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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 from patients' blood, almost all were *T. 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 rRNA 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′-AGCTGCTGCCAATGCTAGCTC-3′) and 5SR (5′-AGCTTGACTTCGCAGATCGG-3′) as described by Sugita et al. [17].

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′-

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 type's exhibit 90.1-93.3% sequence similarity (Figure 1).

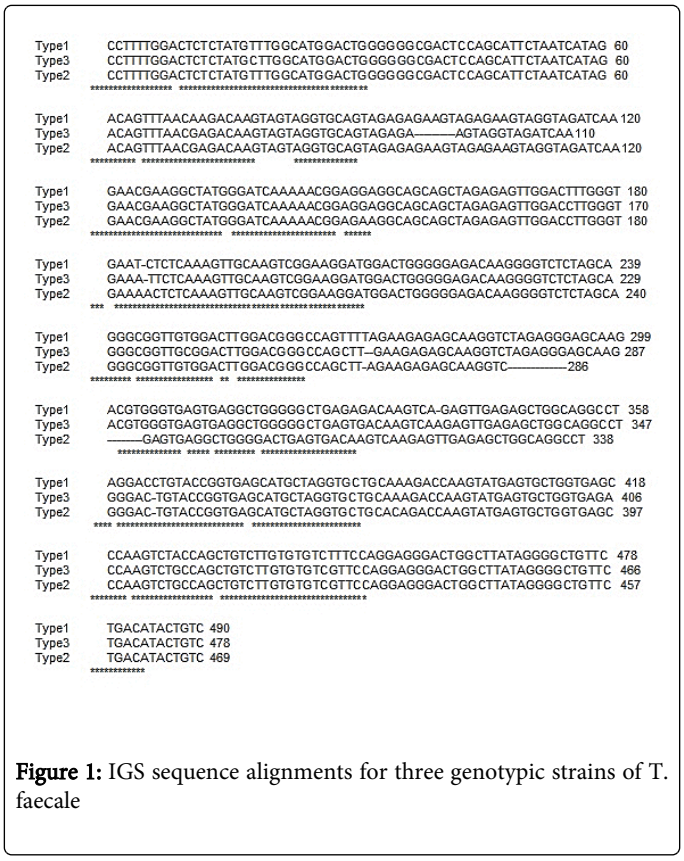

Figure 1: IGS sequence alignments for three genotypic strains of T. faecale

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].

Genotype	Origine of the specimens	Number of strains in this study	Origin of the country	GenBank accession number	Reference
Genotype 1	Skin wound	7	Japan		This study
	Skin in healthy subjects	13	Japan		This study
	House of patients with summer-type hypersentive pneumonitis	3	Japan		This study
	Soil	5	Japan		This study
	Faces (CBS 4828)a)		Unknown	AB066413	[17]
	Wound		Taiwan	FJ153607	[16]
	Oropharyngeal secretion (B3963)		Brazil		[12]
	Nail (CNM-CL6505)		Brazil	FJ153599	[13]
	Nail (CNM-CL6515)		Brazil	FJ153607	[13]
	Nail (CNM-CL6507)		Brazil	FJ153601	[13]
	Skin (CNM-CL6506)		Brazil	FJ153600	[13]
Genotype 2	Urine (IFM 48045)		Brazil		[12]
	Wound		Turkey	AB439004	[15]
Genotype 3	Sputum	1	Thailand		This study
	Pus	1	Thailand		This study
	Unknown substrate (JCM2390)	1	Unknown		This study
	Oral cavity (IFM 48951)		Brazil		[12]
	Broncho alveolar lavage		Turkey	AB439006	[15]
	Nail (CNM-CL6102)		Brazil	FJ153600	[13]
	Unknown substrate (CNM-CL4327)		Brazil	FJ153587	[13]
	Unknown substrate (CNM-CL4551)		Brazil	FJ153888	[13]

Strain numbers are shown parensis.

Unknown genotypic strains are not included in this table.

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

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

1. Sugita T, Ikeda R, Nishikawa A, Takashima M, Mekaha N, Poonwan N, Kalkanci A, Kustimur S. Chapter 77 Trichosporon, In Molecular Detection of Human Fungal Pathogens, edited by Liu D, pp. 669-674, 2011, CRC Press, New York
2. Walsh TJ, Groll A, Hiemenz J, Fleming R, Roilides E, et al. (2004) Infections due to emerging and uncommon medically important fungal pathogens. Clin Microbiol Infect 10 Suppl 1: 48-66.
3. Guého E, Improvisi L, de Hoog GS, Dupont B (1994) Trichosporon on humans: a practical account. Mycoses 37: 3-10.
4. Sugita T Trichosporon Behrend (1890), In The Yeasts, A Taxonomic Study 5th edition, pp. 2015-2062, 2011, edited by Kurtzman CP, Fell JW, Boekhout T, ELISVIR, New York
5. Fleming RV, Walsh TJ, Anaissie EJ (2002) Emerging and less common fungal pathogens. Infect Dis Clin North Am 16:915-933.
6. Bayramoglu G, Sonmez M, Tosun I, Aydin K, Aydin F (2008) Breakthrough Trichosporon asahii fungemia in neutropenic patient with acute leukemia while receiving caspofungin. Infection 36: 68-70.
7. Sugita T, Nishikawa A, Shinoda T, Kume H (1995) Taxonomic position of deep-seated, mucosa-associated, and superficial isolates of Trichosporon cutaneum from trichosporonosis patients. J Clin Microbiol 33: 1368-1370.
8. Yoshida K, Ando M, Sakata T, Araki S (1988) Environmental mycological studies on the causative agent of summer-type hypersensitivity pneumonitis. J Allergy Clin Immunol 81: 475-483.
9. Ando M, Arima K, Yoneda R, Tamura M (1991) Japanese summer-type hypersensitivity pneumonitis. Geographic distribution, home environment, and clinical characteristics of 621 cases. Am Rev Respir Dis 144: 765-769.
10. Sugita T, Ikeda R, Nishikawa A (2004) Analysis of Trichosporon isolates obtained from the houses of patients with summer-type hypersensitivity pneumonitis. J Clin Microbiol 42: 5467-5471.
11. Rodriguez-Tudela JL, Diaz-Guerra TM, Mellado E, Cano V, Tapia C, Perkins A, Gomez-Lopez A, Rodero L, Cuenca-Estrella M (2005) Susceptibility patterns and molecular identification of Trichosporon species. Antimicrob Agents Chemother 49:4026-4034.
12. Zeng J, de Souza Motta CM, Fukushima K, Takizawa K, Magalhes OM, et al. (2009) Identification of Trichosporon spp. strains by sequencing D1/D2 region and sub-typing by sequencing ribosomal intergenic spacer region of ribosomal DNA. J Huazhong Univ Sci Technolog Med Sci 29: 655-658.
13. Araujo Ribeiro M, Alastruey-Izquierdo A, Gomez-Lopez A, Rodriguez-Tudela JL, Cuenca-Estrella M (2008) Molecular identification and susceptibility testing of Trichosporon isolates from a Brazilian hospital. Rev Iberoam Micol. 25:221-225.
14. Taj-Aldeen SJ, Al-Ansari N, El Shafei S, Meis JF, Curfs-Breuker I, et al. (2009) Molecular identification and susceptibility of Trichosporon species isolated from clinical specimens in Qatar: isolation of Trichosporon dohaense Taj-Aldeen, Meis & Boekhout sp. nov. J Clin Microbiol 47: 1791-1799.
15. Kalkanci A, Sugita T, Arikan S, Yucesoy M, Ener B, et al. (2010) Molecular identification, genotyping, and drug susceptibility of the basidiomycetous yeast pathogen Trichosporon isolated from Turkish patients. Med Mycol 48: 141-146.
16. Ruan SY, Chien JY, Hsueh PR (2009) Invasive trichosporonosis caused by Trichosporon asahii and other unusual Trichosporon species at a medical center in Taiwan. Clin Infect Dis 49: e11-17.
17. Sugita T, Nakajima M, Ikeda R, Matsushima T, Shinoda T (2002) Sequence analysis of the ribosomal DNA intergenic spacer 1 regions of Trichosporon species. J Clin Microbiol 40: 1826-1830.
18. Sugita T, Suzuki M, Goto S, Nishikawa A, Hiruma M, et al. (2010) Quantitative analysis of the cutaneous Malassezia microbiota in 770 healthy Japanese by age and gender using a real-time PCR assay. Med Mycol 48: 229-233.
19. Sugita T, Kodama M, Saito M, Ito T, Kato Y, Tsuboi R, Nishikawa A (2003) Sequence diversity of the intergenic spacer region of the rRNA gene of Malassezia globosa colonizing the skin of patients with atopic dermatitis and healthy individuals. J Clin Microbiol 41:3022-3027.
20. Sugita T, Tajima M, Amaya M, Tsuboi R, Nishikawa A (2004) Genotype analysis of Malassezia restricta as the major cutaneous flora in patients with atopic dermatitis and healthy subjects. Microbiol Immunol 48: 755-759.
21. Meyer W, Aanensen DM, Boekhout T, Cogliati M, Diaz MR, et al. (2009) Consensus multi-locus sequence typing scheme for Cryptococcus neoformans and Cryptococcus gattii. Med Mycol 47:561-570.