MYCOSCIENCE 57 (2016) 171–180

Available online at www.sciencedirect.com

MYCOSCIENCE

ISSN 1340-3540 (print), 1618-2545 (online)

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

Full paper

Structure of phyllosphere fungal communities in a tropical dipterocarp plantation: A massively parallel next-generation sequencing analysis

Ayako Izuno [a,*], *Akifumi S. Tanabe* [b,c], *Hirokazu Toju* [d], *Michimasa Yamasaki* [a], *Sapto Indrioko* [e], *Yuji Isagi* [a]

[a] *Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan*
[b] *Graduate School of Global Environmental Studies, Kyoto University, Yoshida Nihonmatsu-cho, Sakyo-ku, Kyoto 606-8501, Japan*
[c] *National Research Institute of Fisheries Science, Fisheries Research Agency, 2-12-4 Fuku-ura, Kanazawa-ku, Yokohama, Kanagawa 236-8648, Japan*
[d] *Graduate School of Human and Environmental Studies, Kyoto University, Yoshida Nihonmatsu-cho, Sakyo-ku, Kyoto 606-8501, Japan*
[e] *Faculty of Forestry, Gadjah Mada University, Jl. Agro No.1 Bulaksumur, Yogyakarta 55281, Indonesia*

ARTICLE INFO

Article history:
Received 24 May 2015
Received in revised form
13 December 2015
Accepted 14 December 2015
Available online 17 March 2016

Keywords:
Ion PGM
Metagenomics
Phyllosphere fungi
Shorea
Tropics

ABSTRACT

Phyllosphere fungi show high species diversity and fulfill important ecological functions not only in natural forests but also in plantations. We sought to estimate the species diversity and community structure of phyllosphere fungi from tropical tree plantation plots in Central Kalimantan, Indonesia. We conducted a massively parallel amplicon sequencing analysis of fungi collected from the leaves of *Shorea leprosula* (Dipterocarpaceae), an ecologically and commercially important tree species. Phyllosphere fungal compositions and spatial variability were investigated for 31 *S. leprosula* trees across four plots within a plantation stand. In total, 488 fungal operational taxonomic units (OTUs) were recognized in 153,194 ribosomal internal transcribed spacer reads at 95% OTU identity level. Rare OTUs accounted for the majority of fungal diversity detected in the study site; 200 OTUs (41%) comprised fewer than 10 reads and 465 OTUs (95%) were found in fewer than half of the samples. Fungal OTU compositions of *S. leprosula* trees were differentiated within a narrow area of the plantation and even between plots that were separated by 15 m. These findings indicate that highly diverse fungal OTUs form spatially structured communities even within a tropical plantation stand of single tree species.

---

* *Corresponding author*. Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan. Tel./fax: +81 75 753 6129.
E-mail address: izunoay@gmail.com (A. Izuno).

http://dx.doi.org/10.1016/j.myc.2015.12.005

1. Introduction

Phyllosphere fungi, which include epiphytes on the surfaces of leaves (Lindow and Brandl 2003), endophytes living asymptomatically within leaf tissues (Rodriguez et al. 2009) and pathogens, exert neutral, negative, or positive influences on their host plants and play various ecological and physiological roles in terrestrial ecosystems. For example, they may reduce the photosynthetic rates of host plants (Pinto et al. 2000), pathogenically attack host plants (Newton et al. 2010), enhance the resistance of hosts to pathogens (Arnold et al. 2003), or act as decomposers of leaf litter and drive nutrient cycles (Osono 2006). Phyllosphere fungi have been recognized to be the most species-rich groups of fungi (Unterseher et al. 2011). The habitats of phyllosphere fungi are likely very large and diverse, as the total global leaf surface area exceeds 4×108 km^2 (Morris and Kinkel 2002), a value approximately 2.7 times larger than the total land area. This likelihood of diversity is further supported by the fact that phyllosphere fungi inhabit all major land plant lineages from the tropics to the Arctic (Arnold 2007). Recent methodological developments based on next-generation sequencing facilitate the sequencing of DNA extracted directly from environmental materials, thereby permitting the estimation of species richness among various populations of microorganisms. Metagenomic amplicon sequence analyses with high-throughput sequencers have begun to uncover the tremendous diversity of fungi from aquatic (Brown et al. 2009; Stoeck et al. 2010) to terrestrial environments, including plant roots (e.g., Davison et al. 2012; Clemmensen et al. 2013; Toju et al. 2013) and leaves (e.g., Jumpponen and Jones 2009; Cordier et al. 2012; Zimmerman and Vitousek 2012). The investigation of community composition, richness and dynamics of phyllosphere fungi via metagenomic amplicon sequence analyses will contribute to the exploration of new fungal bioresources and understanding of the nature of the ecological interactions between fungi and plants.

Many factors determine fungal diversity and community structures. Differences in abiotic conditions, along with environmental gradients, such as elevation (Davey et al. 2013), continental-scale climate conditions (U'Ren et al. 2012) and the degree of urbanization (Jumpponen and Jones 2009), generate spatial variation in phyllosphere fungal communities. Although dispersal can counteract this spatial variation, topological or anthropogenic factors that limit dispersal among sites are likely to cause small-scale geographic variations in community structure (Lomolino et al. 2006; Adams et al. 2013). The effects of these factors on fungal community structures can differ both spatially and temporally (Matulich et al. 2015). Next-generation sequencing is an effective and comprehensive method of investigating the phyllosphere fungal community structure (Jumpponen and Jones 2009; Cordier et al. 2012), but to our knowledge few studies have used high-throughput meta-barcoding analysis to reveal the assemblies of phyllosphere fungi in the tropics.

Southeast Asia contains one of the most diverse floras in the world. It comprises 29,332 endemic vascular plant species (about 10% of approximately 300,000 global vascular plant species; Myers et al. 2000). In view of this diversity, fungi that interact with those plants (such as phyllosphere fungi and mycorrhizal fungi) are likely to be highly diverse. Nonetheless, the richness of fungal species in this region remains poorly investigated (Hawksworth and Rossman 1997; Webb et al. 2010; but see; Peay et al. 2010). Within this region, plantations of native tree species represent an important land use because they contribute to sustainable timber production without further accelerated deforestation, which would inevitably result in a substantial loss of biodiversity. Indonesia has established commercial timber production by planting trees after cutting (Hardiansyah et al. 2006; Ministry of Forestry 2009). However, pathogens, pests and diseases interfere with commercial plantation forestry (van Staden et al. 2004). Plantation forests contribute to the maintenance of biodiversity in the region as well as in primary forests (Barlow et al. 2007; Meijaard and Sheil 2007). For this reason, the accumulation of knowledge regarding the richness and spatial distribution patterns of fungal species is important for the sustainable management of biodiversity and commercial plantations.

In the present study, we addressed the question "How diverse are the phyllosphere fungi inhabiting in the Southeast Asian tropics?" We used massively parallel next-generation sequencing to characterize the diversity and spatial variability of phyllosphere fungal communities of managed *Shorea leprosula* Miq. (Dipterocarpaceae), which is commonly distributed in the lowland rainforests of Southeast Asia and is an important timber species called red meranti. We investigated the phyllosphere fungal assemblage of trees grown in plantation stands, evaluated total fungal richness, and then compared fungal community compositions among spatially distant tree individuals.

2. Materials and methods

2.1. *Study site and plant materials*

In Jul 2012, leaves were collected at *S. leprosula* plantation stands managed by PT. Sari Bumi Kusuma (SBK), a private forestry company in Central Kalimantan, Indonesia (0°35.5′S, 112°14.2′E; Fig. 1A). In SBK-managed plantation stands, young *S. leprosula* trees had been planted at 2.5-m intervals and had grown to approximately 10 m in height and 15 cm in diam at breast height during a six-year period. To evaluate the effects of geographic locations on fungal assemblage, we established four plots comprising seven to eight adjoining trees that had originated from different mother trees (Fig. 1B). The distances separating the four plots ranged from 15 m to 3.5 km (Fig. 1B). A total of 31 *S. leprosula* trees were selected as target plant individuals (Supplementary Data S1). To minimize the effect of the heterogeneous distribution of fungi within an individual plant on the observed species diversity, 10 leaves from three branches growing in different directions were collected from each plant. The collected leaves were then dried in silica gel until DNA extraction was performed.

2.2. *DNA extraction*

A 25-mm^2 piece was cut from a dried leaf with a disposable knife, and 10 leaf pieces from the 10 leaves originating on the