C for 90 s, followed by 30 cycles of 95 °C for 30 s, 42 °C for 60 s, 72 °C for 60 s, and a final extension at 72 °C for 3 min. The PCR products were purified using a QIAquick PCR purification kit (Qiagen) and then sequenced using the ABI PRISM BigDye Terminator cycle sequencing kit and an Applied Biosystems model 310 automatic DNA sequencer (Applied Biosystems). The sequence data were assembled using SeqMan DNASTART (Madison, WI) and compared with rDNA sequences available in GenBank using the BLAST program to determine their approximate phylogenetic affiliations. Reference sequences obtained from GenBank were also included in this analysis. Phylogenetic analysis was conducted based on ITS and 5.8S gene data using Neighbor-Joining (NJ) with the Jukes–Cantor correction in the MEGA 4 package [34].

3.5. Definitions and statistical analyses

The percentage of Myelochroa pieces with single and multiple infections was computed using Microsoft Excel. The similarity between endolichenic fungal assemblages on different Myelochroa substrata was expressed based on the percentage of shared species and the Bray–Curtis similarity index [5]. Patterns from the resulting similarity matrix were examined using Nonmetric Multidimensional Scaling (NMDS) ordination in PRIMER v.6 [7].

The overlap and complementarity of endolichenic fungi from different Myelochroa species were calculated as follows [8]:

Overlap (%) = (number of taxa shared between A and B × 100) / (total number of taxa observed in A and B)

Complementarity (%) = 100 - overlap

Where A denotes the number of endolichenic fungi species in one Myelochroa species and B represents the number of endolichenic fungi species in another Myelochroa species (or elevation or site).

4. Results

4.1. Isolation

The morphological diversity of the isolated endolichenic fungi growing on PDA and water agar medium clearly demonstrated that there were several different species inside the lichen thalli. A total of 56 isolates were obtained from the lichen thalli of four Myelochra species. Among them, 19, 21, 12 and 4 stains were isolated from M. coreana, M. rhytidodes, M. irrugans and M. auralenta, respectively (Table 2). There was no significant difference in morphological characters of the isolates cultured on water agar and PDA with the same Myelochroa species (P=0.05), however, PDA yielded a significantly higher number of isolates than WA (P=0.05).

Table 2: GenBank accession numbers and the closest matching sequences during a BLAST search of the endolichenic fungi isolated from Myelochroa species.

Fungal Strain	Number of Isolates	Accession No.	Blast match sequence		
			Reference accession No.	Cov. (%)	Max. ident. (%)
JP14-1	1	GQ906953	*Chaetomium globosum* GQ221865.1	89	99
JW14-1	1	GQ906969	*Daldinia childiae* AM292043.1	96	99
JP14-2	1	GQ906969	*Daldinia childiae* AM292043.1	96	99
JW14-2	1	GQ906965	*Daldinia childiae* AM292043.1	95	99
JP14-3	2	GQ906954	*Xylaria acuta* isolate AY544676.1	59	99
JP14-4	1	GQ906954	*Xylaria acuta* isolate AY544676.1	59	99
JP28-1-1	1	GQ906956	*Nemania aenea* AF201704.1	72	98
JW28-1-1	1	GQ906955	*Creosphaeria sassafras* AJ390424.1	64	99
JP28-1-2	1	GQ906970	*Anthostomella leucospermi* EU552100.1	100	92
JP28-1-3	1	GQ906955	*Creosphaeria sassafras* AJ390424.1	64	99
JP28-1	1	GQ906952	*Nemania aenea* AF201704.1	72	97
JW28-1	1	GQ906952	*Nemania aenea* AF201704.1	72	97
JP28-2	1	GQ906952	*Xylaria acuta* AY544676.1	60	99
JW28-2	1	GQ906957	*Creosphaeria sassafras* AJ390424.1	63	99
JP28-3	1	GQ906958	*Xylaria polymorpha* AB512310.1	98	90
JP38-1	2	GQ906965	*Daldinia childiae* AM292043.1	95	99
JW38-1	1	GQ906960	*Bjerkandera adusta* strain xsd08025	93	99
JP38-2	2	GQ906952	*Xylaria acuta* AY544676.1	60	99
JW38-2	1	GQ906969	*Daldinia childiae* AM292043.1	96	99
JP38-3	4	GQ906969	*Daldinia childiae* AM292043.1	96	99
JW38-3	1	GQ906952	*Xylaria acuta* isolate AY544676.1	60	99
JP38-4	1	GQ906969	*Daldinia childiae* AM292043.1	96	99
JW38-4	1	GQ906959	*Anthostomella leucospermi* EU552100.1	100	91
JP38-5	1	GQ906969	*Daldinia childiae* AM292043.1	96	99
JW38-5	1	GQ906952	*Xylaria acuta* isolate AY544676.1	60	99
JP38-6	1	GQ906966	*Daldinia childiae* AM292043.1	95	99
JP40-1	1	GQ906945	*Hypocrea lutea* AB027384.1	100	99
JW40-1	1	GQ906952	*Xylaria acuta* isolate AY544676.1	60	99
JW40-2	1	GQ906942	*Cordyceps sinensis* EF488439.1	86	99
JW40-3	1	GQ906943	*Bjerkandera adusta* AY089741.1	94	99
JW40-4	1	GQ906944	*Xylaria* sp. DQ480358.1	81	96
JP 60-1	1	GQ906967	*Daldinia childiae AM292044.1*	94	97
JW60-1	1	GQ906951	*Phaeoacremonium rubrigenum AB278173.1*	77	90
JP60-2	1	GQ906949	*Biscogniauxia sp. EU009960.1*	78	95
JW60-2	3	GQ906952	*Xylaria acuta isolate AY544676.1*	60	99
JP60-3	1	GQ906948	*Lecythophora sp. AY219880.1*	88	96
JW60-3	1	GQ906971	*Anthostomella proteae EU552101.1*	92	95
JP60-4	1	GQ906950	*Camillea obularia AF201714*	72	94
JW60-4	1	GQ906947	*Xylaria arbuscula AF163028.1*	87	97
JP60-5	1	GQ906952	*Xylaria acuta isolate AY544676.1*	60	99
JW60-5	1	GQ906952	*Xylaria acuta isolate AY544676.1*	60	99
JW60-6	1	GQ906952	*Xylaria acuta isolate AY544676.1*	60	99
JW60-7	1	GQ906946	*Ophiocordyceps sinensis FN386283.1*	78	100
JP89-1	1	GQ906943	*Bjerkandera adusta AY089741.1*	94	99
JW89-1	1	GQ906954	*Xylaria acuta isolate AY544676.1*	59	99
JP89-2	1	GQ906961	*Xylaria venosula AB462757.1*	71	98
JW89-2	1	GQ906952	*Xylaria acuta isolate AY544676.1*	60	99
JP89-3	1	GQ906969	*Daldinia childiae AM292043.1*	96	99
JW89-3	1	GQ906969	*Daldinia childiae AM292043.1*	96	99
JP89-4	1	GQ906941	*Nemania aenea AF201704.1*	72	97
JW89-4	1	GQ906962	*Xylaria polymorpha AB512310.1*	98	90
JP89-5	1	GQ906943	*Bjerkandera adusta AY089741.1*	94	99
JW89-5	1	GQ906963	*Cordyceps dipterigena AY245629.1*	72	93
JP 94-1	1	GQ906964	*Anthostomella leucospermi AY544676.1*	60	98
JW 94-1	1	GQ906964	*Anthostomella leucospermi AY544676.1*	60	98
JP 94-2	1	GQ906968	*Biscogniauxia capnodes EF026131.1*	91	96

4.2. Morphological characteristics of endolichenic fungi

The endolichenic fungi obtained in this study could be separated into four distinct groups based on morphological obeservation. The representative isolates of these groups (designated Groups A to D) were chosen for further study (Table 2). The Group A isolates (JP14-3, JP28-1-2, JW40-4, JP60-1, JP60-4, JW60-4, JP60-5, JW89-2 and JW89-1) produced light-colored mycelium bearing ascomata with light colored bases, similar to Xylaria. The Group B isolates (JW89-4 and JP28-3) produced light brown to greyish-white colonies and ascomata with black ascomatal necks.

The Group C isolates (JW14-2, JW14-1, JP14-2, JP38-1, JP38-6, JW38-2, JP38-3, JP38-4, JP38-5, JP89-3 and JW89-3) produced dark grey to greenish colonies similar to Hansfordia. The Group D isolates (JW38-1, JW40-4) produced white-colored colonies that turned light brown with age and were similar to Paecilomyces.

4.3. Phylogenetic analysis of representative endolichenic fungi

The sequences of the ITS1-5.8S-ITS2 region of these isolates were compared with 23 corresponding sequences of reference fungal taxa in the NCBI database. In Figure 1, all of the isolates from Myelochroa lichens could be classified as Sordariomycetes and included Sordariales (Group A), Hypocreales (Group B) and Xylariales (Group C), and Xylariales, Hypocreales, Coniochaetales and Sordariales formed momophyletic groups. The unidentified isolates were positioned in all of these groups, which indirectly indicated their affiliations with respect to the previously identified taxa. Within the Sordariales (Group A), A BLAST search showed that Chaetomium globosum GQ221865.1 was closely related to JP14-1. In Hypocreales (Group B), Hypocrea lutea AB027384.1 was most closely related to JW60-1 and JP60-3 by high bootstrap values. Xylariaceae (in group C) including Nemania, Thuemenella, Anthostomella, Daldinia, Biscogniauxia, Creosphaeria and Camillea were well characterized in a single group, although they were divided into several clades. The BLAST searches of the ITS sequences revealed no clear relationship between JW14-1, JP38-1, and JP38-6 and any fungal groups that have been reported to date, suggesting that these fungi may be potential novel endophyte species.

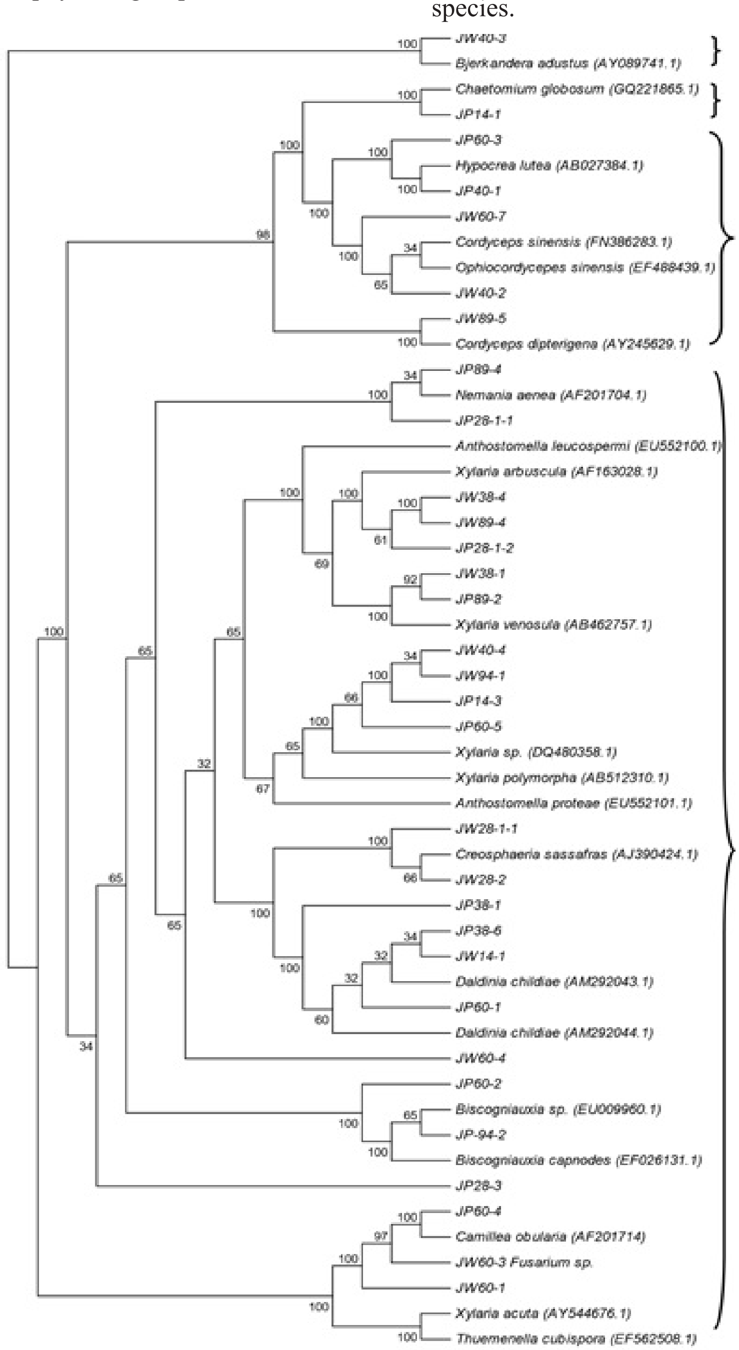

Figure 1: Phylogenetic analysis of the endolichenic fungal isolates based on the ITS sequence. Bootstrap values (>50%) from 1000 replicates are included at the internodes.

4.4. Identification of novel endophyte species

Colonies on PDA at 25 °C up to 50 mm diam in 7 d, effuse, hairy or velvety, pale grayish brown to brown or dark brown. Mycelia superficial, partly immersed in substratum, composed of smooth, branched, septate, pale brown hyphae, 1.5–4.0 μm wide. Conidiophores macronematous, mononematous, erect, branched, straight, very pale brown to pale brown, smooth, apices not setiform, of indeterminate length, 2.5–4.0 μm wide. Conidia apiculate at one end, Conidial ontogeny holoblastic, apical wall building, matured conidia synchronous with conidial cells. Conidial secession rhexolytic by fracture of the wall of a small separating cell. Proliferation holoblastic. Conidia aerogenous, solitary, later acropleurogenous, obovoid to broadly ellipsoid, appearing smooth or minutely echinulate, non-septate, very pale brown, 5.0–10.0 μm long, 4.5–8.0 μm wide, with a minute basal frill derived from the apex of the separating cell (Figure 2).

This fungus differs from H. alba in that it has no basal scars and conidiophores which are macronematous only on the base. It is also different from H. biophila because it usually produces only one conidium on conidiophores, while the latter usually produces several conidia on conidiophores [23]. Other species of Hansfordia were isolated from soil and treated as members of soil fungi [29]. Hansfordia is very closely related to Dicyma Boulanger, Arx [2] and Hu and Guo [17] treated Dicyma as a synonym of Hansfordia. However, Hansfordia species do not produce conidia from sides of conidiophores. The morphological identification of this fungus is confirmed by phylogenetic analyses of its ITS sequence (Accession No. GQ906969). The species formed a clade with many species of Daldinia (Figure 3). It is suggested that JW14-1 as Hansfordia pauciconidia sp. nov., MYCOBANK MB 517708.

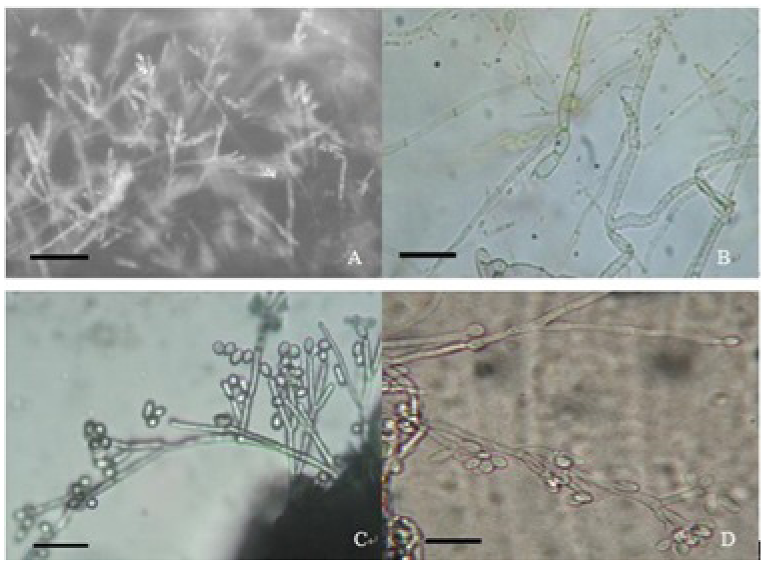

Figure 2: Hansfordia pauciconidia. A: conidiophores viewed on PDA, B: chlamydospore-like cells, C: conidiophores and conidia, Bars A: 100 μm; B, C: 25 μm; D: 15 μm.

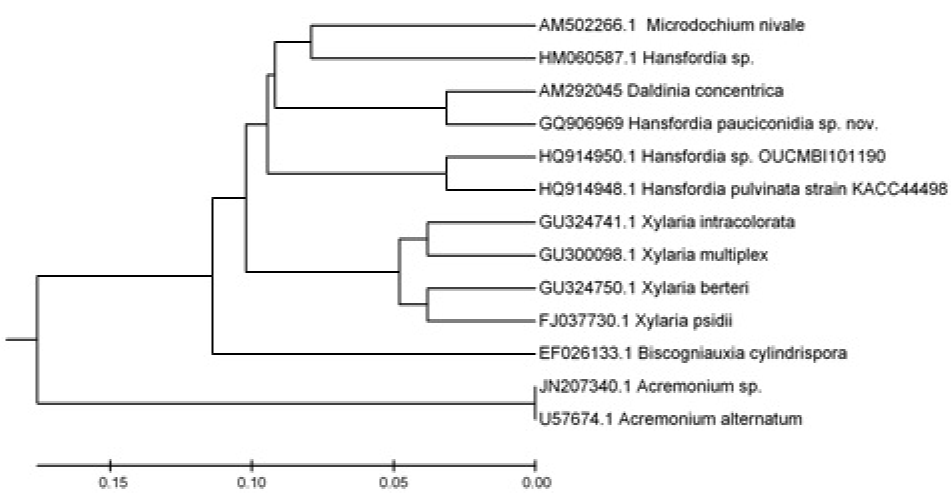

Figure 3: The dendrogram generated based on ITS sequence of Hansfordia paucicondia and related fungi blasted in GenBank

4.5. Determination the relationship between Endolichenic fungal and Myelochroa spp.

A total of 56 species of endolichenic fungal were obtained from four Myelochroa spp. These findings suggest that the actual number of species isolated from Myleochroa spp. may be underestimated. Because the observation of species richness underestimates the actual number of species, statistical methods have been developed to reduce this bias. Non-parametric extrapolation methods are efficient for predicting population richness from samples. The main non-parametric estimators are the first-(Jack1) and second-order Jackknife (Jack2), and two Chao estimators (Chao1 and Chao2). These estimators differ in the way in which rare species are tailed to correct the observation of species richness. Observations of species richness ranged from 4 species (M. auralenta) to 21 species (M. rhytidodes) in our samples. The Chao2 Estimator suggested that 5 to 51 species could be isolated from one species of lichens (Figure 4). In the Bootstrap (Bootstrap estimator based on the proportion of quadrats containing each species) diversity profile, samples were found to have high diversity. Except for Chao1, the sample size impacted all other non-parametric estimators of different Myelochroa species. For Chao2, the correction of Chao1 showed similar behaviour to the Jack-knife estimators in Myelochroa. The value of Jack2 is generally higher than that of Jack1. This indicates that one Myelochroa species is more abundant than two Myelochroa species and that when the number of Myelochroa species increases, new taxa belonging to the rare species is added.

4.6. Differences between Myelochroa species, locality and elevation

The endolichenic fungal assemblages of Myelochroa were also strongly shaped by the Myelochroa species, as shown in Figure 4. The MDS graph revealed that the endolichenic fungal assemblages from the same host species were clustered together. A stress level of 0.15 indicated that the MDS plots provided a satisfactory representation of the data. The statistical significance of assemblage differences in Myelochroa species was confirmed by cumulative species count, which was in agreement with the results shown in Figure 5. Endolichenic fungal assemblages isolated from different Myelochroa species differed significantly in all pairwise comparisons (Figure 5). The overlap of endolichenic fungi in pairwise comparisons of Myelochroa species was low (0–20%), indicating that few endolichenic fungi were shared by different Myelochroa species (Table 2). Although several fungal taxa were common to Myelochroa species, including Xylaria species and Hansfordia species, no species were isolated from all four Myelochroa species.

The locality at different altitudes was also a major factor that shaped the endolichenic fungi assemblages in Myelochroa. In the MDS plots, endolichenic fungal assemblages from the same site clustered together (Figure 5). Many of the isolated endolichenic fungi appeared to be species not previously reported in Korea.

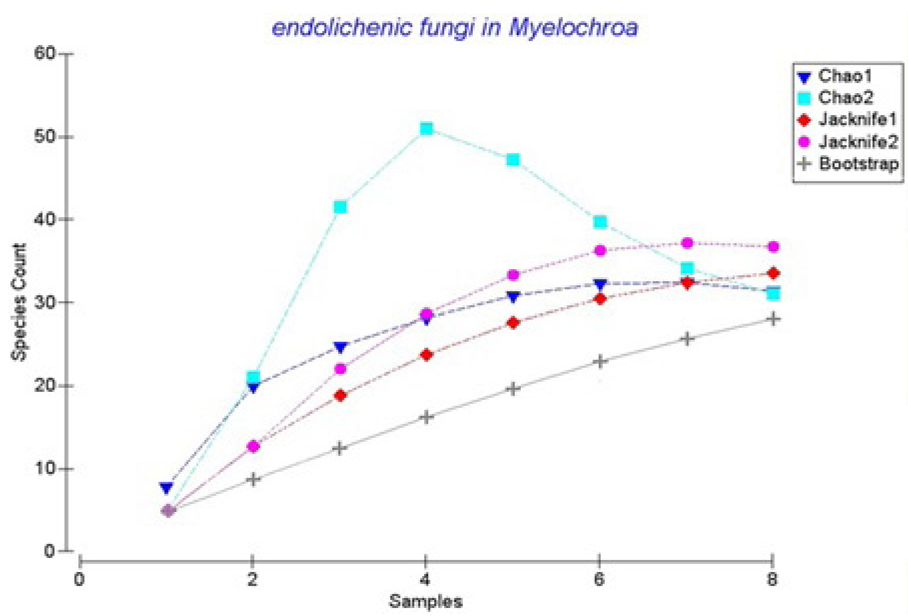

Figure 4: Plots of cumulative species count in the relation with changes in Mylochroa sample numbers.

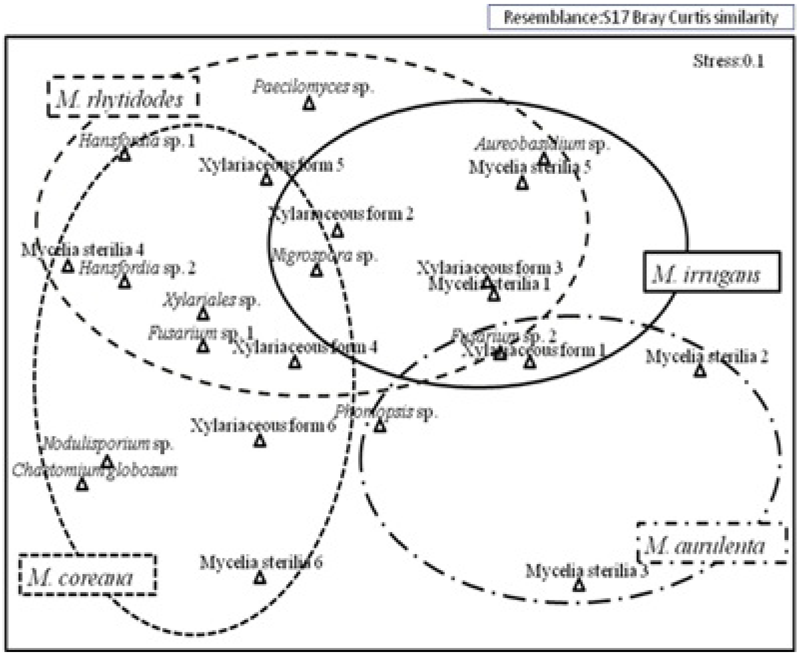

Figure 5: The endolichenic fungal assemblages clustered together in the same host species with non-metric Multi-Dimensional Scaling analysis (Stress=0.15).

5. Discussion

The endophytes in the lichen were established based on the isolation from Parmelia taractica, Peltigera praetextata, Cladonia coniocraea, Dermatocarpon miniatum, Melanelia sorediata, Parmelia sp., Punctelia borreri, Ramalina sinensis and Xanthoria mandschurica [4, 19, 22, 33]. We relied on a combination of morphological and molecular methods for fungal identification, and there was a good consistence between the datasets generated using these methods. The endolichenic fungi isolated from Myelochroa sp. mainly belonged to Xylaria and Hansfordia, and there were different species in each genus. For example, Xylariaceous form isolates obtained from the same Myelochroa sp., JW89-1, JP89-2, and JW89-2, had different ITS1-5.8S-ITS2 sequences, suggesting that they belonged to different fungal taxa. However, 20–30% of the sequences available in GenBank for comparative analysis might not be accurately identified, therefore, additional studies are necessary to correct their identification (Hyde & Soytong, 2007). Nevertheless, we can use data generated by molecular methods as a reference for identification because they facilitate identification of individual species among other fungal taxa.

Xylaria species are common endophytes in many tropical plants, including palms, orchids, bromeliads, aroids and ferns (Dreyfuss & Petrni, 1984; Rodrigues, 1994; Richardson & Currah, 1995). In Puerto Rico, Xylaria endophytes have been found in rain forest trees (Lodge, Fisher, & Sutton, 1996) and orchids (Bayman, Lebron, Tremblay, & Lodge, 1997). Because Myelochroa thalli used in our study were exclusively collected from the bark of trees, it was interesting to compare the diversity of endolichenic fungi that originated from lichens with that of endophytes from plants. Hansfordia species comprised a small proportion of the fungal biota. This genus is most closely related to Dicyma, Ascotricha, Geniculosporium, Nodulisporium and Calcarisporium (Arx, 1981). The natural habitats of Hansfordia included soil and the dead stems of trees and herbaceous plants, especially dead wood in the tropics (Paulus, Kanowski, Gadek, & Hyde, 2006). No information was available regarding health effects or toxicity to its habitat plant. This was the first report of Hansfordia from lichens. Therefore, it will be interesting to determine the origin and relationship of Hansfordia endolichenic fungi with other endophytes inhabiting in the same host tree. Moreover, investigation of the function of these endolichenic fungi in lichens and/or host trees will provide valuable information. Accordingly, the ecological relationship between Hansfordia and Myelochroa should be concerned in further study.

In this study, several endolichenic fungi isolated from Myelochroa were identified and their diversity was also investigated. The endolichenic fungi were expected to play different roles in ecological functions, for example, Chaetomium globosum is the most common species of Chaetomium which can produce several bioactive compounds (Attaur, 2005; Wang et al., 2006; Wijeratne, Turbyville, Fritz, Whitesell, & Gunatilaka, 2006; Fogle, Douglas, Jumper, & Straus, 2007). Additionally, Chaetomium globosum is the most common endophytic genus found in lichens, however, the relevance and function of the endolichenic fungi and host lichen species were still not studied to date.

Endolichenic fungi assemblages in the four Myelochroa spp. evaluated in this study were strongly shaped by host species. This was indicated by the clear separation of groups corresponding to Myelochroa taxa in the NMDS plots (Figure 4). Among four Myelochroa spp., the highest endolichenic fungal colonization rate occurred in M. rhytidodes. Additionally, most xylariaceous taxa, including some host-restricted species identified in the present study exhibited Myelochroa preference. Other studies showed that endophyte assemblages reflected host relationships at the species level (Carroll & Carroll, 1978; Arnold, 2007). Although our study revealed the diversity of endolichenic fungi in Myelochroa, more work is still required to elucidate fungal-lichen affiliations such as the function of endolichenic fungi in lichens symbiosis. While host-related factors were the strongest influencing factor, locality (site) also affected the endolichenic fungal assemblages. Additionally, even though all samples were collected from the same island, endophyte assemblages were influenced by season (Shankar, Shashikala, & Krishnamurthy, 2008). Finally, systematic characterizations of secondary metabolites are required to elucidate the function involved in host and tissue specificity.

6. Funding

The National Key Research and Development Program of China (2020YFD1000304, 2018YFD1001000), and the Program of Introducing Talents of Discipline to Universities (D17014) supported this study.

7. Acknowledgments

The authors also thank Dr. Xinyu Wang for his assistance with the identification of Myelochroa.

References

1. Arnold A. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biology Reviews. 2007; 21(2-3): 51-66.
2. Arx JAV. On Monilia sitophila and some families of Ascomycetes. Sydowia. 1981; 34: 13-29.
3. Attaur R. Cumulative general subject index volumes 1-30. In Studies in Natural Products Chemistry. 2005; 31: 3-869.
4. Bayman P, Lebron LL, Tremblay RL, Lodge DJ. Variation in endophytic fungi from roots and leaves of Lepanthes Orchidaceae. New Phytologist. 1997; 135(1): 143-9.
5. Bray JR, Curtis JT. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs. 1957; 27(4): 325-49.
6. Carroll GC, Carroll FE. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Canadian Journal of Botany. 1978; 56(56): 3034-43.
7. Clarke KR, Warwick RM. Change in marine communities: an approach to statistical analysis and interpretation, p.172. Second ed. Plymouth, UK, PRIMER-E Ltd. 2001.
8. Colwell RK, Coddington JA. Estimating terrestrial biodiversity through extrapolation. Philos. Trans. R. Soc. Sec. B. 1994; 345(1311): 101-18.
9. Dreyfuss M, Petrni O. Further investigations on the occurrence and distribution of endophytic fungi in tropical plants. Botanic a Helvetica. 1984; 94: 33-40.
10. Fogle MR, Douglas DR, Jumper CA, Straus DC. Growth and mycotoxin production by Chaetomium globosum. Mycopathologia. 2007; 164(1): 49-56.
11. Fukasawa Y, Ando Y, Song Z. Comparison of fungal communities associated with spruce seedling roots and bryophyte carpets on logs in an old-growth subalpine coniferous forest in Japan. Fungal Ecology. 2017; 30: 122-31.
12. Gange AC, Eschen R, Wearn JA, Thawer A, Sutton BC. Differential effects of foliar endophytic fungi on insect herbivores attacking a herbaceous plant. Oecologia. 2012; 168: 1023-31.
13. Gao FK, Yong YH, Dai CC. Effects of endophytic fungal elicitor on two kinds of terpenoids production and physiological indexes in Euphorbia pekinensis suspension cells. Journal of Medicinal Plants Research. 2011; 5: 4418-25.
14. Gene J, Mercado-Sierra A, Guarro J. Dactylaria cazorlii and Hansfordia catalonica, two new hyphomycetes from litter in Spain. Mycological Research. 2000; 104(11): 1404-7.
15. Girlanda M, Isocrono D, Luppi-Mosca AM. Two foliose lichens as microfungal ecological niches. Mycologia. 1997; 89(4): 531-6.
16. Masumoto H, Degawa Y. The effect of surface sterilization and the type of sterilizer on the genus composition of lichen-inhabiting fungi with notes on some frequently isolated genera. Mycoscience. 2019; 60(6): 331-42.
17. Hu KX, Guo SX. A new species of Hansfordia, an endophyte from Anoectochilus roxburghii. Mycotaxon. 2007; 102: 253-6.
18. Hyde KD, Soytong K. Understanding microfungal diversity - a critique. Cryptogamie, Mycologie. 2007; 28(4): 281-9.
19. Li WC, Zhou J, Guo SY, Guo LD. Endophytic fungi associated with lichens in Baihua mountin of Beijing, China. Fungal Diversity. 2007; 25: 69-80.
20. Lodge DJ, Fisher PJ, Sutton BC. Endophytic fungi of Manilkara bidentata leaves in Puerto Rico. Mycologia. 1996; 88(5): 733-8.
21. Paulus BC, Kanowski J, Gadek PA, Hyde KD. Diversity and distribution of saprobic microfungi in leaf litter of an Australian tropical rainforest. Mycological Research. 2006; 110(12): 1441-54.
22. Petrini O, Hake U, Dreyfuss MM. An analysis of fungal communities isolated from fruticose lichens. Mycologia. 1990; 82(4): 444-51.
23. Rao PR, Rao S. A new Hansfordia Hughes from India. Current Science. 1980; 49: 447-7.
24. Richardson KA, Currah RS. The fungal community associated with the roots of some rainforest epiphytes of Costa Rica. Selbyana. 1995; 16(1): 49-73.
25. Robert L, Brendan PH, Steven DL. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota –Approaching one thousand genera. The Bryologist. 2016; 119(4): 361-416.
26. Rodrigues KF. The foliar fungal endophytes of the Amazonian palm Euterpe oleracea. Mycologia. 1994; 86(3): 376-85.
27. Shankar NB, Shashikala J, Krishnamurthy YL. Diversity of fungal endophytes in shrubby medicinal plants of Malnad region, Western Ghats, Southern India. Fungal Ecology. 2008; 1(2): 89-93.

28. Shivankar A, Sunil K, Deshmukh M, Sudhakara R, Ram P, Mayurika G. Endolichenic fungi: A hidden source of bioactive metabolites, South African Journal of Botany. 2020; 134: 163-86.

29. Stone JK, Bacon CW, White JF. An overview of endophytic microbes: endophytism defined. 2000; 3-29.

30. Bacon CW, White JF. editors. Microbial endophytes. Marcel Dekker, New York USA.

31. Sun X, Guo LD. Endophytic fungal community of Illicium verum with different star anise yields in southern China. Mycosystema. 2015; 34(5): 996-1006.

32. Suryanarayanan TS, Thirunavukkarasu N. Endolichenic fungi: the lesser known fungal associates of lichens. Mycology. 2017; 8(3): 189-96.

33. Suryanarayanan TS, Thirunavukkarasu N, Hariharan GN, Balaji P. Occurrence of non–obligate microfungi inside lichen thalli. Sydowia. 2005; 57(1): 120-30.

34. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 2007; 24(24): 1596-9.

35. Wang S, Li XM, Teuscher F, Li DL, Diesel A, Ebel R, et al. Chaetopyranin, a benzaldehyde derivative, and other related metabolites from Chaetomium globosum, an endophytic fungus derived from the marine red alga Polysiphonia urceolata. Journal of Natural Products. 2006; 69(11): 1622-5.

36. Wijeratne EM, Turbyville TJ, Fritz A, Whitesell L, Gunatilaka AA. A new dihydroxanthenone from a plant-associated strain of the fungus Chaetomium globosum demonstrates anticancer activity. Bioorg Med Chem. 2006; 14(23): 7917-23.