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Low DNA Sequence Diversity of the Intergenic Spacer 1 Region in the Human Skin Commensal Fungi *Malassezia sympodialis* and *M. dermatis* Isolated from Patients with *Malassezia*-Associated Skin Diseases and Healthy Subjects

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Abstract As DNA sequences of the intergenic spacer (IGS) region in the rRNA gene show remarkable intraspecies diversity compared with the small subunit, large subunit, and internal transcribed spacer region, the IGS region has been used as an epidemiological tool in studies on *Malassezia globosa* and *M. restricta*, which are responsible for the exacerbation of atopic dermatitis (AD) and seborrheic dermatitis (SD). However, the IGS regions of *M. sympodialis* and *M. dermatis* obtained from the skin of patients with AD and SD, as well as healthy subjects, lacked sequence diversity. Of the 105 *M. sympodialis* strains and the 40 *M. dermatis* strains, the sequences of 103 (98.1 %) and 39 (97.5 %), respectively, were identical. Thus, given the lack of intraspecies diversity in the IGS regions of *M. sympodialis* and *M. dermatis*, studies of the diversity of these species should be performed using appropriate genes and not the IGS.

Keywords *Malassezia sympodialis* · *Malassezia dermatis* · Intergenic spacer · rRNA gene

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Introduction

Malassezia species are skin commensal yeasts in healthy humans; they also colonize the skin of non-human animals. All of them, with the exception of *M. pachydermatis*, require fatty acids for their growth, which accounts for their preferential colonization of areas of the skin that are rich in sebaceous glands [1]. Currently, the genus *Malassezia* comprises 14 species, nine of which have an affinity for human skin and five an affinity for the skin of non-human animals. In humans, *Malassezia* is associated with the development or exacerbation of atopic dermatitis (AD), seborrheic dermatitis (SD), and pityriasis versicolor, despite being skin commensal microorganisms [1]. Among *Malassezia* species, both *M. globosa* and *M. restricta* are the most frequently found on human skin, diseased or healthy, followed by *M. sympodialis* and *M. dermatis*; the colonization rates of the other five species (*M. furfur*, *M. japonica*, *M. obtusa*, *M. slooffiae*, and *M. yamatoensis*) are relatively low [2, 3].

The fungal rRNA gene consists of a small subunit (SSU), the 5.8S subunit, a large subunit (LSU), the 5S subunit, and two spacer regions, those of the internal transcribed spacer (ITS) and the intergenic spacer (IGS) [4]. While DNA sequence analyses of the ITS regions and the D1/D2 regions of the LSU have been widely used for fungal species identification, the remarkable intraspecies diversity of the DNA sequences of the IGS regions has their use in epidemiology. We earlier showed that the IGSs of

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M. globosa and *M. restricta* colonizing the skin of patients with AD and SD exhibited remarkable sequence diversity; we suggested that the specific IGS sequences of these microorganisms play significant roles in AD or SD [5–7].

In the present study, we determined the IGS sequences of 145 *M. sympodialis* and *M. dermatis* strains obtained from patients with AD and SD and from healthy subjects.

Materials and Methods

Strains Used

The 105 *M. sympodialis* and 40 *M. dermatis* strains analyzed in this study were isolated from the skin of patients with AD and SD and from healthy subjects (HSs), and maintained in our laboratory culture collection (Table 1). The strains were identified based on a DNA sequence analysis of the ITS region of the rRNA gene [8].

PCR Primer Design

Based on the genomic data of *M. sympodialis* and *M. dermatis* [9], the forward primer (5′-TGCTAC GATCTGCTGAGGCT-3′), recognizing the end of the LSU, and the reverse primer (5′-AGCTTGACTGCG CAGATCGGA-3′), recognizing the 5S subunit, were designed and used to amplify the IGS1 region.

Results and Discussion

Among the 105 *M. sympodialis* strains, two types of IGS1 sequences were determined, I and II, with lengths of 573 base pairs (bp) and 620 bp, respectively (Table 1). Type I was found in 98.1 % (103/105) of the strains, whereas type II was detected in only two strains (1.9 %) from HS. Two IGS1 sequences types, I and II, were also identified among the 40 *M. dermatis* strains, with lengths of 611 and 601 bp, respectively. Similar to *M. sympodialis*, the majority of the strains were type I (39/40, 97.5 %) and only one strain was type II (2.5 %). The DNA sequences of the representative strains have been deposited in the DNA Data Bank of Japan (DDBJ), as shown in Table 1.

Sugita and coworkers investigated the diversity of the IGS regions of *Malassezia globosa*, *M. restricta*, and *M. pachydermatis* [4–7, 10], *Cryptococcus albidus*, *C. neoformans*, and *C. gattii* [11, 12], and *Trichosporon asahii* [13]. Among skin commensal fungi, *Malassezia* is known to exacerbate AD and SD. The most predominant species found on the skin of these patients are *M. globosa* and *M. restricta*. The DNA sequences of the IGS regions of the respective strains obtained from AD and SD patients and from HS showed remarkable intraspecies diversity. In previous studies, 25 IGS types from *M. globosa* and 52 IGS types from *M. restricta* were identified in the skin specimens of AD patients and healthy individuals [5]. Similarly, 32 IGS types of *M. globosa* and 15 of *M. restricta* were detected in dandruff patients [4]. These findings suggested the existence of disease-specific IGS types (genotypic strains) and provided further support for the use of IGS sequence analysis in elucidating the relationship between fungal strain and disease. However, in the present study, sequencing of the IGS regions of 105 *M. sympodialis* strains and 40 *M. dermatis* strains obtained from patients with AD and SD and from HS showed almost no intraspecies diversity. *M. sympodialis* and *M. dermatis* were the second most common skin commensal fungi, followed

Table 1 Strains used and their intergenic spacer (IGS) sequence types

Species	IGS type	Length (bp)	Source and number of strains			DDBJ accession number
			AD	SD	HS	IGS1
M. sympodialis	I	573	36	29	38	LC151467 (CBS 7222)[a]
	II	620	0	0	2	LC151468 (MPU-SY83)
M. dermatis	I	611	9	23	7	LC151469 (JCM 11348)
	II	617	0	0	1	LC151470 (MPU-DR41)

AD atopic dermatitis; *SD* seborrheic dermatitis; *HS* healthy subject

[a] Representative strain was shown in parenthesis

by *M. globosa* and *M. restricta*, in the skin samples of AD and SD patients and in those from HS.

The skin environment differs among individuals. The skin of AD patients is typically dry and of neutral pH, while that of SD patients is oily. Our interest in the IGS sequences of *M. sympodialis* and *M. dermatis* was based on our expectation that, like *M. globosa* and *M. restricta*, the IGS sequences of these microorganisms would show broad intraspecies diversity and that specific genotypic strains could be attributed to diseased and healthy skin. Why specific genotypes of *M. globosa* and *M. restricta* predominate in patients with *Malassezia*-associated skin diseases is unknown, but probably reflects their compatibility with the pH, water content, and amount of sebum that characterize the skin of these patients and the latter's antibiotics history. *Trichosporon asahii*, which is the major causative agent of disseminated trichosporonosis, has more than 10 genotypes according to its IGS region [14]. In Japanese patients, the major genotype was type I, whereas in patients from the USA, type III predominates [13]. *M. sympodialis* and *M. dermatis* are positioned within the same clade on the phylogenetic tree [15]. This clade also includes *M. equina*, *M. caprae*, and *M. nana*, isolated only from non-human animals. de Bellis et al. [16] analyzed the IGS sequence of *M. nana* and reported the absence of diversity in the IGS sequence in strains obtained from cats and dogs from Japan, the UK, and Spain. Whether this species, as well as *M. sympodialis* and *M. dermatis*, has unique biological characteristics that obviate the need for IGS sequence diversity remains to be determined. The almost complete absence of intraspecies diversity in the IGS regions of *M. sympodialis*, *M. dermatis*, and *M. nana* suggests that this is a feature specific to species belonging to this clade.

In conclusion, our study demonstrated the absence of intraspecies diversity in the IGS region of the skin commensal fungi *M. sympodialis* and *M. dermatis* and the lack of specific genotypic strains in patients with AD and SD versus HS. Thus, strain-based analyses of these two species require the use of genes showing intraspecies diversity, as the IGS region is not an appropriate tool for this purpose.

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Compliance with Ethical Standards

Conflict of interest None.

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