Biodiversity of cultivable fungi in hair samples from tree shrews

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Accepted 29 November, 2013

Diverse fungal species live on the surfaces of plant tissues, some of which presumably occur in a mutualistic association. Some fungal species are widespread and can be found in many different animal species, whereas others are highly specific to single hosts. In this study, we investigated the taxonomic identities and phylogenetic relationships of fungal species isolated from tree shrew hair samples, using a combination of morphological and molecular approaches. Morphological differences among the seventy-one fungal isolates indicate that diverse distinct morphotypes might be present on the hosts. Seven representative isolate taxa were selected for further molecular phylogenetic analysis using nuclear ribosomal internal transcribed spacer (ITS1 and ITS2) DNA sequencing. The 71 endophytes were identified to the species level based on fungal sequences with known identities in GenBank. Our results suggest that 7 fungal genera are the dominant fungal parasites on the tree hairs.

Key words: Tree shrew, cultivable fungi, phylogeny, taxonomy.

INTRODUCTION

The tree shrews are small animals belonging to the family Tupaiidae, mainly found in Southern China, India, and Southeast Asia. The 17 known species of tree shrews are classified in genus Tupaiia, family Tupaiidae. Among them, northern tree shrew (Tupaia belangeri) is typical species. Tree shrews are now being tried as possible models for medical and biological research. Because of the susceptibility of the tree shrews (T. belangeri) and their hepatocytes to infection with human hepatitis B virus (HBV) in vivo and in vitro, these animals have been used to establish human hepatitis virus-induced hepatitis models. As these animals are phylogenetically close to Primates in evolution and have a well-developed visual system and color vision in some species, they have been utilized to establish myopia models. In brief, the tree shrews holds significant promise as research models and great use could be made of these animals in biomedical research (Novacek et al., 1992; Cao, 2003).

In natural or domesticated state, tree shrew (T. belangeri) is proved to be infected with several bacteria, viruses, and parasites (Bahr and Darai, 2001; Tidona et al., 1999; Saitou and Nei, 1987). Based on these facts, when tree shrews are used as experimental model, microbiological quality control over tree shrews is compulsory. In this report, we describe the isolation and identification of seven fungal species from the hair samples of adult T. belangeri tree shrews from wild situations.

MATERIALS AND METHODS

Animal and fungus isolation

Forty wild adult tree shrews were captured from urban mountains of...
Morphological identification of fungal isolates

Morphological identification of the 71 fungal isolates from 40 tree shrews was first carried out according to colony or hyphal morphology of the fungal cultures, characteristics of the spores, and reproductive structures if discernible (Barnett and Hunter, 1998; Carille et al., 2001; Watanabe, 2002; Gadd et al., 2007). Based on these features, the 72 isolates could be classified into 7 different morphological taxa. Using the traditional morphological techniques, only a few of the fungal isolates could be identified to the genus level.

Molecular identification of fungal isolates

DNA extraction and amplification fungi

A total of 7 representative isolates of various morphological fungi isolated from tree shrew hair samples were selected for molecular identification. Fungal genomic DNA was prepared using Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China). The DNAs were transferred to the new tubes and stored at -20°C respectively.

DNA detection concentration was performed by electrophoresis on a 2% (wt/vol) agarose gel stained with ethidium bromide. A volume of 10 μl of DNA and 2 μl of Ficoll dye was loaded in each lane. Electrophoresis condition was 110 V for 50 min in 1× TE buffer.

TaKaRa fungi Identification PCR Kit (Code No. D317) was used in amplifying the internal transcribed spacer (ITS) region of 18 to 26S nuclear ribosomal DNA with primers ITS4 (5'-TCCTCCGCTTATTGATATGS-3') and ITS5 (5'-GGAATTTAAA GTGCT AACAGG-3'). An initial denaturing step (94°C for 5 min) was followed by 30 cycles (with each cycle consisting of DNA denaturing at 94°C for 30 s, primer annealing at 55°C for 30 s, and elongation at 72°C for 1 min and a final extension step at 72°C for 5 min. A no-template negative control was included in each PCR run. The PCR products were purified with a TaKaRa Agarose Gel Purification Kit Ver.2.0 (Code No. D805A, TaKaRa Biotechnology (Dalian) Co., Ltd.).

Sequencing and bioinformational analysis of fungal ITS

Sequencing and bioinformational analysis of fungal ITS was performed for each of the 7 representative fungal sequences against the non-redundant database maintained by the National Center for Biotechnology Information using the BLAST algorithm (http://www.ncbi.nlm.nih.gov). Alignment search tool (BLAST) at the National Center for Biotechnology Information, and percent homology scores were generated to identify fungi. Fungi with amplified ITS sequences >98% similar were considered to be the same phylotype. Each ITS DNA sequence was compared by using the BLAST alignment program with data available from GenBank at the National Institutes of Health. The computer alignment provides a list of matching organisms, ranked in order of similarity between the unknown sequence and the sequence of the corresponding organism from the database. The percentage and absolute number of matched base pairs from each BLAST match were reported.

RESULTS AND DISCUSSION

Seventy one pure fungal isolates from hair samples of tree shrew (T. belangeri), which were tentatively identified, based on phenotypic analysis by colonial character, pigment, stain and fungal morphology, and then these isolates are classified into 7 morphological taxa of fungi: F5, F12-14, F14-122, F16-9, F24-1, 24-121, and F25-7 (summarized in Table 1).

Genomic DNAs of Seven morphological taxa of fungi were extracted and its ITS regions were amplified by PCR and sequenced separately. Sequence similarity searches for ITS region DNA were performed for each of the 7 representative fungal sequences against the non-redundant database maintained by the National Center for Biotechnology Information using the BLAST algorithm. Each ITS DNA sequence was compared by using the BLAST alignment program with data available from GenBank at the National Institutes of Health. The computer alignment provides a list of matching organisms, ranked in order of similarity between the unknown sequence and the sequence of the corresponding organism from the database. Fungi with amplified ITS sequences >98% similar were considered to be the same phylotype (Table 2).

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Table 1. Morphological taxa of fungi from tree shrew hair.

<table>
<thead>
<tr>
<th>Taxa name</th>
<th>Number of isolates</th>
<th>Detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>11</td>
<td>17.74</td>
</tr>
<tr>
<td>F12-14</td>
<td>19</td>
<td>12.40</td>
</tr>
<tr>
<td>F14-122</td>
<td>8</td>
<td>12.90</td>
</tr>
<tr>
<td>F16-9</td>
<td>12</td>
<td>19.35</td>
</tr>
<tr>
<td>F24-1</td>
<td>5</td>
<td>16.13</td>
</tr>
<tr>
<td>24-121</td>
<td>9</td>
<td>14.52</td>
</tr>
<tr>
<td>F25-7</td>
<td>7</td>
<td>11.29</td>
</tr>
</tbody>
</table>

Figure 1. Agarose gel electrophoresis of PCR-amplified ITS regions of 28s rDNA. All products were electrophoresed in 3% agarose gels. M: DL 2,000 DNA marker; (1) Taxa F5 PCR product; (2) F12-14 PCR product; (3) F14-122 PCR product; (4) F16-9 PCR product; (5) F24-1 PCR product; (6) F24-121 PCR product; (7) F25-7 PCR product; + = Positive control; - = Negative control.

Table 2. Molecular identification of fungi from tree shrew hair.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5-1</td>
<td>Chaetomium</td>
<td>Chaetomium globosum</td>
</tr>
<tr>
<td>F12-14</td>
<td>Trichosporon</td>
<td>Trichosporon mucoides</td>
</tr>
<tr>
<td>F14-122</td>
<td>Corynascus</td>
<td>Corynascus kuwaitiensis</td>
</tr>
<tr>
<td>F16-9</td>
<td>Ascochyta</td>
<td>Ascochyta rabiei</td>
</tr>
<tr>
<td>F24-1</td>
<td>Alternaria</td>
<td>Alternaria alternata</td>
</tr>
<tr>
<td>24-121</td>
<td>Retroconis</td>
<td>Retroconis fusiformis</td>
</tr>
<tr>
<td>F25-7</td>
<td>Debaryomyces</td>
<td>Debaryomyces hansenii</td>
</tr>
</tbody>
</table>

We thank Ruwen Liu and Jianlin Jie for their assistance.
during the studies. This work was supported by the Planned Science and Technology Project of Yunnan Province “Project for Establishing Yunnan Facilities and Information Infrastructure for Science and Technology”, China, grant no. 2006PT07-1.

REFERENCES


