Acta Ecologica Sinica 34 (2014) 160–164

Contents lists available at ScienceDirect

Acta Ecologica Sinica

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

Diversity of endophytic mycobiota in *Fortunearia sinensis*

Zhenying Wu, Shuzhen Yan, Shengliang Zhou, Shuanglin Chen *

College of Life Sciences, Nanjing Normal University, Nanjing 210023, China

ARTICLE INFO

Article history:
Received 22 October 2012
Revised 27 November 2012
Accepted 5 June 2013

Keywords:
Fortunearia sinensis
Endophytic fungi
Species accumulation curves
Relative abundance
Dominant population

ABSTRACT

Fortunearia sinensis is an endemic species of arbor to China. The diversity of endophytic fungi of *F. sinensis* was preliminarily studied in order to understand the composition and change of mycobiota. 1436 strains of endophytic fungi were isolated from healthy laminae, petioles and twigs of *F. sinensis* located in Baohua mountain of Jiangsu province separately in spring and autumn. The isolation rate amounted to 53%. The isolates were identified and classified into 33 genera based on the morphological characters and ITS sequences. *Alternaria*, *Fusarium* and *Pestalotiopsis* were three dominant genera with the relative abundance of 21.52%, 19.64% and 13.16%, respectively. 498 isolates belonging to 27 genera of endophytic fungi were obtained from tested petiole samples of *F. sinensis*. The Shannon–Wiener index and Margalef index of endophytic fungi in the petiole were respectively 2.77 and 2.90 which were larger than those in the lamina and twig. 1076 isolates belonging to 27 genera of endophytic fungi were obtained in tissue samples of *F. sinensis* collected in autumn. Endophytic fungi of *F. sinensis* in autumn with the Shannon–Wiener index of 2.69 and Margalef index of 2.58 were more diverse than in spring.

1. Introduction

Fortunearia sinensis Rehd. et Wils., a species of woody plant in Hamamelidaceae, is unique to China. Its leaves contain bergenin, fortunearioside and other medicinal ingredients [1]. Bergenin has some effects including antitussive, expectorant, anti-inflammatory, hepatoprotection, immunity enhancement, anti-lipid peroxidation, scavenging various free radicals and so on [2–3]. In recent years, the wild medicinal plant resources producing bergenin has been decreasing rapidly. It is necessary for these plants to protect and propagate [4]. Endophytic fungi are common in living tissues of plants and have an abundant species diversity [5]. They can generally improve their host's resistance to severe environment and thus enhance the abilities of ecological competition [6]. On the other hand, endophytic fungi can produce the same or similar active natural products as the host plants. They are important resources from which natural products with the new structure or new activities can be obtained [7]. It is an important foundation to understand the community composition for the studies of ecological value, plant adverse resistance, natural active ingredients and so on. Consequently, the study for the endophytic fungi diversity of *F. sinensis* contributes to deeply explore the influence of endophytic fungi on the plant growth and seek endophytic fungi resources produced bergenin.

2. Materials and methods

2.1. Sampling

Samples were collected from one experimental plot located in Baohua Mountain Nature Reserve in Jurong City west of Jiangsu Province, China (32°10′N, 119°05′E). The vegetation type of the area is mixed evergreen/deciduous broadleaved forest. Our sampling site was situated in a natural community of *F. sinensis*. According to the linear trapezoidal sampling method, six individual trees were chosen as a sample based on the trapezoid (2 for up, 4 for below). Twigs, petioles and laminae were collected from three locations (upper, middle and lower of each tree) and were stored in ziplock bags, transported to our laboratory, and preprocessed within 48 h.

2.2. Isolation media

Potato Dextrose Agar (PDA): potato 200 g, dextrose 20 g, water 1000 mL, agar 15–20 g.

Czapek Dox Agar (CDA): $NaNO_3$ 3.0 g/L, KH_2PO_4 1.0 g/L, KCl 0.5 g/L, $MgSO_4$ 0.5 g/L, $FeSO_4$ 0.01 g/L, sucrose 30 g/L, agar 15–20 g, pH 6.7.

Corn Meal Agar (CMA): corn flour 200 g, agar 17 g, water 1000 mL, 70°C water bath for 1 h.

Malt Extract Agar (MEA): malt 200 g, saccharified for 3–4 h with 65 °C water bath and filtered, pH 6.4, agar 15–20 g/L.

* Corresponding author.
E-mail address: chenshuanglin@njnu.edu.cn (S. Chen).

http://dx.doi.org/10.1016/j.chnaes.2014.03.005
1872-2032/

Potato and *F. sinensis* Extract Agar (PFEA): potato 100 g/L were boiled for 20 min, and then *F. sinensis* leaf 100 g/L were boiled for 10 min. The filtrates were mixed, added dextrose 15 g/L and agar 15–20 g/L, and sterilized with 121 °C for 20 min.

Note: The above-mentioned 5 kinds of isolation media should be added 30 mL/L of 0.05% streptomycin and 0.05% penicillin before being used.

2.3. Separation and purification of endophytic fungi

Endophytic fungi were isolated from *F. sinensis* used tissue cultivation. To kill fungal propagules adhering to cuticle, fresh materials chopped in proper pieces were surface-sterilized by immersion in 3% NaClO solution for 1–2 min and 75% ethanol for different time (the leaf for 40 s, petioles and twigs for 90 s), before being rined in distilled water for 2–3 times. The laminae were cut into 5 mm × 5 mm in size, the petioles and twigs into 5 mm long. Five pieces were placed onto the same culture medium. Meanwhile, proper "sterile water" used to wash plant tissue for the last time was spreaded a blank culture medium. In addition, another fresh culture medium was placed in super-clean bench for 10 min. All mediums were cultured at 26 ± 1 °C for 3–5 d, until mycelia appeared at the cut of plant tissues. The mycelia of colony edge was taken with inoculating needle and continued cultivation. To get a single species, the above method should be repeated 2–3 times.

2.4. Identification of endophytic fungi

After isolation, species were classified to the genus according to morphological characteristics of their sporulating structures, spores (e.g. size, shape, color) and mycelium form using some identification manuals or monographs including Wei [8], Barnett and Hunter [9], Sutton [10] and Seifeit et al. [11]. Nomenclature to followed Kirk et al. [12].

391 strains were isolated from *F. sinensis* which could not produce spores, and were divided into 14 types of mycobiota on the basis of culture features. Genomic DNA from mycelia was extracted applying improved CTAB method. The rDNA ITS region was amplified with ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGA AGTAAAAGTCGTAACAAGG-3′) [13] primers. Sequence alignment and rearrangement were used Clustalx 1.81 software. The phylogenetic tree was built with Neighbor-jointing (NJ) arithmetic, and the confidence level of branch was estimated by bootstrap test repeated 1000 times.

2.5. Date analysis

To estimate the completeness of the survey in terms of the recorded species, species accumulation curve were constructed using the program EstimateS software. Diversity indices and calculation formulas:

Isolation rate (IR): the percentage of the amount of a particular type of endophytic fungi isolated to the total mass of tissue samples.

Relative abundance (RA): the percentage of the amount of a particular type of endophytic fungi isolated to the total isolates.

Shannon–Wiener index: $H' = -\Sigma(P_i \mathrm{Ln} P_i)$, in this formula: P_i is relative abundance of the i, given $P_i = N_i/N$; N_i is Number of i individual species, $i = 1, 2, 3, 4 \ldots S$.

Margalef index: $R = (S - 1)/\log_2(N)$, in this formula: S is number of species; N is the total number of individuals.

Sorenson index (C_S): $C_S = 2j/(a + b)$, j is the owned number of species or genus by two kinds of tissues or seasons; a is the number of species or genus of the endophytic fungi isolated from the one type of tissue or season and b is the number of species or genus of the endophytic fungi isolated from the other type of tissue or season.

3. Results and analysis

3.1. Sufficiency judgment of sampling quantity associated with the diversity in the endophytic fungi isolated from F. sinensis

The isolates were sampled by the following methods: two seasons (autumn and spring), three kinds of tissues (laminae, petioles and twigs) and six individual trees of *F. sinensis* were conducted. A total of 36 samples were obtained. Species accumulation curve was rising sharply and then shading into an asymptote and slow rise (Fig. 1). It showed that sampling effort was sufficient and effective [15]. The results indicated that species richness of endophytic fungi isolated from *F. sinensis* was calculated by Abundance-base Coverage Estimator (ACE) and Incidence-based Coverage Estimator (ICE), which was 43.65 and 42.33, respectively. In other words, the composition of isolates from this community of *F. sinensis* was about 44 and 43 genera. From the results of sampling, the isolates were actually 33 genera, which was 75–77% complete. It also indicated that the major species had been acquired from this community of *F. sinensis* ($\geqslant 3/4$).

3.2. The composition of endophytic fungi community from F. sinensis

In this study, 1436 strains of endophytic fungi were isolated from 2700 pieces including laminae, petioles and twigs of *F. sinensis* (Table 1). For two seasons, isolation rate of endophytic fungi in autumn (79.70%) was higher than that in spring. For different tissue, isolation rate of endophytic fungi from petioles was the highest (83.00%). According to morphological characteristics and rDNA-ITS sequences, the isolates from *F. sinensis* were classified into 33 genera, all which belonged to ascomycetes. From isolation rate and relative abundance, the percentage of anamorph ascomycetes was much greater than teleomorph ascomycetes. The hyphomycetes of mitosporic ascomycetes were dominant fungal community and relative abundance was 68.40%. The hyphomycetes of mitosporic ascomycetes were classified into 13 genera and isolation rate was 36.37%; the coelomycetes of mitosporic ascomycetes belonged to 14 genera and isolation rate was 11.37%; 6 genera were teleomorphs ascomycetes and isolation rate was 5.44%. If relative abundance exceeding 10% was taken as a difference index, *Alternaria*, *Fusarium* and *Pestalotiopsis* were dominant fungal genera, of which relative abundance were 21.52%, 19.64% and 13.16%, respectively (Table 2).

3.3. The effects of different media on isolation of endophytic fungi from F. sinensis

Endophytic fungi isolated from different media were little different in the number of genera (Table 3). Seventeen genera were isolated by Czapek Dox Agar (CDA); 19 genera were obtained respectively from Potato Dextrose Agar (PDA), Malt Extract Agar

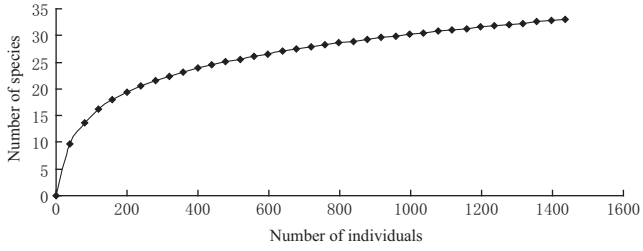

Fig. 1. Species accumulation curves of endophytic fungi from *Fortunearia sinensis*.