Postprint: Resources and Species Diversity of Phyllosphere Yeasts in the Yandang Mountains, Zhejiang

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Date: 2018-06-09T00:00:00+00:00

Abstract

An investigation of leaf-dwelling yeast resources was conducted across the vast regions of North Yandang Mountain, Central Yandang Mountain, South Yandang Mountain, West Yandang Mountain, and East Yandang Mountain, with the aim of elucidating the resources and diversity of leaf-dwelling yeasts in the Yandang Mountain range. PDA plates were utilized to isolate and purify leaf-dwelling yeast resources from the Yandang Mountain region, the taxonomic status of the yeasts was determined based on the 26S rDNA D1/D2 sequences and phylogenetic relationships of yeast cultures, and the diversity, composition, and distribution of leaf-dwelling yeast communities in this region were analyzed. The results revealed that 1,118 strains of leaf-dwelling yeasts isolated from the Yandang Mountain range could be identified as 56 known taxa and 19 potential new species, among which 38 yeast species were relatively common in this region. Among the common yeast taxa, these included 5 species of the genus Derxomyces, 3 species of Sporobolomyces, 2 species each of the genera Bannoa, Bulleribasidium, Rhodosporidiobolus, Symmetrospora, Taphrina, Tilletiopsi, and Udeniomyces, and 1 species each of the genera Bullera, Coniochaeta, Coniosporium, Cryptococcus, Elsinoe, Erythrobasidium, Fellozyma, Golubevia, Kockovaella, Kondoa, Leucosporidium, Moesziomyces, Oberwinklerozyma, Phyllozyma, Ruinenia, and Saitozyma. Based on the relative frequency of common yeast taxa, Derxomyces mrakii exhibited a distinct distribution advantage among the leaf-dwelling yeasts isolated from this region, and the stability and variation amplitude of 26S rDNA D1/D2 sequences among Derxomyces mrakii strains were investigated. Based on the composition and similarity of common leaf-dwelling yeasts in the Yandang Mountain region, it can be inferred that geographically adjacent areas exhibit greater similarity in yeast species distribution. The species accumulation curve drawn based on the species and quantities of common leaf-dwelling yeasts in the Yandang Mountain

range demonstrates that the vast majority of leaf-dwelling yeast species in the Yandang Mountain region have been isolated, and the research results can accurately reflect the existing status of leaf-dwelling yeast resources and species diversity in this region.

Full Text

Resources and Species Diversity of Phyllosphere Yeasts from the Yandang Mountains in Zhejiang Province

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Abstract

Phyllosphere yeasts from the Yandang Mountains were investigated to reveal their resources and species diversity. Yeasts were isolated and purified from the phyllosphere using potato dextrose agar (PDA) plates, and their classification status was defined according to 26S rDNA D1/D2 sequences and phylogenetic relationships. The composition, distribution, and diversity of phyllosphere yeast communities were then analyzed in detail. In total, 1,118 phyllosphere yeast strains were isolated from the Yandang Mountains and identified as 56 known groups and 19 proposed new species, among which 38 groups were common. The common yeast species were widely distributed across 25 genera. Of these, the genus *Derxomyces* consisted of five species, *Kockovaella* and *Kondoa* each consisted of three species, while *Rhodosporidiobolus*, *Symmetrospora*, *Taphrina*, *Tilletiopsis*, *Udeniomyces*, *Coniochaeta*, *Bullera*, *Leucosporidium*, *Coniosporium*, *Moesziomyces*, *Cryptococcus*, *Oberwinklerozyma*, *Phyllozyma*, *Bannoa*, *Bulleribasidium*, *Elsinoe*, *Erythrobasidium*, *Fellozyma*, *Ruinenia*, and *Saitozyma* each consisted of one species. According to the relative frequency of common groups, *Derxomyces mrakii* showed obvious distribution dominance. The stability and variation range of 26S rDNA D1/D2 sequences among different strains were discussed. Based on a comparison of the composition and similarity of common phyllosphere yeasts in the Yandang Mountains, it can be speculated that yeast species distribution within geographically close regions might have more similarities. The majority of phyllosphere yeasts in the Yandang Mountains were isolated through analyzing species accumulation curves based on the species and numbers of common phyllosphere yeasts. Therefore, this study reflects the actual resources and species diversity of phyllosphere yeasts from the Yandang Mountains.

Keywords: yeast; diversity; Yandang Mountain; 26S rDNA D1/D2; *Derxomyces mrakii*

Introduction

Yeasts are primarily defined by their morphology rather than taxonomy and represent a group of unicellular fungi that mainly reproduce asexually through budding or fission without forming fruiting bodies [1]. Various yeasts are widely distributed in diverse habitats including seawater and are closely related to human life. Given their important roles in industry, food production, basic research, and ecological functions [1], investigating and isolating yeast resources from various habitats has become an important foundation for biotechnology development [3]. Consequently, the distribution of yeast communities in specific habitats has received widespread attention. Research findings on yeast resources in environments such as alcoholic beverage brewing [4-7], traditional food fermentation [8-10], water [11], soil [12], plant surfaces [3,13-15], human body [16], infected sites [17-18], extreme environments [19], and other habitats [20] have fully demonstrated that numerous yeast species remain to be discovered in nature.

The Yandang Mountain range, located in southeastern Zhejiang Province and extending for hundreds of kilometers, can be divided into North Yandang, South Yandang, West Yandang, East Yandang, and Middle Yandang (including Dongtou Banping Mountain). The region has a mild and humid climate suitable for yeast habitation and reproduction. However, no studies on wild yeast resources in this area have been reported to date. Using classical yeast isolation methods and modern molecular identification techniques, we investigated phyllosphere yeast resources across the vast Yandang Mountain region to clarify the diversity, composition, and structure of phyllosphere yeast communities and to discover special yeast groups to further enrich China' s yeast resource repository.

1. Sampling Sites

The study area focused on the Yandang Mountain region in southeastern Zhejiang Province, including North Yandang, South Yandang, West Yandang, East Yandang, and Dongtou Banping Mountain. The region has a subtropical maritime climate with an average annual temperature of 13.5°C, average annual rainfall of 1,935.6 mm, and an average frost-free period of 269 days.

2. Sampling Methods

Semi-withered leaves were collected from trees in the Yandang Mountains and temporarily stored in sterile ziplock bags. Samples were transported to the laboratory as soon as possible for phyllosphere yeast isolation and purification. During sampling, detailed records were made of host plant information, latitude, and longitude.

3. Yeast Isolation

The method of Nakase and Takashima [21] was used. Collected semi-withered leaves were attached to the inner side of petri dish lids with Vaseline and inverted for incubation until colonies gradually became visible. Morphologically distinct yeast colonies were selected and streaked on plates to obtain pure cultures of phyllosphere yeasts. Fresh medium was changed daily during this period.

4. Yeast Purification

Purification was performed using the method of Makimura et al. [22].

5. 26S rDNA D1/D2 Amplification

Small amounts of vigorously growing young yeast cells from slant cultures were used for amplification. Primers NL1 (5 -GCATATCAATAAGCGGAGGAAAAG-3) and NL4 (5 -GGTCCGTGTTTCAAGACGG-3) were used with the following reaction conditions: 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min. Synthesis of 26S rDNA D1/D2 was completed by Sangon Biotech, and amplification products were sequenced by Beijing Liuhe Huada Gene Technology Co., Ltd.

6. Sequence Analysis

The obtained yeast 26S rDNA D1/D2 sequences were subjected to homology searches in the GenBank nucleotide sequence database (BLAST search). Clustal X software was used to align sequences of type strains with experimental strains [24], and molecular phylogenetic trees were constructed using MEGA5 Neighbor-Joining to clarify the taxonomic status of relevant yeasts [25-26]. Based on accession numbers annotated on the phylogenetic trees, corresponding rDNA D1/D2 sequences were downloaded from GeneBank.

7. Data Analysis

Relative frequency (RF) refers to the percentage of a particular phyllosphere yeast species isolated from the Yandang Mountain region relative to the total number of phyllosphere yeasts in that region. Species with RF 1% are considered common species. The Shannon-Weiner diversity index (H) was calculated using the formula: $H = -\Sigma(Pi \times \ln Pi)$, where k is the number of common phyllosphere yeast species isolated from a Yandang region and Pi is the percentage of a particular yeast species relative to the total number of phyllosphere yeast strains in that region. Richness (R) refers to the total number of common phyllosphere yeast species isolated from a Yandang region. Similarity coefficients (Sorensen) were calculated using the formula: $S = 2j/(a+b)$, where j is the number of common phyllosphere yeast species that can be isolated from both regions of the Yandang Mountains, a is the number of common phyllosphere yeast species from one region, and b is the number from another region. EstimateS software was used to complete bootstrap analysis and calculations. Excel was used to plot species accumulation curves for phyllosphere yeasts from the Yandang Mountains.

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2. Results and Analysis

2.1 Known Taxa of Phyllosphere Yeasts from the Yandang Mountains Semi-withered leaf specimens were collected from North, West, and East Yandang Mountains, yielding 144, 209, 257, and 337 yeast isolates respectively. Homology searches (BLAST search) and Clustal X comparisons with 26S rDNA D1/D2 sequences of type strains were performed, and molecular phylogenetic trees were constructed with typical strains. Sequence similarity was analyzed.

2.2 Potential New Species of Phyllosphere Yeasts from the Yandang Mountains Among the yeast strains isolated from the Yandang Mountains, 30, 32, 30, and 31 strains had special 26S rDNA D1/D2 sequences showing more than a certain number of base differences from known type strains. According to current international yeast classification standards [27-29], these are suspected to represent new yeast taxa. The 26S rDNA D1/D2 sequences of these potential new species showed significant differences from known species. A total of 19 strains were isolated from North, South, and East Yandang Mountains.

[Figure 1: see original paper] Phylogenetic tree based on 26S rDNA D1/D2 sequences showing relationships among representative strains and their close relatives. E, S, W, N indicate strains isolated from East, South, West, and North Yandang Mountains respectively.

Clustal X software was used for comparison and alignment with corresponding type strains. The results included both ascomycetous and basidiomycetous yeasts.

Comparison of 26S rDNA D1/D2 sequences between proposed new species and their closest known yeast type species.

2.3 Distribution Characteristics of Phyllosphere Yeasts from the Yandang Mountains In East Yandang Mountain, there were [number] common taxa, accounting for 86.8% of phyllosphere yeasts in that region. In West Yandang Mountain, there were [number] common taxa, accounting for 95.4% of the regional phyllosphere yeasts. In South Yandang Mountain, [number] common taxa accounted for 94.4%, while in North Yandang Mountain, [number] common taxa accounted for 93.3%. Only *Derxomyces mrakii* and *Derxomyces pseudoschimicola* were found in [region]. Except for North Yandang Mountain where the dominant yeast was *Derxomyces* [species], *Derxomyces mrakii* showed obvious distribution dominance in phyllosphere yeasts isolated from other regions. The relative frequency of *Derxomyces mrakii* among common phyllosphere yeasts in this region could reach 55%.

Relative frequency (RF) of commonly occurring wild yeasts from the Yandang Mountain area (%, RF 1%)

Rare taxa (RF < 1%) were also identified in various regions: - East Yandang Mountain: *Microsporomyces magnisporus*, *Naganishia albida*, *Bulleribasidium foliicola*, *Tilletiopsis*, *Bannoa hahajimensis* - West Yandang Mountain: *Cystobasidiomycetes*, *Kockovaella corallina*, *Hannaella surugaensis*, *Meira nashicola*, *Jaminaea angkorensis*, *Rhodotorula*, *Sporobolomyces koalae* - North Yandang Mountain: *Bannoa ogasawarensis*, *Oberwinklerozyma yarrowii*, *Carlosrosaea* - South Yandang Mountain: *Derxomyces boekhoutii*, *Derxomyces* sp., *Kockovaella vietnamensis*, *Chrysozyma*, *Dioszegia zsoltii*, *Microsporomyces magnisporus* sp. 1, *Exobasidium symploci-japonicae* sp. 2, *Microstroma phylloplanum*, *Colacogloea falcata*, *Fellozyma*, *Nielozyma formosana*, *Chrysozyma*, *Colacogloea* sp. 1, *Phyllozyma*, *Golubevia pallens* sp. 2, *Sporobolomyces pararoseus*, *Symmetrospora coprosmae*, *Cryptococcus*, *Bulleribasidium*, *Takashimella formosensis*, *Derxomyces bambusicola*, *Dioszegia zsoltii*, *Piskurozyma cylindricus*, *Colacogloea*, *Colacogloea falcata*, *Rhodotorula*, *Saitozyma* - Middle Yandang Mountain: *Kondoa*, *Oberwinklerozyma yarrowii*, *Papiliotrema aurea*, *Rhodotorula* sp. 1, *Tilletiopsis*, *Coniosporium*, *Cryptococcus*, *Derxomyces boekhoutii*, *Dioszegia zsoltii*, *Gjaerumia minor* sp. 2

The diversity index reflects the species diversity level of phyllosphere yeasts in the region. Phyllosphere yeasts distributed across the vast Yandang Mountain area showed rich diversity. Based on abundance values, the diversity index ranking was: North Yandang > Middle Yandang > South Yandang > West Yandang > East Yandang. The diversity indices also showed that phyllosphere yeast species from North, Middle, and South Yandang Mountains were relatively [diverse].

Diversity index and richness of commonly occurring wild yeasts from the Yandang Mountain area

Similarity coefficients can compare the similarity of phyllosphere yeast species composition between two regions of the Yandang Mountains. The composition of common phyllosphere yeasts in Middle Yandang Mountain showed high similarity coefficients with East and North Yandang Mountains. Middle Yandang Mountain and East Yandang Mountain had the most similar phyllosphere yeast composition, with similarity coefficients reaching relatively high levels of [value]. However, similarity coefficients between West Yandang Mountain and

South/North Yandang Mountains were lower, at 0.21. South Yandang Mountain and North Yandang Mountain showed similarity coefficients of 0.4-0.52.

Similarity coefficients of commonly occurring wild yeasts from the Yandang Mountain area

2.4 Sequence Variation of Phyllosphere Yeasts from the Yandang Mountains Using *Derxomyces mrakii* (found in East, North, and Middle Yandang Mountains) as a representative, the 26S rDNA D1/D2 sequences of this widespread species from the Yandang Mountains were analyzed and compared. Based on comparison with the type strain, various genotypes and their relative frequencies in different Yandang regions were identified. Among them, the genotype with identical D1/D2 sequence to the type strain accounted for 87.4%-98.2% of *Derxomyces mrakii* strains across various Yandang regions.

Relative frequency of various genotypes of *Derxomyces mrakii* and sequence identities with the type strain

2.5 Species Accumulation Curves for Phyllosphere Yeasts from the Yandang Mountains Based on distribution data for common phyllosphere yeasts from East, North, and Middle Yandang Mountains, EstimateS software was used to analyze and calculate yeast diversity in the region and plot species accumulation curves. According to characteristics of the species accumulation curves and EstimateS software calculations, bootstrap analysis estimated that 80.94% of phyllosphere yeast species in the Yandang Mountain region have been isolated in this study, with a value of 46.95.

[Figure 2: see original paper] Species accumulation curves for yeasts from the Yandang Mountain area. The curves show upper and lower confidence intervals.

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3. Discussion

The Yandang Mountains are one of the important mountain ranges in East China, with rich resources that have received widespread attention in biodiversity research [30]. Environmental factors such as temperature and humidity in this region are suitable for wild yeast growth. Comprehensive study of yeast resources in the Yandang Mountain area therefore has certain representativeness.

In wild yeast research, plant leaf surfaces are a habitat of great interest [31-32], and mature plant leaves in autumn generally harbor the most abundant yeast species and quantities [33]. Compared with soil and other habitats, leaf surfaces may be more likely to yield special yeast species. Currently, the main basis for yeast identification internationally is the sequence composition of the 26S rRNA D1/D2 region [23,27]. Sequence differences among different strains within a species should be less than 1% for this region; if inter-strain base differences exceed this threshold, they can generally be considered different species [28].

Yeast classification systems have undergone considerable changes in recent years [34-36]. When performing sequence alignment and phylogenetic analysis of phyllosphere yeasts from the Yandang Mountain region, we used the most current yeast classification system to make necessary corrections to identification results. The 1,118 phyllosphere yeast strains isolated from the Yandang Mountains were identified as 56 known groups and 19 potential new species through 26S rDNA D1/D2 sequence alignment and phylogenetic analysis. The results of 26S rDNA D1/D2 sequence alignment and phylogenetic analysis can basically confirm the taxonomic status of these strains.

Although some strains showed 26S rRNA D1/D2 sequences that differed significantly from known species, and further proof of their new species status would require analysis of sequence characteristics, physiological and biochemical characteristics, cell morphology, and culture characteristics, the relatively common occurrence of potential new species suggests that the Yandang Mountain region harbors rich phyllosphere yeast resources.

There are 38 common phyllosphere yeast groups in the Yandang Mountain region, including the genera *Derxomyces*, *Sporobolomyces*, *Bannoa*, *Bulleribasidium*, *Rhodosporidiobolus*, *Symmetrospora*, *Taphrina*, *Coniochaeta*, *Leucosporidium*, *Coniosporium*, *Moesziomyces*, *Cryptococcus*, *Oberwinklerozyma*, *Phyllozyma*, *Elsinoe*, *Erythrobasidium*, *Fellozyma*, *Ruinenia*, and *Saitozyma*, each represented by one species, plus *Golubevia*, *Kockovaella*, *Tilletiopsis*, and *Udeniomyces* each represented by one species, and *Bullera* and *Kondoa* each represented by multiple species. Among these, *Derxomyces mrakii* showed obvious distribution dominance among phyllosphere yeasts isolated from this region.

Derxomyces mrakii was first isolated by Hamamoto and Nakase [37] from three plant species (*Leptospermum scoparium*, *Nothofagus fusca*, and *Pseudowintera colorata*) in New Zealand and was initially identified as a new *Bullera* species. When the genus *Derxomyces* was established, it was recombined into this genus [38]. Analysis and comparison of 26S rDNA D1/D2 sequences of *Derxomyces mrakii* from the Yandang Mountain region revealed that numerous cultures did not have sequences completely identical to the type strain, indicating a certain degree of genetic diversity.

Based on the composition and similarity of common phyllosphere yeasts from different regions, Middle Yandang Mountain showed high similarity coefficients with East and North Yandang Mountains. The species composition of phyllosphere yeasts from Middle Yandang Mountain was most similar to that from East Yandang Mountain, with similarity coefficients reaching high levels. In contrast, species composition similarity was lower between West Yandang Mountain and South/North Yandang Mountains (0.21), and between South and North Yandang Mountains (0.4-0.52). This suggests that geographically close regions may have more similar yeast species distributions, a speculation that can provide reference for future wild yeast resource surveys.

In biodiversity research, species accumulation curves are widely used to assess sample adequacy and estimate species richness [39]. Based on species accumulation curves and EstimateS software calculations, this study has isolated the vast majority of phyllosphere yeast species from the Yandang Mountain region. The results can truly reflect the actual status of phyllosphere yeast resources and species diversity in this area.

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