

NOTE

Sequence diversity of the intergenic spacer region of the rRNA gene of *Cryptococcus albidus* isolated from the skin of patients with atopic dermatitis and healthy individuals

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ABSTRACT

The yeast species *Cryptococcus albidus* var. *albidus* was found to more often colonize the skin surface of patients with atopic dermatitis (77.0%, 47/61) than that of healthy subjects (37.5%, 15/40). The intergenic spacer 1 region of the rRNA gene of this species consists of four sequence types: I, II, III and IV. Types I and II were predominant among healthy subjects and atopic dermatitis patients, respectively.

Key words atopic dermatitis, *Cryptococcus albidus*, intergenic spacer, microbiome.

A wide variety of microorganisms, including bacteria and fungi, cover human skin. These skin microorganisms play a significant role in maintaining a healthy status by inhibiting invasion of external pathogens and maintaining a weakly acidic pH on the skin surface. Generally, they are harmless to humans; however, they sometimes cause various types of skin diseases (1, 2).

Dermatitis with pruritus characterizes atopic dermatitis, a chronic disease that typically has repeated cycles of remission and deterioration. Various environmental factors together with hypersensitivity to dry skin and ready production of IgE antibodies related to allergy contribute to the pathogenesis of this condition. AD patients produce specific anti-IgE antibodies against several skin microorganisms. For example, anti *Malassezia*-specific IgE antibody, which is not produced by healthy subjects, has been identified in serum from AD patients and the symptoms improve following administration of antifungal agents (3).

While analyzing the cutaneous fungal microbiomes of patients with AD, we isolated large amounts of the basidiomycetes yeast *C. albidus* from the skin of patients and

healthy individuals. This species is distributed widely in the environment (4) and sometimes causes opportunistic infections in immunocompromised hosts (5).

In this study, we compared the frequency of isolation of *C. albidus* in healthy individuals and patients with AD and investigated the significance of the genotype.

This study included 61 AD outpatients attending Tokyo Medical University Hospital and 40 healthy subjects. We diagnosed AD according to the criteria proposed by Hanifin and Rajka (6). We collected samples from lesional regions on the face and neck of patients and from normal skin on the face and neck of healthy subjects. Prior to sampling, patients were receiving routine skin care, including intermittent applications of mild steroid ointment or petrolatum. Our Institutional Review Board approved the study and each subject gave informed consent. We strapped OpSite transparent dressings (3 × 7 cm; Smith and Nephew Medical, Hull, UK) to the skin (3) and then transferred the straps onto modified Sabouraud dextrose agar (20 g glucose, 10 g polypeptone, 5 g yeast extract, 15 g agar) containing 50 µg/mL chloramphenicol

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List of Abbreviations: AD, atopic dermatitis; *C. albidus*, *Cryptococcus albidus*; CBS, Centraalbureau voor Schimmelcultures; D1/D2, domain 1 and domain 2; DDBJ, DNA Data Bank of Japan; IGS, intergenic spacer; LSU, large subunit; *M. globosa*, *Malassezia globosa*; *M. restricta*, *Malassezia restricta*; rRNA, ribosomal ribonucleic acid.

Table 1. *Cryptococcus albidus* isolated from the skin of patients with atopic dermatitis and healthy subjects

Source	Number of subject examined	Number of subjects whose samples yielded <i>C. albidus</i>	Variety <i>albidus</i>				Variety <i>kuezingii</i>	Variety <i>ovalis</i>
			Type I	Type II	Type III	Type IV		
Patients with mild AD	26	18 (69.2%)	2 (11.1%)	14 (77.8%)	1 (5.6%)	1 (5.6%)	0	0
Patients with moderate AD	24	19 (79.2%)	3 (15.8%)	15 (78.9%)	0	1 (5.3%)	0	0
Patients with severe AD	11	10 (90.9%)	1 (10.0%)	9 (90.0%)	0	0	0	0
Total	61	47 (77.0%)	6 (12.3%)	38 (80.9%)	1 (2.1%)	1 (2.1%)	0	0
Healthy subjects	40	15 (37.5%)	10 (66.7%)	2 (13.3%)	3 (20.0%)	0	0	0

(Daiichi-Sankyo, Tokyo, Japan). We incubated the plates at 32°C for 5 days.

We selected yeast colonies that were cream to pinkish-cream or yellowish-cream in color, butyrous to soft or soft to slightly mucoid in consistency and had slightly to moderately glossy and smooth surfaces for analysis (4). We sequenced the D1/D2 regions of the LSUs of the rRNA genes of the strains. We identified strains with D1/D2 LSU sequence similarity of 99% or more of that of the type strains of three varieties of *C. albidus* (GenBank accession numbers: AF075474 [variety *albidus*], AF137602 [variety *kuezingii*] and AF137605 [variety *ovalis*]) as conspecific (7). We further analyzed the strains identified as *C. albidus* by analysis of the D1/D2 LSU sequence in the IGS 1 region of the rRNA gene using the primers 26SBF (5'-AGCTGCTGCCAATGCTAGCTC-3') and Crpt-5SR (5'-ACCCAGGATTCACGTCGTCC-3') (8). We also sequenced the IGS 1 region of strains of three varieties of *C. albidus*: CBS 142 (type strain of variety *albidus*); CBS 945, CBS 969, CBS 1925, CBS 5592, and CBS 7015 (variety *albidus*); CBS 1926 (type strain of variety *kuezingii*); and CBS 5810 (type strain of variety *ovalis*).

We found *C. albidus* strains in 47/61 cases (77.0%) (Table 1). In terms of clinical severity, we classified the AD of 69.2%, 79.2%, and 90.9% of patients as mild, moderate, and severe, respectively. We found no remarkable differences between the three clinical severities. In the 40 healthy subjects, we found *C. albidus* strains in 15 cases (37.5%); the detection frequency was less than that in AD patients. The number of *C. albidus* colonies detected on agar medium ranged from one to three for all cases.

The D1/D2 LSU of all strains recovered from the medium had 100% sequence similarity to that of the type strain of *C. albidus* var. *albidus*. We therefore identified all strains obtained from the samples of patients and healthy individuals as *C. albidus* var. *albidus*. We detected no *C. albidus* var. *kuezingii* or var. *ovalis* in any scale sample. The IGS 1 region of variety *albidus* consists of four sequence types: I, II, III and IV. These four types of IGS 1 regions had > 75.6% DNA sequence similarities with one another and the type strains of the three varieties

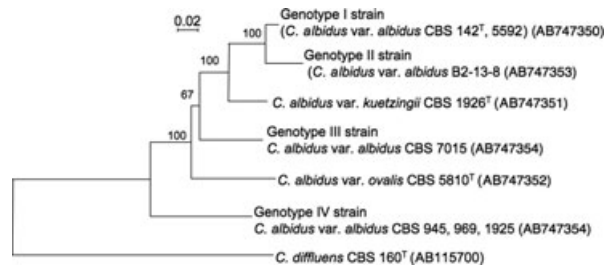


Fig. 1. Molecular phylogenetic tree constructed using IGS 1 sequences. The sequences were aligned using the Clustal W software (15). For neighbor-joining analysis (16), the distances between sequences were calculated using Kimura's two-parameter model (17). A bootstrap analysis was conducted with 100 replications (18). The IGS 1 sequences determined in this study have been deposited in DDBJ, their accession numbers are shown in parentheses. Six CBS stock strains of *C. albidus* var. *albidus* were assigned to types I, III and IV. T, type strain.

had 82.1–91.4% DNA sequence similarities. Figure 1 depicts the phylogenetic relationship among IGS 1 sequences of the three varieties and four sequence types of variety *albidus*.

In the AD patients, type II (80.9%) was predominant, followed by types I (12.3%), III (2.1%) and IV (2.1%). We found no remarkable differences in distribution frequency of the types among the three clinical severities. We recovered multiple colonies from some scale samples; however, in every case, all colonies showed identical IGS 1 sequences. In healthy individuals, type I (66.7%) was predominant, followed by types III (20.0%) and II (13.3%). Based on our findings, we conclude that the predominant type of *C. albidus* that colonizes the skin surface of AD patients is different from that of healthy individuals.

There are few published reports concerning the human skin fungal microbiome. Yang *et al.* analyzed 323 subjects and determined that *C. albidus* is rare in healthy human skin (9). We have also conducted a comprehensive analysis of skin fungal microbiota of AD patients and healthy individuals using the clone library method as a

culture-independent method. In that study, we found no *C. albidus* in skin scale samples from either group (10). It is possible that the clone library method was insufficiently sensitive to detect *C. albidus* because the proportion of this fungus in the fungal microbiome of skin scale was very low. It is easier to grow predominant yeast species on medium using the culture-dependent method employed in the current study.

In this study, we detected *C. albidus* more frequently in AD patients than in healthy individuals. Healthy skin is weakly acidic, suggesting that healthy skin prevents invasion of external pathogens. However, because the skin of patients with AD is closer to neutrality, these patients have an increased risk of infection. If cutaneous *C. albidus* is responsible for exacerbating AD, a specific IgE antibody against this species should be present in the sera of AD patients.

Koivikko et al. investigated common allergenic properties of yeast allergen extracts of 18 yeast species (including *C. albidus*). They found significant multiple sensitivities to extracts of *Candida albicans*, *C. utilis*, *C. albidus*, *Rhodotorula rubra*, and *Saccharomyces cerevisiae* when skin-prick testing atopic patients, suggesting the presence of one or more common skin-reactive allergens (11). Although IgE antibody against *C. albidus* is present in the sera of AD patients, no IgE was detected in that of healthy individuals (12). Therefore, cutaneous *C. albidus* might be one exacerbating factor for AD.

In our study, the predominant IGS1 sequence of *C. albidus* obtained from AD patients differed from that of the *C. albidus* isolated from healthy individuals. Similar examples reportedly include *M. globosa* and *M. restricta* colonizing the skin surfaces of AD patients and healthy subjects; even though these two species were predominant in both study groups, the IGS 1 DNA sequence types differed significantly between the two groups (13, 14). Taken together, these results indicate that the skin microbiotas of AD patients and healthy individuals differ at the strain level, whereas the qualitative skin microbiomes of these two groups of subjects are similar.

We believe that our findings will prove useful in elucidating the relationship between the skin microbiome and exacerbation of AD.

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Disclosure

The authors have no disclosures or conflicts of interest to report.

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