Fungal Ecology 20 (2016) 124–131

Contents lists available at ScienceDirect

Fungal Ecology

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

Richness of cultivable endophytic fungi along an altitudinal gradient in wet forests of Costa Rica

Keilor Rojas-Jimenez [a, b, *], Myriam Hernandez [a], Jorge Blanco [a], Luis Diego Vargas [a], Luis Guillermo Acosta-Vargas [a], Giselle Tamayo [a, c]

[a] Instituto Nacional de Biodiversidad, Apdo. 22-3100, Santo Domingo de Heredia, Costa Rica
[b] Universidad Latina de Costa Rica, Campus San Pedro, Apdo. 10138-1000, San José, Costa Rica
[c] Escuela de Química, Universidad de Costa Rica, Apdo. 11501-2060, San José, Costa Rica

ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form
1 December 2015
Accepted 21 December 2015
Available online xxx

Corresponding editor: Barbara Schulz

Keywords:
Fungal endophytes
Altitude
Biodiversity
Tropical wet forest
Braulio Carrillo
Costa Rica

ABSTRACT

We collected various plant species along an altitudinal gradient ranging from 400 to 2900 m in tropical wet forests of Costa Rica, isolated the associated endophytic fungi, and performed bioinformatic analyses to determine whether changes in altitude are related to changes in their richness and community structure. We showed that the richness of endophytic fungi varied along the altitudinal gradient, being higher in the lowest stratum and decreasing as elevation increases. Each stratum presented a particular composition and diversity of endophytes, although the whole population was characterized by the presence of a few dominant and apparently ubiquitous species, coexisting with a number of less abundant species that presented a more limited host range. These results have important implications for better understanding the role of altitude on the distribution and composition of endophytic fungal populations in tropical forests, but also for maximizing the number and diversity of endophytic isolates in bioprospecting campaigns.

1. Introduction

Fungal endophytes represent a significant component of plant communities, colonizing the inter and intracellular spaces of healthy plant tissues and often performing important functions for their hosts by providing nutrients, enhancing growth, stimulating systemic resistance against pathogens as well as buffering external stresses and microbial competition (Clay and Schardl, 2002; Redman et al., 2002; Arnold et al., 2003; Schulz and Boyle, 2005). Many of these beneficial functions are coupled with the production of biologically active secondary metabolites of medicinal, agricultural, and industrial interest. Consequently, the isolation, cultivation and characterization of endophytic fungi has received increasing attention over the last two decades, leading to the discovery of novel antibiotics, antimycotics, pesticides, immunosuppressants, and anticancer compounds (Strobel and Daisy, 2003; Maheshwari, 2006; Kusari et al., 2012; Deshmukh et al., 2014).

Endophytic fungi seem to be ubiquitous as they have been isolated from every plant species examined to date. However, important differences in their diversity and richness were reported among latitudes, ecosystems, sites, hosts, and tissue types (Suryanarayanan et al., 2002; Piercey et al., 2004; Arnold, 2007; Verma et al., 2007; Rodriguez et al., 2009). For example, Arnold and Lutzoni (2007) found that endophytes isolated from tropical forests were more abundant than in temperate forests and that assemblages were composed of a large number of rare species, suggesting that tropical sites are hyperdiverse. Conversely, the knowledge about the richness of endophytic fungi along altitudinal gradients is much more limited in comparison to other variables.

In general, it is expected that the species richness of plants, animals and insects on mountaintops should be lower than in lowland areas (Stevens, 1992). In fungal populations, specifically saprotrophic, one of the first studies addressing this issue was carried out by Weir (1918), who found that certain species of fungi in temperate forests disappeared with increasing elevation, while some were associated with particular zones and few were cosmopolitan. In the tropical forests of Costa Rica, Lieberman

* Corresponding author. Andre Pican Str. 17, 16515 Oranienburg, Germany.
E-mail address: keilor.rojas@gmail.com (K. Rojas-Jimenez).

http://dx.doi.org/10.1016/j.funeco.2015.12.006
1754-5048/

et al. (1996) analyzed the composition and structure of tree populations along an altitudinal transect, documenting a progressive decrease in plant species richness, species diversity, number of families and number of species per family with increasing altitude.

In this study, we collected various plant species along an altitudinal gradient in Braulio Carrillo National Park, Costa Rica, which is considered the last remaining strip of undisturbed forest in Central America with a wide range of altitudes (Norman, 1985). We isolated the associated endophytes and performed molecular and bioinformatic analyses to determine whether changes in altitude are related to changes in the richness and community structure of the fungal endophytes.

Our working hypothesis was that the species richness of endophytic fungi varies among altitudes, being higher at lower altitudes and decreasing with increasing elevation. The results of this study have important implications for better understanding the ecological distribution of fungal endophytes along altitudinal gradients but also for bioprospecting purposes, since more precise data about the expected richness among strata can reorient sampling efforts, under the rationale that a larger diversity of endophytic isolates can provide a larger diversity of secondary metabolites and derived applications.

2. Materials and methods

2.1. Study site and plant sampling

This study was conducted in the Braulio Carrillo National Park with the respective permit resolutions R-012-2005-OT-CONAGEBIO, R-CM-INBio-06-2006-OT and R-CM-INBio-059-2008-OT of the National Authority of the Ministry of Environment. The protected area is located in the central part of Costa Rica, between the Central Mountain Range and the Caribbean coast (10°09′35.64″N; 83°58′27.93″W). It is covered mainly by primary forest and protects nearly 6000 plant species as well as a large number of animal species. The park possesses a wide altitudinal range, from 32 m in the north boundary up to 2906 m in the Barva Volcano, comprising several life zones with annual mean precipitations ranging from 2600 mm to 5734 mm and annual mean temperatures between 12 °C to 25 °C (SINAC, 2005). We carried out 25 collecting field trips to different sites of the national park from November 2005 to March 2009, covering an altitudinal range from 400 m to 2900 m (Fig. 1). In each site, several kilometers of natural trails were explored looking for trees, shrubs and herbs that were only in their fertile stage. This phenological condition limited the amount of plants that could be collected but was strictly necessary for the proper taxonomical identification and voucher preservation in the herbarium of the National Institute of Biodiversity. Each plant sample consisted of some plant branches that were placed in polyethylene bags, the fertile voucher with inflorescences and/or fruits properly mounted, and the related information including microhabitat description, associated species, and location coordinates. The initial identification was performed on site by a trained collector and later confirmed by the botanists of the National Biodiversity Institute (only two out of the 92 plant species were repeated). For the purpose of this study, all plant samples were assigned to three major life zones (Table 1). Each life zone associated with a different range of altitude, precipitation, temperature, evapotranspiration and vegetation type, according to the Holdridge classification system (Holdridge, 1947, 1967). Plants located from 400 to 1400 m elevation were assigned to tropical premontane moist forest, from 1400 to 2400 m to tropical lower montane moist forest and from 2400 to 2900 m to tropical montane wet forest.

2.2. Isolation and selection of fungal endophytes

Once in the laboratory, the plant samples were washed thoroughly in running water, then healthy and physically-undamaged tissues (three leaves, 1 petiole, 1 branch, and one root when possible) were selected and surface sterilized with ethanol 70% for 2 min, with sodium hypochlorite 5% for 4 min, and rinsed twice with sterile deionized water for 1 min. The efficacy of the surface sterilization was verified by imprinting the tissues on media plates (Schulz et al., 1998). Under aseptic conditions, the basal, mid and distal parts of the tissues were sliced into 2 mm^2 segments and plated on potato-carrot agar medium (20 g l^{-1} potatoes, 20 g l^{-1} carrots, 15 g l^{-1} agar, pH 7.0) amended with 120 mg^{-1} of chlortetracycline and 120 mg^{-1} of streptomycin. Every sample consisted of 6 plates containing 8 tissue segments each, which were incubated at 25 °C with a photoperiod of 8 h. For up to 6 weeks, the plates were checked regularly for the appearance of endophytes, where each emerging fungus was transferred to a new plate of potato-dextrose agar (DIFCO, MD, USA) amended with the same antibiotics mentioned above. Since the aim of our bioprospecting department was to maximize the diversity of cultivable endophytes and hence the possible number of secondary metabolites produced, we performed a stringent screening step to discard redundant isolates from the same sample, comparing characteristics such as color, texture, shape, border type, mycelial density, presence-absence of secretions, and growth rate. The resulting isolates, generally few morphotypes per sample, were included in a database with the associated information and were preserved in the National Biodiversity Institute's culture collection. In total, we were able to isolate 346 fungal endophytes. For the molecular taxonomy we used a stratified random sampling; first we divided the population into three subgroups corresponding to the altitudinal range of the life zones mentioned above and then selected at random 33% of the isolates from each stratum. The selected sample consisted of 40 endophytes from altitudes 400–1400 m, 40 from 1400 to 2400 m and 35 from 2400 to 2900 m. These 115 endophytic fungi were isolated from 92 plant species belonging to 47 families (nearly 1.25 isolates selected per plant species). The distribution of the selected isolates according to plant families and altitudes is shown in Fig. 2.

2.3. Molecular analyses and taxonomic assignation

The selected endophytes were grown in petri dishes containing the same media used for its preservation. For the DNA extraction, 400 mg of mycelia were ground with mortar and pestle in liquid nitrogen and further extracted using the DNeasy Plant kit (Qiagen, USA) including a pretreatment step consisting of the incubation at 60 °C for 1 h with 400 μl of the kit's lysis buffer and 30 μl of Proteinase K (20 mg/ml, Sigma Aldrich, USA). The ITS1-5.8S-ITS2 region was amplified using primers ITS1 and ITS4 (White et al., 1990) with the following reaction conditions: 95 °C for 10 min, 35 cycles at 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min and additional extension at 72 °C for 10 min. The products were purified using the Nucleospin Extract II kit (Macherey–Nagel, Germany) according to the manufacturer's protocol. Sanger sequencing was performed at the sequencing facility of the Dana Farber Cancer Institute at the Harvard University, Boston, Massachusetts, using the above-mentioned primers. Sequences were assembled using Seqman program of DNASTAR Lasergene 8.0 (GenBank accessions: **KR534639-KR534753**). The fungal identification was performed using a Bayesian Classifier and the Fungal Warcup training set of Internal Transcribed Spacer sequences implemented in the