Fungal Ecology 21 (2016) 43–49

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Fungicolous xylariaceous fungi in coralloid basidiomata

Wan-Rou Lin, Te-Yu Gu, Pi-Han Wang*

Department of Life Science, Tunghai University, No. 1727, Sec. 4, Taiwan Boulevard, Taichung 40704, Taiwan, ROC

ARTICLE INFO

Article history:
Received 23 June 2015
Received in revised form
25 November 2015
Accepted 16 February 2016

Corresponding editor: Anne Pringle

Keywords:
Fungicolous fungi
Commensalism
Scytinopogon
Xylaria
Xylariales

ABSTRACT

Fifteen species of xylariaceous fungi were isolated from more than 100 *Scytinopogon* sp. basidiomata in three forests and identified by rDNA ITS sequencing. Xylariaceous fungi were present in the lower part of the basidiomata, occurred less often in the upper part and were absent in the tips of its coralloid basidiomata. These results indicated that xylariaceous fungi grow continuously in the basidiomata and that the apical growth of *Scytinopogon* sp. basidiomata was faster than the growth of xylariaceous fungi in its host. The results suggested that these xylariaceous fungi established a stable coexistence with *Scytinopogon* sp. in the forest. Five species of xylariaceous fungi were endophytes in nearby plants. Our study suggested that these endophytic xylariaceous fungi were sources of fungicolous fungi in *Scytinopogon* sp. in the forest.

© 2016 Elsevier Ltd and The British Mycological Society. All rights reserved.

1. Introduction

Fungicolous fungi represent species that regularly accompany other fungi (Jeffries, 1995). This term can be applied to any inter-fungal relationship, including parasites, commensals or saprobionts (Kirk et al., 2008). Most studies report fungicolous fungi growing on sporocarps of other fungi, but not inside them. Only two reports have described the presence of basidiomycete hyphae in the ascocarp of truffles (Ceruti, 1988; Pacioni et al., 2007), and one study isolated *Cryptococcus victoriae* from the inner tissue of *Paxillus* (Yurkov et al., 2012). These fungi are present in healthy fruit bodies, suggesting that they may play significant roles by interacting with the developing host sporocarp.

Recently, most research on fungicolous fungi has focused on biochemical innovation (Shim et al., 2011; Hwang et al., 2015) and the interactions between fungicolous fungi and their host (He et al., 2006; Pacioni et al., 2007). For example, some mycelia have been observed in the sections of truffle ascomata, and they grow attached to the hyphal wall of the mycelium of *Tuber borchii* when in dual culture (Pacioni et al., 2007). Most of the fungicolous fungi reported were microfungi. Few studies have investigated the diversity of a coexisting fungicolous fungi in a host species in forest ecosystems nor the source of these fungicolous fungi. In this study, we examined the association of a coralloid basidiomata and ascomycetous xylariaceous fungi and explored the above questions.

2. Materials and methods

2.1. Study site

The study was conducted in three separate areas: two located in central Taiwan and one in northern Taiwan. The Zen-Len area spans the region from 23°28′ N to 23°55′ N latitude and from 120°48′ E to 121°09′ E longitude. The elevation ranges between 1300–1500 m. The average annual temperature and rainfall are 15.78 °C and 2628 mm, respectively. Most rainfall at this site occurs during the summer, from June to September. Some rainfall also occurs during the spring, from March to May; there is no obvious dry season. The main vegetation in this area is Japanese cedar, *Cryptomeria japonica*. The Lienhuachih area ranges from 500–900 m in altitude, and the main type of flora is the lauro-fagaceous forest (Su, 1984). The mean annual temperature and rainfall is 20.8 °C and 2285 mm with seasonality (Lu et al., 2008). More than half of the rain falls between May and September. Guanwu (121°07′E, 24°31′N) is a subtropical montane forest and ranges from 2000–2250 m in altitude. It is dominated by *C. japonica* with a few *Taiwania cryptomerioides*, *Chamaecyparis formosensis* and *Cunninghamia konishii*.

* Corresponding author.
E-mail address: phwang@thu.edu.tw (P.-H. Wang).

http://dx.doi.org/10.1016/j.funeco.2016.02.004
1754-5048/© 2016 Elsevier Ltd and The British Mycological Society. All rights reserved.

2.2. Isolation of xylariaceous fungi

In our previous study, an undescribed *Scytinopogon* species was found to be widely distributed in Japanese cedar plantations of central Taiwan and exhibited a long fruiting season from April to October (Lin et al., 2015). *Scytinopogon* sp. basidiomata were collected from 2006 to 2012 and xylariaceous fungi were isolated from more than 100 *Scytinopogon* sp. sporocarps (data not shown). Mature and healthy basidiomata of *Scytinopogon* sp. were collected from the three study areas in 2010. Basidiomata were cut into 1 cm pieces. The process of sterilization was modified from the procedures of Guo et al. (2001). The sporocarp segments were surface sterilized with 1.05% hydrogen peroxide for 1 min and finally rinsed in sterile water for 45 s. The segments were dried with sterilized tissue paper and placed onto potato dextrose agar. When hyphae grew from the cross sections of the segments, the hyphal tip was isolated and purified on a potato dextrose agar (difco) plate. The cultures were incubated at room temperature.

Elatostema lineolatum, *Diplazium dilatatum* and *C. japonica* were dominant plants in the Zen-Len area. Fresh leaves of *E. lineolatum* and *D. dilatatum*, root, bark and dead leaves of *C. japonica*, and soil samples were collected from the Zen-Len area with *Scytinopogon* sp. basidiomata. Xylariaceous fungi from these samples were also isolated, as described above.

2.3. DNA isolation and ITS amplification

Mycelia of xylariaceous fungi were harvested from the plates and stored at -20 °C. Total DNA from the mycelium was extracted by the CTAB method (Doyle and Doyle, 1990). The PCR amplification of the rDNA ITS region was undertaken using the universal primers ITS5/ITS4 or primers ITS1/ITS4 (White et al., 1990). PCR products were directly sequenced in an ABI PRISM 3730 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

2.4. Sequence analysis

Sequence data for the ITS regions were analyzed together with the outgroup *Sordaria fimicola* (GenBank accession no. AY681188). Moreover, several species of *Xylaria*, 2 species of *Eutypella*, and one species each of *Hypoxylon* and *Nemania* were used in ITS analysis. These species from GenBank were analyzed with the isolates in this study (Table 1). Sequence data for the ITS region were initially aligned and subsequent manual adjustments were made using the BioEdit Sequence Alignment Editor (Hall, 2011) and Clustal X 1.83 (Thompson et al., 1997). Using the same aligned datasets, parsimony analysis was performed with the default settings and parsimony bootstrap values were generated with 1000 replicate heuristic searches to estimate support for the clade stability of the consensus tree, with 1000 replicates in PAUP (Phylogenetic Analysis Using Parsimony) v4.0b10 (Swofford, 2003).

2.5. Detection and isolation of Xylaria spp. from Scytinopogon sp. basidiomata

To determine the distribution of *Xylaria*, 20 basidiomata of *Scytinopogon* sp. were cut into 1 cm segments from top to bottom and were individually placed into 1.5 ml microcentrifuge tubes. Ten basidiomata were analyzed by isolation and 10 underwent PCR detection. The isolation of *Xylaria* was performed as described previously (modified from Guo et al., 2001). The presence of xylariaceous fungi was recorded. Isolation rate was defined as the number of fragments that xylariaceous fungi isolated, divided by the total number of fragments isolated.

Xylaria-specific primers were designed for PCR detection. Sequences of *Xylaria* spp. from GenBank were aligned with the same regions of xylariaceous fungal isolates and related species using Clustal X 1.83 (Thompson et al., 1997). Primers were designed from the variable regions of the ITS. To examine the specificity of the primers, Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information was used to search for species with sequences homologous to the primers from GenBank. One oligonucleotide primer pair Xf1 (5′-GGGACATTCTGGGATGGGACATCC-3′) and Xr2 (5′-ACACACAACACGGCCAGGGGAC-3′), which targeted the appropriate *Xylaria* spp. rDNA sequences, were designed, and these primers shared little or no homology with *Scytinopogon* sp. rDNA sequences. Based on the sequencing information, the primer pair was *Xylaria* genus-specific, and the predicted amplification size of the *Xylaria* spp. product was approximately 400–500 bp. Primers were synthesized by Mission Biotech (Taipei, Taiwan). DNA from specimens of 1 cm tissues was extracted by the CTAB method (Doyle and Doyle, 1990) and PCR amplification with *Xylaria*-specific primer pairs was used to detect the presence of *Xylaria*. Detection rate was defined as the number of fragments that *Xylaria* detected, divided by the total number of fragments detected.

3. Results

3.1. Isolation

A total of 53 xylariaceous fungal isolates were isolated from fresh, healthy and intact basidiomata of *Scytinopogon* sp (Table 2). Xylariaceous fungal hyphae grew from tissues of the *Scytinopogon* sp. sample on a PDA plate in 24–48 h (Fig. 1). Pure cultures of these fungi formed white colonies with radial hyphal strands and black pigmentation. After 2–4 weeks, stromata were produced in rays. A total of 7 xylariaceous isolates belonging to 5 *Xylaria* spp. were isolated from plants which located near to *Scytinopogon* sp. in plots. *Scytinopogon* sp. was unculturable on 7 types of culture media (data not shown).

3.2. Diversity of xylariaceous fungi in basidiomata of Scytinopogon sp. and other substrata

The internal transcribed spacer (ITS) rDNA sequences of approximately 600 bp were obtained from 53 xylariaceous fungal isolates. Twenty-two reference sequences representing 14 fungal species were obtained by BLAST (Table 1).

According to ecological studies of endophytic fungi (Arnold et al., 2009; Okane et al., 2012), 90–95% ITS sequence similarity is often used as a species boundary in fungi. The ITS-5.8S rDNA gene dataset contained 85 taxa with 754 characteristics. One hundred and ninety-two base pairs of ambiguous aligned regions were excluded from parsimony. The phylogenetic tree from the maximum parsimony analysis is shown in Fig. 2. The newly isolated xylariaceous fungi were clustered into four clades. Clade A included 5 isolates of *Xylaria* sp. with high branch support (MP = 100%). In Clade B, isolates ZLX7-3 and ZLX7-4 from *Scytinopogon* sp. basidiomata clustered with *Nemania bipapillata* with strong bootstrap values (MP = 100%) and ITS sequence similarity (93%). Clade C included two clusters; GWX-1 was identified as *Hypoxylon monticulosum* with 97% ITS sequence similarity; isolates ZLX7-1 and ZLX7-2 had 95% ITS similarity to *Eutypella* sp., and they were different from the reference sequence of *Eutypella cerviculate*. Clade D consisted of several species of *Xylaria* supported by strong bootstrap values, including *Xylaria* spp., *Xylaria apoda*, *Xylaria adscendens*, *Xylaria bambusicola*, *Xylaria curta*, *Xylaria feejeensis*, *Xylaria grammica*, *Xylaria laevis*, *Xylaria multiplex*, and *Xylaria papulis*.

Download English Version:

https://daneshyari.com/en/article/8384420

Download Persian Version:

https://daneshyari.com/article/8384420

Daneshyari.com