ABSTRACT

Candida is a genus of opportunistic yeast that are usually harmless residents of the oral cavity, but they become pathogenic under conditions which allow them to increase their proportion to other members of the oral microflora. Correct and accurate identification of the candidal species infecting an oral candidiasis patient is important due to antifungal drug resistences. The region in the fungal genome that contains the gene that codes for rRNA, also known as rDNA, has been found to be useful for phylogenetic studies and species identification. The purpose of this study is to compare the candidal loads of denture wearers, periodontal disease patients, and a control group, in addition to differentiating isolated oral *Candida* sp. based on rDNA, in order to assess the effectiveness of using the rDNA region for candidal species identification. Samples from the saliva and the surfaces of the palate, tongue and cheek mucosa were collected from 45 individuals consisting of three target groups: periodontal disease patients, denture wearers with healthy oral cavity, and non-denture wearers with healthy oral cavity as the control group. The samples were subjected to serial dilution and spread on agar plates, which were then scored for Colony-Forming Units (CFUs). Next, fifteen random candidal colonies were isolated and subjected to genomic DNA extraction based on glass beads disruption. ITS1, ITS2, ITS3 and ITS4 primers were used to amplify regions in the rDNA, and the ITSI-5.8S-ITSII region was then digested by *Hinf*I and *Msp*I restriction enzymes. The microbial loads on all the sites of denture wearers groups was found to be significantly higher than the control group, while only the microbial loads on the tongue surface of the periodontal disease group was significantly higher than in the control group, while at all the other sites there was no significant difference. Comparing the restriction fragment lengths of the clinical samples to that of seven ATCC control species allowed the identification of *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida dubliniensis* and *Candida glabrata* (*C. krusei* and *C. lusitaniae* were also among the

ATCC control species and could be differentiated, but none of the clinical samples were of those species). One clinical sample had a unique band and restriction fragment pattern that could not be matched to any of the control species, however based on the band sizes it is most likely to be *Candida famata*. The *Msp*I restriction digest was not able to distinguish between *C. albicans* and *C. dubliniensis*, whereas the *Hinf*I digest could not distinguish between *C. tropicalis* and *C. parapsilosis*. In conclusion, candidal colonization in denture wearers, while there appears to be no significant difference in periodontal patients other than on the tongue surface. Furthermore, restriction enzyme digestion of the candidal rDNA region is potentially useful for candidal species identification.

ABSTRAK

Candida merupakan genus yis yang opportunis, lazimnya terdapat di ruang mulut dan tidak membahayakan, namun menjadi patogenik di bawah keadaan yang membenarkan mereka membiak dan meningkatkan kadar nisbah mereka berbanding dengan ahli mikro-flora yang lain. Pengecaman tepat dan jitu spesies *Candida* yang menjangkiti pesakit kandidiasi mulut (*oral candidiasis*) adalah penting kerana wujudnya kerintangan terhadap ubat antikulat. Kawasan di dalam genom kulat yang mengandungi gen yang mengkod rRNA, juga dikenali sebagai rDNA, telah pun didapati bermanfaat dalam kajian filogenetik dan pengenalan spesies. Tujuan kajian ini adalah untuk membandingkan kandungan *Candida* di dalam pemakai *denture*, pesakit periodontal dan kumpulan kawalan, serta membezakan antara spesies *Candida* yang diasingkan dari mulut berdasarkan rDNA untuk mengkaji keberkesanan penggunaan kawasan rDNA bagi tujuan pengenalan spesies *Candida*. Sampel dari air liur, permukaan lelangit mulut, lidah dan mukosa pipi diambil dari 45 individual yang terdiri dari 3 kumpulan sasaran; pesakit periodontal, pemakai dentur yang mempunyai rongga mulut yang sihat dan bukan pemakai dentur yang mempunyai rongga mulut yang sihat sebagai kawalan. Pencairan bersiri dijalankan ke atas sampel yang kemudiannya dikira Unit Pembentukan Koloni (CFUs). Kemudian, 15 koloni *Candida* dipilih secara rawak dan diasingkan sebelum melalui proses pengekstrakan DNA genomik berdasarkan penggunaan manik kaca. Primer ITS1, ITS2, ITS3 dan ITS4 digunakan untuk amplifikasi kawasan rDNA, dan kawasan ITSI-5.8S-ITSII kemudiannya dipotong oleh enzim restriksi *Hinfl* dan *Mspl*. Kandungan mikrobial di kesemua kawasan pemakai dentur didapati lebih tinggi dengan signifikan daripada kumpulan kawalan, manakala kandungan mikrobial di permukaan lidah pesakit perodontal adalah lebih tinggi secara signifikan berbanding kumpulan kawalan sementara di kawasan lain tiada perbezaan yang signifikan. Perbandingan antara saiz fragmen

sampel klinikal dengan tujuh spesies kawalan ATCC membolehkan identifikasi *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida dubliniensis* dan *Candida glabrata* (*C. krusei* dan *C. lusitaniae* juga dikalangan tujuh spesies kawalan ATCC yang boleh dibezakan, namun sampel klinikal tidak mengandungi spesies tersebut). Satu sampel klinikal mempunyai saiz jalur dan fragmen yang unik yang tidak boleh dipadankan dengan mana-mana spesies kawalan. Namun, berdasarkan saiz jalur, sampel tersebut berkemungkinan adalah *Candida famata*. Pemotongan oleh *Msp*I tidak dapat membezakan antara *C. albicans* dengan *C. dubliniensis* manakala pemotongan *Hinf*I tidak dapat membezakan antara *C. tropicalis* dengan *C. parapsilosis.* Kesimpulannya, pertumbuhan *Candida* lebih tinggi secara signifikan bagi pemakai dentur, manakala tiada perbezaan ketara bagi pesakit peridontal selain daripada permukaan lidah. Tambahan itu, pemotongan enzim restriksi di kawasan rDNA *Candida* mempunyai potensi digunakan bagi tujuan pengenalan spesies *Candida*.

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ABBREVIATIONS

%	:	percentage
°C	:	degree centigrade
g	:	gram
µg	:	microgram
µm	:	micrometer
L	:	liter
mL	:	milliliter
µL	:	microliter
DNA	:	deoxyribonucleic acid
RNA	:	ribonucleic acid
PCR	:	polymerase chain reaction
M	:	Molar
ed.	:	edition
et al.	:	et alia (and others)
no.	:	number
sp.	:	species
CFU	:	colony forming unit