Genotypic Analysis of a New Fungal Pathogen, *Trichosporon faecale*, Isolated from Japanese Subjects

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Abstract

The basidiomycetous yeast *Trichosporon faecale* is considered to be a non-pathogenic fungus; however, the microorganism has been isolated from clinical specimens in several countries. *Trichosporon faecale* is classified as type I, II, or III depending on the sequence of the intergenic spacer (IGS) region in its rRNA gene. In this study, 28 T. faecale strains obtained from Japanese subjects and environmental samples were found to be type I. In addition, T. faecale was detected by PCR in 32 scale samples from 146 Japanese healthy subjects, and all 32 samples were found to be type I. Our findings suggest a lack of intraspecific diversity among *T. faecale* samples originating from Japanese subjects and that *T. faecale* is part of the skin fungal microbiome.

Keywords: *Trichosporon faecale*; Intergenic spacer; rRNA

Introduction

Trichosporonosis is clinically classified as a superficial or deep-seated fungal infection [1-3]. The genus *Trichosporon* includes 38 species [4], approximately one-third of which have been isolated from clinical specimens; however, the major causative agent of trichosporonosis is *Trichosporon asahii* [4]. Deep-seated *Trichosporon* infections are lethal opportunistic infections seen occasionally in immunocompromised patients, particularly those who are neutropenic due to hematological disease or cytotoxic therapy [2,5]. The prognosis of this infection is poor; its mortality rate is approximately 70% [6]. This is higher than that of candidiasis, which carries a mortality rate of 40%. Of the isolates obtained previously [2,5], the major causative antigen of *Trichosporon asahii* [3,7]. Meanwhile, *Trichosporon* is also widely distributed in the environment, including the soil and air [4]. Type III or IV allergies can develop through the repeated inhalation of these microorganisms. This allergic response is called summer-type hypersensitivity pneumonitis (SHP) and is mainly observed in western or southern Japan during the hot, humid, and rainy months of summer [8,9]. These conditions favor the growth of *Trichosporon* species. Interestingly, the major causative antigen of SHP is also *T. asahii* [10]. *Trichosporon faecale* is an environmental fungus found mainly in soil [4]. Although *T. faecale* is generally considered to be non-pathogenic, it has been isolated from clinical specimens in Argentina [11], Brazil [12,13], Egypt [14], Qatar [14], Turkey [15], Spain [11], and Taiwan [16]. In the present study, we found that *T. faecale* was part of the fungal skin microbiome in Japanese subjects and that the genotype of the microorganism was of a single type.

Materials and Methods

Strains used

Twenty-eight *T. faecale* strains were examined in this study. They were obtained from skin wounds (n=7), healthy skin (n=13), soil (n=5), and the homes of Japanese patients with SHP (n=3).

DNA sequencing of the intergenic spacer (IGS) region of the rRNA gene

Fungal RNA genes consist of four subunits: a small subunit, large subunit, 5S, and 5.8S. Two spacer regions, an internal transcribed spacer and IGS, are located between these subunits. In this study, the DNA sequence of the IGS 1 region from 28 strains was determined using two primers: 26SBF (5′-AGCTTGACTTCGCAGATCGG-3′) and 26SB (5′-AGCTGCTGCCAATGCTAGCTC-3′). In addition, T. faecale was detected by PCR in 32 scale samples from 146 Japanese healthy subjects, and all 32 samples were found to be type I. Our findings suggest a lack of intraspecific diversity among *T. faecale* samples originating from Japanese subjects and that *T. faecale* is part of the skin fungal microbiome.

Detection from *T. faecale* in Scale Samples from Healthy Japanese Subjects

To elucidate whether *T. faecale* is part of the skin fungal microbiome, we screened for *T. faecale* in skin samples from 146 healthy subjects ranges between 20 and 70 years old using a culture-independent method: nested PCR. The skin scale samples used in this study were the same as those used in the study of Sugita et al. [18]. Nested PCR was conducted using two sets of primers. *Trichosporon faecale*-specific primers were derived from the IGS 1 sequences in GenBank (accession numbers AB066413, AB43004, and AB43006). The PCR procedure consisted of denaturation at 94°C for 3 min, followed by 30 cycles of 30 s at 94°C, 30 s at 52°C, and 20 s at 72°C, with a final extension at 72°C for 10 min using the primers TFF1 (5′-CGACTCCAGCATTCTAATCA-3′) and TFR1 (5′-CGACTCCAGCATTCTAATCA-3′) and TFR1 (5′-CGACTCCAGCATTCTAATCA-3′).
CTATAAGCCAGTCCCTCCTG-3'). In the nested PCR step, 1 μl of the first amplification product was added to a new reaction mixture with the same composition as the first. The PCR procedure consisted of denaturation at 94°C for 3 min, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C, and 20 s at 72°C, with a final extension at 72°C for 10 min using the primers TFF2 (5′-AACGAGACAAGTAGTAGGTGC-3′) and TFR2 (5′-TAAGCCAGTCCCTCCTGGAA-3′). All amplicons were directly sequenced using the primers TFF2 and TFR2.

Results and Discussion

We previously demonstrated that the IGS 1 region of T. faecale can be classified into three types: I, II, and III [15]. The length of the IGS 1 region is 490, 469, and 475 bp, respectively, and the types' exhibit 90.1-93.3% sequence similarity (Figure 1).

![Figure 1: IGS sequence alignments for three genotypic strains of T. faecale](image)

The sequences of all 28 strains obtained from the Japanese subjects and environmental samples were of the same type (type I). Genotypic analyses of T. faecale strains have been conducted in several countries [11-13,15,16], and they have identified sequences belonging to all three types. The two Thai isolates examined in this study were of types II and III (Table 1). Most of the strains originated from non-blood specimens, suggesting that T. faecale is part of the normal skin microbiome. To confirm this, we screened for T. faecale in 146 scale samples by nested PCR. Trichosporon faecale was detected in 32 of the samples and all were found to be type I.

While the majority of fungi are identified using either the intergenic transcribed spacer (ITS) regions or the D1/D2 region of the large rRNA subunit, differentiation between T. faecale, T. asahii, and T. coremiiforme is more commonly performed using IGS sequences. This distinction is primarily due to the high degree of homology in both the ITS and D1/D2 regions, with only one to three nucleotide differences (>99% similarity) between species [4].

**Table 1: IGS 1 genotype of Trichosporon faecale isolated in Japan and other countries**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Origin of the specimens</th>
<th>Number of strains in this study</th>
<th>Origin of the country</th>
<th>GenBank accession number</th>
<th>Reference</th>
</tr>
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<td>Type 1</td>
<td>Skin wound</td>
<td>7</td>
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<td>This study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin in healthy subjects</td>
<td>13</td>
<td>Japan</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>House of patients with summer-type hypersensitive pneumonitis</td>
<td>3</td>
<td>Japan</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>5</td>
<td>Japan</td>
<td>This study</td>
<td></td>
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<tr>
<td></td>
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<td>AB08641</td>
<td>[17]</td>
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<td>Taiwan</td>
<td>FJ153607</td>
<td>[16]</td>
<td></td>
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<td>AB43900</td>
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Strain numbers are shown parenthes. Unknown genotypic strains are not included in this table.
In contrast, the fungal IGS region exhibits remarkable intraspecific sequence diversity, allowing for clear differentiation between these three species. IGS 1 of *T. asahii* consists of several genotypes, and there is a geographical substructure [17]. Based on a study of samples taken from patients with *Malassezia*-related skin diseases and healthy individuals, the predominant fungi on human skin, *Malassezia globosa* and *Malassezia restricta*, can be distinguished based on their IGS sequences [19,20]. Similarly, IGS sequencing has been used in the multi-locus sequence typing of *Cryptococcus neoformans* and *Cryptococcus gattii* [21].

In a study of the antifungal susceptibility of 15 *T. faecale* strains isolated from Turkish patients, Kalkanci et al. [15] showed that the minimum inhibitory concentrations of amphotericin B, fluconazole, and voriconazole were slightly lower than those reported for *T. asahii*.

Our findings indicate that *T. faecale* originating from Japanese subjects did not exhibit intraspecific diversity in their IGS 1 region and that this microorganism is part of the skin fungal microbiome in humans.

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References