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Distribution of *Candida albicans* and non-albicans *Candida* species in oral candidiasis patients: Correlation between cell surface hydrophobicity and biofilm forming activities

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ABSTRACT

Objectives: The purposes of this investigation were to study the prevalence of *Candida albicans* and non-albicans *Candida* (NAC) species from oral candidiasis patients and evaluate the cell surface hydrophobicity (CSH) and biofilm forming capacity of the clinical isolates *Candida* species from oral cavity.

Design: This study identified a total of 250 *Candida* strains isolated from 207 oral candidiasis patients with PCR-RFLP technique. CSH value, total biomass of biofilm and biofilm forming ability of 117 oral *Candida* isolates were evaluated.

Results: *C. albicans* (61.6%) was still the predominant species in oral candidiasis patients with and without denture wearer, respectively, followed by *C. glabrata* (15.2%), *C. tropicalis* (10.4%), *C. parapsilosis* (3.2%), *C. kefyr* (3.6%), *C. dubliniensis* (2%), *C. lusitaniae* (2%), *C. krusei* (1.6%), and *C. guilliermondii* (0.4%). The proportion of mixed colonization with more than one *Candida* species was 18% from total cases. The relative CSH value and biofilm biomass of NAC species were greater than *C. albicans* ($p < 0.001$). Ninety-two percent of oral isolates NAC species had biofilm forming ability, whereas 78% of *C. albicans* were biofilm formers. Furthermore, the significant difference of relative CSH values between biofilm formers and non-biofilm formers was observed in the NAC species ($p < 0.005$), whereas the difference was not statistically significant in *C. albicans*.

Conclusion: The frequency of the NAC species colonization in oral cavity was gradually increasing. The possible contributing factors might be high cell surface hydrophobicity and biofilm forming ability. The relative CSH value could be a putative factor for determining biofilm formation ability of the non-albicans *Candida* species.

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1. Introduction

Candida spp. is an important and common causative organism of human fungal infection. It can exist not only as a commensal organism but also as an opportunistic pathogen in the human body. Oral candidiasis is a superficial candida infection which is more common in elderly age, immunocompromised host, denture wearer, and people with xerostomia. It has many clinical manifestations such as pseudomembranous candidiasis, erythematous candidiasis, hyperplastic candidiasis, denture stomatitis, angular cheilitis and burning sensation.[1] Among these, *Candida albicans* is the most frequently isolated species,[2,3] followed by *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, *C. guilliermondii*, *C. krusei*, and *C. kefyr* are responsible for oral infection.[4,5]

Presently, there are many identification methods for *Candida* species, for example, phenotypic and molecular techniques.[6,7] The phenotypic method includes chromogenic medium and carbohydrate assimilation. The molecular techniques for discrimination of *Candida* species have been continuously developed such as multiplex PCR, PCR-RFLP, and real-time PCR. Identification of the *Candida* species is beneficial for choosing the proper treatment and management of the oral candidiasis. Related to this, non-albicans *Candida* (NAC) species have been increasingly found associated with antifungal drug resistance especially *C. glabrata*, *C. tropicalis*, and *C. krusei*.[8,9]

The increasing frequency of NAC species has been continuously reported[10–12]; they are considered emerging significant pathogens in the oral cavity, bloodstream, and genitourinary system. The growing number of NAC species might be associated with previous exposure to azole and polyene drugs, malignancy disease and indwelling medical devices.[13–15] Nevertheless, the distribution of NAC species may vary according to age groups and geographic areas.[16] For example, *C. glabrata* has high incidence in America, North and Central Europe. *C. tropicalis* is commonly isolated in South America and Asia. Moreover, *C. glabrata* predominates in the elderly.[17] *C. parapsilosis* is a significant pathogen in neonate bloodstream infection, especially prematurity and low birth weight.[18] *C. dubliniensis* is associated with oral opportunistic pathogen in patients infected with human immunodeficiency virus.[19]

Cell surface hydrophobicity (CSH) and biofilm formation are identified as an important virulence factors that have significant roles in the pathogenesis of *Candida* spp. Previous studies[20–22] have shown positive correlation between CSH and adhesion of *Candida* to the buccal epithelial cells and inert polymeric surface such as denture prostheses. The adhesion of *Candida* spp. is an initial and critical step in biofilm formation. Candida biofilms are communities of mixture cell types including yeast form and/or filament form that adhere to each other. They are embedded in the extracellular matrix.[23] The *Candida* biofilms can occur on medical devices and host mucosal surface. Biofilm forming ability and matrix composition highly vary among various *Candida* species.[24] The purposes of this investigation are to study the prevalence of *C. albicans* and non-albicans *Candida* species (NAC) from oral candidiasis patients and the correlation between biofilm forming capacity and CSH of clinical isolates *Candida* species recovered from oral cavity.

2. Materials and methods

2.1. Clinical samples

A total of 250 clinical candida isolates were collected from 207 Thai patients with oral candidiasis attending the Dental Clinic at the Faculty of Dentistry, Mahidol University, during the period from 2008 to 2010 and 2013. The study was approved by the Institutional Review Board of the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Thailand (MU-DT/PY – IRB 2013/063.0810).

2.2. Identification of Candida spp.

All clinical samples were grown on Sabouraud Dextrose Agar (SDA) overnight at 37 °C. *Candida* spp. was identified by colony morphology on SDA and microscopic morphology showing budding yeast cells. Each colony morphology of *Candida* spp. was stored in 15% glycerol at −20 °C.

2.3. DNA extraction

Candida isolates were cultured for 18 h at 30 °C in Sabouraud Dextrose broth. Cell pellet was collected by centrifugation at 8000 rpm for 2 min. The pellet was resuspended with lysis buffer containing 100 mM NaCl, 1 mM EDTA, 10 mM Tris–HCl (pH 8.0), 1% SDS, and 2% Triton X-100, and then boiled for 5 min. Following phenol:chloroform:isoamyl alcohol extraction, ethanol precipitation of DNA was done. The DNA pellet was allowed to dry and dissolved in 50 μl of sterile water. The extracted DNA was kept at −20 °C for further use.

2.4. Amplification of ITS sequence

Internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified with fungal-specific primer ITS1 (5′-TCC GTA GGT GAA CGT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′).[25] PCR reaction was performed in a 50 μl reaction volume composed of 10X PCR buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphates, 0.5 μM each primer, 2U of *Taq* DNA polymerase and 50 ng of genomic DNA. The PCR programme was done according to the following conditions: denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 sec, annealing at 56 °C for 30 sec, and extension at 72 °C for 1 min, with a final extension step of 8 min at 72 °C.

2.5. RFLP analysis

The ITS1-ITS4 sequence of various *Candida* spp. was used in restriction digestion reaction. 10 μl of amplified PCR products were digested with 5U of *Msp*I restriction enzyme (Thermo Scientific) in 10X Fastdigest buffer. The reaction was incubated for 3 h at 37 °C. The restriction enzyme products were separated on 2% agarose gel run in TAE buffer at 100 V for 45 min and stained with ethidium bromide. Sizes of DNA

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