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ORIGINAL ARTICLE

Genotypes of *Candida albicans* isolated from healthy individuals and their distribution in patients with oral candidiasis

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Abstract For the study of *Candida albicans* genotypes involved in development of candidiasis, *Candida albicans* isolates were collected from healthy volunteers and patients with oral candidiasis and genotyped on the basis of 25S rDNA and microsatellite polymorphisms. In the microsatellite analysis using two microsatellite markers (CDC3 and CAI), 63 healthy volunteer isolates were classified into 35 genotypes (allelic relations to CDC3 alleles 1:2/CAI alleles 1:2), among which genotypes II (115:119/23:23), III (115:123/18:27), and V (123:127/32:41) were found at frequencies of 12.7 %, 7.9 %, and 7.9 %, respectively. In 68 oral candidiasis isolates classified into 39 genotypes, genotypes II and III were identified in 4.4 % and 20.6 % of the isolates, respectively. The frequency of genotype III was higher in the candidiasis isolates than in the healthy isolates ($p < 0.05$). These results suggest that genotype III *C. albicans* assigned by CDC3/CAI is related to the development of oral candidiasis.

Keywords *Candida albicans* · Genotype · Oral candidiasis · Healthy individuals · Microsatellite · 25S rDNA

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Introduction

Candidiasis occurs in both nonimmunocompromised and immunocompromised individuals. Oral candidiasis is an opportunistic fungal infection that occurs in immunocompromised hosts and is caused by several *Candida* species which inhabit the oral cavity as a commensal population. *Candida albicans* is the most important etiological species of candidiasis because this fungus was the species most frequently isolated from candidiasis patients [1].

Several molecular techniques have been used to study the genotypes of *C. albicans*, some of which have shown a relationship between genotypes and the development of candidiasis [2–8]. The genotypes of *C. albicans* derived from patients with vulvovaginal candidiasis reportedly differed from those of commensal *C. albicans* [9]. Further, *C. albicans* genotypes were related to the severity of vulvovaginal candidiasis [10].

Recently, we studied the major genotype distribution of *C. albicans* isolated from patients with and without candidiasis and reported that microsatellite-based genotyping of commensal *C. albicans* from the oral cavity of patients

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without candidiasis using CDC3 and CAI assigned five major genotypes (genotypes I, II, III, IV, and V). Of these, genotypes II and III *C. albicans* were commonly isolated from both commensal and infective sites of patients with skin cutaneous candidiasis, vulvovaginal candidiasis, and candidemia. In contrast, genotype V was identified only in the commensal isolates of patients without candidiasis [11]. Unfortunately, *C. albicans* isolates from patients with oral candidiasis and from a commensal site of healthy individuals have not been subjected to genotype analysis. Most cases of candidiasis are endogenously caused by commensal *C. albicans* in the oral cavity [12, 13]. Therefore, *C. albicans* isolates from patients with oral candidiasis are useful for understanding the relationship between genotypes II, III, and V and candidiasis. Additionally, *C. albicans* isolates from healthy individuals are necessary to understand the genotype distribution of commensal *C. albicans*. If genotype V *C. albicans* defined by CDC3/CAI is widely distributed in the oral cavity but is less likely to cause either superficial or systemic candidiasis, it is expected that genotype V is not identified in isolates form the infection site of patients with oral candidiasis. In this study, for the relationship between genotypes and candidiasis, genotypes of commensal *C. albicans* from healthy individuals were analyzed and their distributions were compared with those of isolates from patients with oral candidiasis.

Materials and methods

Subjects

Commensal *Candida* species were isolated from 88 healthy volunteers (37 males and 51 females; mean age, 37.8 years) from the Aichi-Gakuin University School of Dentistry (HV group). Infective *Candida* species were isolated from 50 patients with oral candidiasis (7 males and 43 females; mean age, 70.5 years) (OC group). After the patients were diagnosed as oral candidiasis, 48 patients received treatment with amphotericin B syrup and the remaining patients were treated with fluconazole in the University Hospital. In all cases, the *Candida* species is isolated before treatment with antifungal agents.

Isolation and identification of *Candida* species

All volunteers from the HV and OC groups gargled 20 ml sterile distilled water, and the samples were centrifuged at 3,000 rpm for 10 min. The pellets were suspended in 400 µl sterile distilled water, and 30 µl of the suspension was spread onto a chromogenic agar (CHROMagar Candida; BD Co., Tokyo, Japan) plate. After stationary culture at 30 °C for 3–4 days, each colony with a different color (green, pink, blue, and white) was separately transferred onto another chromogenic agar plate and cultured for 2–3 days at 30 °C to isolate single colonies. To study the genotype homogeneity of *C. albicans*, 16 green colonies from each group were transferred onto new plates. Additionally, non-green colonies were also transferred to identify the *Candida* species. A total of 2,755 isolates (1,117 isolates from the HV group and 1,638 isolates from the OC group) were obtained.

Species identification of the isolates was performed by polymerase chain reaction (PCR) targeting the DNA topoisomerase II gene (*TOP2*) [14, 15]. To identify *Candida dubliniensis*, primer sets specific to *C. dubliniensis TOP2* and 25S rDNA (see following) were used [14]. Yeasts that were not identified by these PCRs are referred to as "other yeast species" in this article.

Using multi-colony isolation, 472 strains of *C. albicans* were obtained from the HV group and 676 strains of *C. albicans* were obtained from the OC group. All the isolates were genotyped at the 25S rDNA and microsatellite levels (described below).

The isolation, identification, and genotyping of *Candida* yeasts (described below) were carried out with informed consent from all the participants.

Purification of genomic DNA

Genomic DNA was purified from the isolates as previously described [11, 16]. Briefly, all isolates were cultured on agar plates containing 2 % glucose, 0.3 % yeast extract, and 1 % peptone for 3–4 days at 30 °C. The yeast cells were suspended in extraction buffer and the genomic DNA was extracted by vortexing with glass beads. After centrifugation, the genomic DNA was purified using a DNA purification kit (FastDNA Kit; Qbiogene, Carlsbad, CA, USA) as described previously [14].

25S rDNA-based genotyping

C. albicans genomic DNAs were amplified with the 25SIN primer set, and the nucleotide sequences of forward and reverse primers and PCR parameters were previously published [17]. From this PCR, *C. albicans* was divided into four groups by the size of the amplified DNA (450 bp for group A, 840 bp for group B, 450 and 840 bp for group C, and 1,080 bp for group E), and C. *dubliniensis* was assigned as group D with a 1,040-bp DNA band [17, 18].

Microsatellite-based genotyping

Fragment analysis of the microsatellites of the *C. albicans* isolates was carried out using four microsatellite marker