Distribution features of yeasts in soils of South Vietnam (case study of the biogeocenoses of the National Park Cát Tiên)

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Abstract. The yeast communities of tropical soils of two biotopes in monsoon tropical forest in the specially protected natural territories of the National Park Cát Tiên were studied. It was possible to establish that the distribution of yeasts in the profile of studied soils differed markedly. Of particular interest was the isolation from the tropical soils of Vietnam the pedobiont yeast species Saitozyma podzolica, which is regularly found in soils of temperate latitudes of Russia and other regions.

1. Introduction

The soil cover is a complex and multifunctional open system, of which microbial communities are a permanent and indispensable component. They play a key role in the circulation of substances in nature and determine the state of other components of the ecosystem [1]. Analysis of the diversity of various groups of soil microorganisms is the basis for understanding the processes of formation and functioning of ecosystems as a whole.

Mycelium fungi in the soils of the tropics are of high diversity. Along with cosmopolitan species, stenobiont unique species are present in tropical soils [2, 3]. Yeasts, unlike mycelial fungi, are an extrataxonomic group of predominantly unicellular micromycetes that have lost their mycelial structure. They are an indispensable component of all the natural communities, including the soils. Soil yeasts are involved in the processes of mineralization, in maintaining the soil structure and the transformation of organic matter, they stimulate plant growth, control phytopathogens. Despite the accumulated knowledge of the soil yeast communities to date, many aspects of the formation, development, and transformation of yeast complexes in the soil remain little or not studied. The data on yeast species diversity in the soils of the tropics is much less than compared to the accumulated data for many years on the soil yeast communities of temperate and northern latitudes [4]. Many novel fungi have been described for the tropical region, but much work remains. Up to 96% of fungi in tropical regions may be novel [5]. Soil yeasts may play an important role in soil aggregate formation and maintenance of soil structure [6, 7]. In this regard, a study of the soils of the tropical forest on the territory of South Vietnam was conducted. The goal of our study was to analyze the profile distribution of soil yeasts for two soil types.
2. Materials and methods
Samples of soil, plant litter and roots were taken in November 2016 on the territory of the National Park Cát Tiên. A total of 29 samples of soil, litter and roots in two biotopes were analyzed. The characteristics of the biotopes and soils are presented in table 1.

Table 1. The studied biotopes’ characteristics.

| Area            | Biotope                                      | Soil (according WRB classifier)                  |
|-----------------|----------------------------------------------|-------------------------------------------------|
| "Prirechnaya"  | Inland area on the bank of Dong Nai river, flat terrain with mild micro-relief Dipterocarpus alatus, Lagstroemia calyculata, Irvingia malayana, spots Aspidistra | Dystric Fluvisol (Arenic, Drainic)               |
| (alluvial plain)|                                              |                                                 |
| "Dipterokarpus"| Top of slate ridge Dipterocarpus turbinatus, Shorea roxburghii, Swintonia floribunda, grass vegetation with projective cover 50%, background - fern Taenitis blechnoides | Dystric Regosol (Clayic)                        |
| (watershed)     |                                              |                                                 |

Soil samples were taken in 10 cm to a depth of 140 cm in the "Prirechnaya" area and 110 cm in the "Dipterocarpus" area. The soil, roots and litter were analyzed during the week after sampling, keeping them in a refrigerator at 5 °C. From each sample, 3 weighed 1 g samples were taken, placed in test tubes and supplemented with sterile water to obtain a 1:10 dilution for litter, 1:20 for roots and 1:5 for soil. Suspensions were vortexed (MultiReax, Heidolph, Germany) for 15 minutes at a speed of 2000 rpm. These suspensions were used to inoculate in two replicates on plates with glucose–peptone–yeast medium of the following composition (g/L): glucose, 20; peptone, 10; yeast extract, 5; agar, 10 with the addition of chloramphenicol (500 mg/L) to prevent bacterial growth. The plates were incubated at room temperature for 5–7 days. The grown colonies of yeasts were divided into morphological types under a dissecting microscope and the number of colonies of each type was counted. The representatives of each colony type were brought into pure cultures. Yeast cultures were identified by morphological and physiological features [8] and by analyzing the nucleotide sequences of the D1/D2 region of the 26S (LSU) rDNA. Species identification of yeasts was carried out based on analysis of the nucleotide sequences of the D1/D2 domains of the 26S region (LSU), as well as, if necessary, of the ITS1-5.8S-ITS2 region of the rDNA. DNA isolation and PCR protocol were performed according to the previously described method [9]. For amplification of the rDNA region the following primers were used: ITS1f (5'-CTT GGT CAT TTA GAG TAA) and NL4 (5'-GGT CCG TCT AAG CAG G). DNA sequencing was performed using primer NL4 and / or ITS1f on an Applied Biosystems 3130xl Genetic Analyzer sequencer in the Syntol Scientific Production Company (Moscow). Species identification was performed using the NCBI genebank data (www.ncbi.nlm.nih.gov) and the MycoID database (www.mycobank.org). For each sample studied, the total number of yeasts and the relative abundance of each species were determined.

3. Results and discussion
The number of yeasts in both biotopes expectedly decreased in the series of the litter-rhizosphere-soil (figure 1). The distribution of yeasts differed markedly in the profile of the studied soils. So, on the site "Dipterokarpus" the yeasts were found at a depth of 40-50 cm, and on the site "Prirechnaya" – up to 130-140 cm (figure 2). The revealed differences in the distribution of yeasts by the profile of the studied soils can be explained by different types of soils at the sites. The "Prirechnaya" site, unlike the
"Dipterokarpus" site, was characterized by sandy soil, where there is an unimpeded transfer of yeast cells to deep horizons. In the soil on shale such a process is difficult, and, consequently, the depth of detection of yeast cells is much less.

Of all the studied samples of soil, litter and roots, 8 species of yeasts were isolated: 5 ascomycete and 3 basidiomycete species (table 2). The same number of species on average is characteristic of the forest biogeocenoses soils of the temperate zone of Russia. However, unlike forest soils, where the share of basidiomycete yeasts exceeds 90%, in the studied tropical soils their share was about 40%.

The pedobiont species Saitozyma podzolica dominated the two soils studied. In the alluvial soil at the "Prirechnaya" site, Filobasidium chernovii was the subdominant (11%), while the remaining species were minor components of the community. The species Aureobasidium pullulans (20%), Readeriellipsoides fuscoporiae (yeast-like state) (18%) and Candida akabanensis (10%) were encountered as subdominants at the "Dipterocarpus" site. At the "Prirechnaya" site, F. chernovii and S. podzolica were isolated from layers at the depth of more than 80 cm.

At the site "Dipterokarpus" yeasts at depth more than 50 cm were not isolated. Thus, at different sites, soil yeast complexes were formed by different species except for the common dominant. In both studied soils, the maximum yeast species diversity was found in the surface layer of soil and litter. Most of the species that were found in the surface soil layers and litter (Aureobasidium pullulans, Candida akabanensis, Hannaella sp., etc.) have never been isolated from the deep soil layers. Simultaneously, the species F. chernovii was found only in the deep layers of the soil. The exception is S. podzolica, at the "Prirechnaya" site, this species was presented throughout the soil profile.
Table 2. Relative abundance (%) of yeast species isolated from tropical soils of various biotopes: 1 - "Dipterokarpus", 2 - "Priirechnaya".

| Species                        | 1       | 2       |
|--------------------------------|---------|---------|
| Aureobasidium pullulans        | 20.00   | -       |
| Candida akabanensis            | 10.00   | -       |
| Candida endomichidarum         | -       | 3.13    |
| Filobasidium chernovii         | -       | 10.71   |
| Saitozyma podzolica            | 51.70   | 79.91   |
| Hannaella sp.                  | -       | 3.13    |
| Readeriellopsis fuscoporiae    | 18.30   | -       |
| Schwanniomyces polymorphus     | -       | 3.13    |

It is worth noting that the highest frequency of occurrence of this species was noted earlier for zonal soils of temperate latitudes of Russia and other regions [4, 10, 11]. For the soils of Vietnam, the isolation of this species is described for the first time.

4. Conclusions
As a result of studying the tropical soils yeast communities of two biotopes in monsoon rainforest on the specially protected natural territories of South Vietnam in the National Park Cát Tiên, it was established that the distribution of yeasts by the profile of the studied soils differed markedly. So, at the site "Dipterokarpus" (brown ferralic soil), yeasts were not found at a depth exceeding 40-50 cm, and at the site "Priirechnaya" – yeasts were found along the profile up to a depth of 130-140 cm, which is explained by the peculiarities of the profiles of these soils. The diversity of yeasts in the studied soils was small. In total, 8 species of yeasts, 5 ascomycete and 3 basidiomycete species were isolated from all samples of soil, litter and roots. The same number of species on average is characteristic of the soils of forest biogeocenoses in the temperate zone. However, unlike forest soils of the temperate zone, where the share of basidiomycete yeast exceeds 90%, in the studied tropical soils their share was about 40%. Of particular interest is the isolation from the tropical soils of the pedobiont species Saitozyma podzolica, which is regularly found in sod-podzolic, gray forest soils in Russia.

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