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Full Length Research Paper

Virulence properties and random amplification of polymorphic DNA (RAPD) fingerprinting of *Candida albicans* isolates obtained from Monastir dental hospital, Tunisia

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Genotypic and phenotypic characterization as well as studies on the virulence factors of *Candida albicans* isolates obtained from oral cavity of patients was carried out using random amplified polymorphic DNA (RAPD) fingerprinting and epithelial cells adherence assay, respectively. RAPD patterns revealed the presence of 13 *C. albicans* genotypes separated into two clusters at 75% of similarity when they were combined. Results also showed the presence of haemolytic protease activity as virulence factors with 88% of the *C. albicans* strains been able to adhere to Caco-2 cells and only 64% to Hep-2. RAPD-polymerase chain reaction (PCR) is a molecular tool used to differentiate the isolates into various genotypes based on their virulence properties.

Key words: *Candida albicans*, stomatitis, random amplified polymorphic DNA, Hep-2, Caco-2 cells.

INTRODUCTION

Candida albicans is a commensal pathogen that lives on the skin and mucosal surfaces of the genital and intestinal tracts as well as the oral cavity (Calderone and Fonzi, 2001). This germ is the principal etiological agent of oral candidal infection (Nikawa et al., 1998; Webb et al., 1998). *Candida* strains have been isolated from 93% of patients with denture stomatitis, which are now considered to be the commonest form of oral candidiasis (Budtz-Jorgensen et al., 1975). This germ present in the oral cavity, mixed with saliva where it can grow and adhere to epithelial cells (Cannon and Chaffin, 1999).

The ability to adhere to epithelial cells and medical biomaterial, secretion of hydrolytic enzymes (extracellular proteinases and phospholipases), and production of haemolytic factor are the principal virulence factors of *Candida* strains contributing to their pathogenicity (Ghannoum, 2000).

Secretion of specific hydrolytic enzymes by *C. albicans* has been suggested as possible virulence factors (Neugnot et al., 2002). Phospholipases are hydrolytic enzymes that attack phospholipids common in all cell membranes. In addition, same authors demonstrated that *C. albicans* strains with increased phospholipase activity are associated with higher virulence in murine models (Ibrahim

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Abbreviations: PCR, Polymerase chain reaction; **RAPD,** random amplified polymorphic DNA; **Sap,** secretory aspartyl proteinases; **PFGE,** pulsed-field gel electrophoresis; **SCA,** sabouraud chloramphenicol agar; **EDTA,** ethylenediaminetetraacetic acid; **SDA,** Sabouraud dextrose agar; **YPD,** yeast peptone dextrose; **Hep-2,** cells from human laryngeal carcinoma; **Caco-2,** cells from human colon adenocarcinoma; **PBS,** phosphate-buffered saline; **MIC,** minimum inhibitory concentration.

et al., 1995). Also, lipases might help a microorganism to grow in environments where lipids are the sole carbon source (Stehr et al., 2003). Secretory aspartyl proteinases (Sap) constitute a family of enzymes that are able to degrade several physiologically important substrates such as albumin, immunoglobulin, and skin proteins (Cassone et al., 1987). Also, haemolysins are known to be putative virulence factors facilitating hyphal invasion in disseminated candidiasis (Luo et al., 2001).

Cotter and Kavanagh (2000) demonstrated that adhesion of *C. albicans* to buccal epithelial cells is proved in cases of oral candidiasis. Candidal adherence studies have been frequently performed using cell monolayers including HeLa and human embryonic kidney epithelial cells, fibroblasts and Hep-2 cells (Bektic et al., 2001).

In fact, the investigation of the genetic relatedness between clinical *Candida* strains may be of great importance in clinical diagnosis, epidemiology, treatment and prevention of candidiasis (Pei et al., 2007).

Techniques based on the morphology and phenotypic characteristics are poorly adapted for this purpose and the relatedness between isolates is best investigated by DNA-based methods due to their much higher discriminatory power and to the genetic polymorphism and plasticity of *Candida*. Many techniques have been developed for differentiating *Candida* strains at the molecular level. The most frequent DNA-based methods used to differentiate yeast strains involve a variety of techniques such as pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) fingerprinting (Hunhreys, 2004; Chen et al., 2005; Foulet et al., 2005). Each technique has proven to be sensitive and highly discriminatory. The random amplified polymorphic DNA assay relies on the use of arbitrary primers which are annealed to genomic DNA in low stringent conditions. This technique has become one of the most commonly used for DNA fingerprinting of medically important *Candida* species (Bello et al., 2002). It is easy to perform, fast and much adapted for investingating identity and relatedness between isolates of *C. albicans* (Del Castillo et al., 1997; Pujol et al., 1997).

The aim of the present work is therefore, to investigate the phenotypic and genotypic relationship between oral *C. albicans* isolates isolated from patients suffering from stomatitis. In a second step, we attempted to evaluate the distribution of some virulence properties such as the ability to adhere to epithelial cells and the potency to secrete hydrolytic enzymes.

MATERIALS AND METHODS

Patients

This study was conducted according to the ethical approval over a period of two years (from March 2006 to October 2007). Twenty two patients from the region of Monastir (Centre of Tunisia) suffering from denture stomatitis were included in this study. Patients were observed for signs or symptoms of oral candidiasis at baseline. The symptoms associated with oral *Candida* infection were ranged from none to a painful burning sensation. The oral *Candida* strains were isolated from the inside of the cheek, tongue and palate. Ten males and five females with a mean age of 52.93 ± 21.03 years were subject of this study. In addition, five of the 15 patients suffering from denture stomatitis were diabetics, eight were smokers, five have prostheses and three demonstrated poor oral hygiene. All patients were not submitted to any antifungal treatment.

Clinical strains, media and growth conditions

A total of 16 clinical *C. albicans* isolates and one *C. albicans* type strain ATCC 90028 were used in the present study (Table 1). Samples were collected from the oral cavity, the gingival sulci and the pharyngeal portion, by using swabbing method. Yeast strains were cultured into sabouraud chloramphenicol agar (SCA) (Bio-rad, France) and incubated for 48 h at 35°C to obtain isolated colonies. All oral isolates were identified by standard microbiological methods: Macroscopic test of culture on SCA, microscopic test by induction of hyphal form on Lee's medium (5 g $(NH_4)_2SO_4$; 0.2 g $MgSO_4.7H_2O$; 2.5 g K_2HPO_4; 5 g NaCl; 12.5 g glucose; 0.5 g proline and 0.001 g biotin). The carbohydrates assimilation was tested using the ID 32 C system (bio-Mérieux, Marcy l'Étoile, France) according to the manufacturer's specification and the results were read using an automated microbiological mini-Api (bio-Mérieux). Strains were stored at 4°C on Sabouraud dextrose broth (Bio-rad, France) supplemented with glycerol at 10% (v/v).

Polymerase chain reaction (PCR) confirmation of *C. albicans* strains

The genomic confirmation of all *C. albicans* strains tested at the species level was done according to the protocol for the amplification of a *CaYST1* gene intron fragment (Baquero et al., 2002).

Rapid DNA extraction from different *Candida* strains was made according to the protocol described by Ausubel et al., (1996) for *Saccharomyces cerevisiae*. Briefly, the cell suspensions were vortexed thoroughly following addition of 0.6 g of glass beads (425-600 mm of diameter) and an equal volume of phenol-chloroform-isoamyl alcohol (24:24:1). After centrifugation, the aqueous phase was collected and precipitated by adding ethanol.

The sequences of oligonucleotides (TIB MOLBIOL, Eresburgstrasse, Berlin, Germany) used were primers INT1 (5'-AAGTATT TGGGAGAAGGGAAAGGG-3') and INT2 (5'-AAAATGGGCATTA AGGAAAAGAGC-3') for *C. albicans* (Baquero et al., 2002). Amplification reaction was performed in a 25 µl reaction mixture containing 1 µl of genomic DNA (500 ng), 2.5 µl of 10x buffer, 1 µl of $MgCl_2$ (50 mmol), 2.5 µl of dNTP (2.5 mmol), 4 µmol of each primer (INT1 and INT2) and 2.5 U of Eco *Taq* polymerase (MBI fermentas) in an automated thermocycler (PTC-150 Minicycler™). The mixture was incubated for 5 min at 95°C, followed by 35 cycles of amplification. Each cycle undergo denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and primer extension at 72°C. A final cycle of extension at 72°C for 10 min was added for complete polymerisation.

Five microliters (5 µl) of the PCR products were analyzed on 1% agarose gel stained with ethidium bromide (0.5 µg/ml) running in Tris-acetate-ethylenediaminetetraacetic acid (EDTA) buffer (TAE) for 1 h at 90 V. The gel was visualised under UV transilluminator and photographed using Gel printer plus apparatus. The 100-bp ladder (Fermentas) was used as a DNA weight marker.

Characterization of *C. albicans* strains

Antifungal resistance profile of *C. albicans* strains was determined

Table 1. Phenotypic and genotypic characteristics of *C. albicans* isolates.

Strain code	Hospital	Body site	Date of isolation	Hydrolytic enzymes activities		RAPD pattern	Adhesion to epithelial cells	
				Phospholipase	proteinase		Hep-2	Caco-2
R_1	ATCC 90028	Blood		Negative	Negative	II B	NA	W
3^1	Dental hospital	Oral cavity	03/2006	High	Very high	II B	S	S
4	Dental hospital	Oral cavity	03/2006	High	Very high	II B	S	W
6	Dental hospital	Oral cavity	04/2006	Very high	Negative	II B	NA	S
7	Dental hospital	Oral cavity	04/2006	High	Low	II B	NA	M
9	Dental hospital	Oral cavity	06/2006	High	Negative	II B	S	W
10	Dental hospital	Oral cavity	07/2006	High	Very high	II B	S	S
11	Dental hospital	Oral cavity	07/2006	High	Very high	I	NA	NA
13	Dental hospital	Oral cavity	09/2006	Negative	Negative	II B	NA	NA
14	Dental hospital	Oral cavity	02/2007	High	Negative	II B	S	S
15_B	Dental hospital	Oral cavity	02/2007	High	Very high	II B	S	M
16	Dental hospital	Oral cavity	04/2007	Negative	Very high	II B	S	S
17*	Dental hospital	Oral cavity	04/2007	Negative	Negative	II B	S	S
17_R*	Dental hospital	Oral cavity	04/2007	Negative	Negative	II A	NA	M
18	Dental hospital	Oral cavity	04/2007	Negative	Negative	II B	S	W
21	Dental hospital	Oral cavity	09/2007	Negative	Negative	II A	S	S
65	Dental hospital	Oral cavity	10/2007	Negative	Negative	II B	S	S

*Isolates 17 and 17_R were obtained from the same patient; Hep-2, cells from human laryngeal carcinoma, Caco-2, cells from human colon adenocarcinoma; Adhesion degree, NA = none adhesive, W = weak adhesion, M = medium adhesion, S = strong adhesion.

according to the ATB FUNGUS 2 (ATBF2) (bio-Mérieux) method, testing its susceptibility to amphotericin B, 5-flucytosine, fluconazole and itraconazole.

Some virulence factors like production of hydrolytic enzymes such as phospholipases, aspartylproteases and haemolysins were tested for oral *C. albicans* isolates in order to better characterize these strains (Price et al., 1982; Aoki et al., 1990; Manns et al., 1994; Pei et al., 2007).

Molecular typing of oral *C. albicans* strains

Preparation of DNA

For DNA extraction, the yeasts were routinely grown on Sabouraud dextrose agar (SDA) plates at 28°C for 48 h according to the protocol described by Ausbel et al. (1996). A single colony was then subcultured overnight on yeast peptone dextrose (YPD) broth (1% yeast extract, 2% peptone, 2% dextrose) at 28°C with shaking at 200 rpm. DNA was extracted from this culture by adaptation of the method described previously by Del Castillo-Agudo et al. (1996). The main modification consists on the cell disruption method in which yeast is carried out by both broken cells wall and strong shaking with glass beads. The pellet was resuspended in TE (10 mM Tris-HCl, 1mM Na_2EDTA, pH 8.0) for PCR analysis. DNA concentrations and A260/A280 ratios were determined by means of a "Gene quant Spectrophotometer" (Pharmacia). An A260/A280 ratio of 1.8 - 2.1 was considered acceptable.

RAPD-DNA PCR analysis

For PCR analysis, two different primers CA1 (8-mers 5'-GCTGGTGG-3') and CA2 (10-mers 5'-GCGATCCCCA-3') (TIB MOLBIOL, Eresburgstrasse, Berlin, Germany) were used (Del Castillo et al., 1997). RAPD analysis was performed as described previously (Del Castillo-Agudo et al., 1995). Briefly, every reaction mixture for RAPD analysis contained 10 - 30 ng of genomic DNA. The amplification was performed in an automated thermocycler (PTC-150 Minicycler™) in a final volume of 25 µl containing the appropriate primer at 0.4 µM; dATP, dCTP, dGTP, and dTTP (Sigma, St. Louis, MO, USA), each at a concentration of 200 µM; 50 mM $MgCl_2$; and 2.5 U of *Taq* DNA polymerase (Ecotaq) and 2.5 µl of 10x PCR buffer provided by the manufacturer (Ecogen). Amplification consisted of 1 cycle at 95°C for 5 min, then 35 cycles as follows: 30 s of denaturation at 95°C, 30 s of annealing at 37°C, and 1 min 30 s of primer extension at 72°C. At the final cycle, an additional 10 min of incubation at 72°C was added for complete polymerization.

The resultant fragments of amplified DNA were analyzed by electrophoresis through 1.5% agarose (brand and type) gel in Tris-acetate EDTA buffer (TAE) at 75 V for 4 h (19). Gels were stained with ethidium bromide and then visualised on an ultraviolet transilluminator. 100-bp and 500-pb ladders (Fermentas) were used as a size marker. The obtained gels were photographed by Gel printer plus. The RAPD analyses were performed twice for each isolate.

Analysis of DNA band patterns

DNA fragments sizes were determined by comparison with 100 and 500 bp DNA markers (MBI Fermentas). Bands were read from fingerprints generated by CA1 and CA2 primers and a data matrix was generated for each primer by giving scores of 0 and 1 for the absence or presence of bands at each band position for all strains. The data matrix of individual primers was finally combined to form a single matrix. A dendrogram was constructed, using the data matrix of all the different yeast isolated, based on unweighted pair-group method with arithmetic means (UPGMA) using the Phylogeny

Inference Package software (PHYLIP, version 3.67) as described previously by Sneath and Sokal (1973) and Felsenstein (1995). Isolates were considered different when the band similarity value was less than 95%. The results of the isolates defined the cut-off points used to group the strains into genetically related clusters.

Adherence assay to human epithelial cells lines: Hep-2 and Caco-2

For adherence assay, two human epithelial cell lines Hep-2 (cells from human laryngeal carcinoma) and Caco-2 (cells from human colon adenocarcinoma) were used in this study. Epithelial cells were grown and maintained in Eagle's minimum essential medium (MEM) medium (Gibco BRL, Life Technologies, Rockville, MD, USA) supplemented with 10% fetal bovine serum (Gibco, BRL) 1000 IU/ml of both penicillin and streptomycin. Twenty-four well tissue trays (Falcon) were seeded with the two cell lines (10^3 cells/well). Plates were incubated for 18 h at 37°C in a humidified atmosphere with 5% of CO_2. The semiconfluent monolayers were washed with fresh MEM containing 1% foetal bovine serum. Adhesion of *Candida* strains to Hep-2 and Caco-2 cells was tested as described previously (Holmes et al., 2002). *Candida* strains were grown at 37°C in YPD medium (10 g of yeast extract, 10 g of peptone and 10 g of glucose in 1000 ml of distilled water).

For adherence assay, 100 µl of 10^7 cells/ml were added to Hep-2 and Caco-2 cells, which were cultured into sterile 24-well plates (Nalgene Nunc, Rochester, NY, USA) and incubated at 37°C for 3 h in 5% CO_2. After being washed three times with phosphate-buffered saline (PBS, pH 7.4), *Candida* cells bound to cell lines were fixed with methanol, stained with Giemsa stain and examined microscopically (magnification x40). Uninoculated cell lines served as negative controls. The number of yeasts adhering to each cell lines was counted. All organisms were tested three times. The adhesion index was interpreted as: NA = non adhesive (0 - 10 *Candida*/cells); W = weak adhesion (10 - 20 *Candida*/cells); M = medium adhesion (20 - 50 *Candida*/cells); S= strong adhesion (50-100 *Candida*/cells).

RESULTS

Yeast characterization

All *Candida albicans* strains tested were successfully identified using ID 32 C strips. This biochemical system was previously used with success to identify *Candida* strains isolated from different body sites. All strains tested were genetically confirmed to belong to *C. albicans* species as their relative 310-pb size fragment corresponds to the *CaYST1* gene intron fragment. *C. albicans* ATCC 90028 reference strain produce a 310-pb region specific for this species.

The results of antifungal susceptibility showed that all *C. albicans* strains were susceptible to the fluconazole, amphotericin B and flucytosin. The minimum inhibitory concentration (MIC) values for fluconazole ranged between 0.25 - 4 µg/ml. For amphotericin B and flucytosin, MIC values were lower than 0.5 µg/ml and ranged from 0.5 - 1 µg/ml, respectively.

Hydrolytic enzymes activities were detected in the majority of *C. albicans* tested strains. Results showed that 9 out of 17 isolates (52.9%) were phospholipase positive. Protease activity was detected in six strains (35.3%). Results also showed that among the 17 *C. albicans* strains, 5 produced both phospholipase and protease, and all *C. albicans* strains were beta haemolytic. Haemolytic activity was distributed normally among all the tested isolates and the quantitative data of haemolytic activity ranged from 1.4 - 1.95 arbitrary units.

Interpretation of the RAPD fingerprints patterns

Two different synthetic primers, CA1 and CA2, were used as primers to generate RAPD products from DNA of *C. albicans* strains. Our results showed that each isolate generated different fingerprints in number and size of bands for a given primer. The RAPD patterns of *C. albicans* isolates with the two primers (CA1 and CA2) are shown in Figures 1A and B. When profiles yielded by CA1 and CA2 primers were combined, 13 *C. albicans* genotypes were generated at 75% of similarity and separated into two clusters: Cluster I and II. The first cluster consisted of a single strain (strain 11), whereas, the cluster II was divided into two; groups A and B (Figure 2). No association between genotypes obtained using RAPD technique, and virulence properties of tested *C. albicans* strains have been established. In fact, only one *C. albicans* isolate (strain 11) harbour several virulence related properties including phospholipase, aspartyl-protease and haemolytic factor such as other oral strains (3^1, 4, 10 and 15_B) grouped in the second cluster.

The number of bands obtained with CA1 primer (8 oligonucleotides) ranged between 11 and 17 for oral *C. albicans* strains (Figure 1A). The CA2 primer (10 oligonucleotides) produced a higher number of bands: 16 - 20 bands for oral *C. albicans* strains (Figure 1B). A dendrogram generated by *C. albicans* strains amplified by CA1 and CA2 primers is shown in Figure 2.

Results also showed that some oral *C. albicans* strains (16 and 17) isolated at the same period (April 2007) showed similar RAPD patterns and clustered in group B (Figure 1A).

A predominant species specific 1.5-kb band was demonstrated in most *C. albicans* isolates and also in *C. albicans* ATCC 90028 (R_1). Additional minor bands represented subfamilies of related patterns for some isolates (strains 3', 65, 16 and 17).

A few RAPD products were polymorphic, such as the example of the 2.2-kb fragment generated by the *C. albicans* strain 17 (cluster II, group A) when it was amplified with CA1 primer and 550 bases fragment for CA2 oligonucleotide.

Adherence to Hep-2 and Caco-2 epithelial cells

The *C. albicans* strains were able to adhere to the two cell lines used in this study with different degrees: 88% to Caco-2 cells and 64% to Hep-2 cells (Table 1). However, a medium (10%) and weak (23%) adhesion was detected

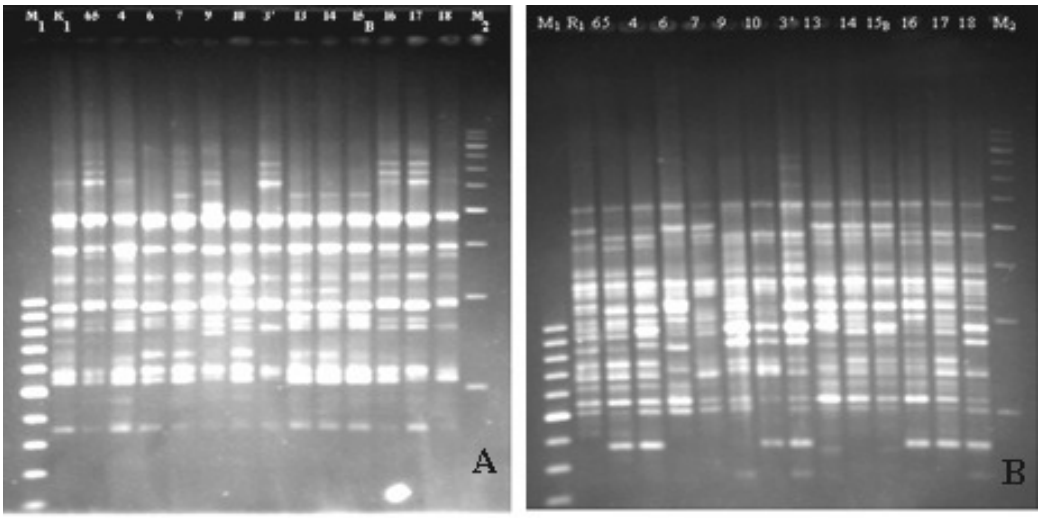

Figure 1: RAPD DNA fingerprints of C. *albicans* isolates: A, CA1 primer; B, CA2 primer. Lane M1 and M2 represent 100-pb and 500-pb molecular size markers respectively.

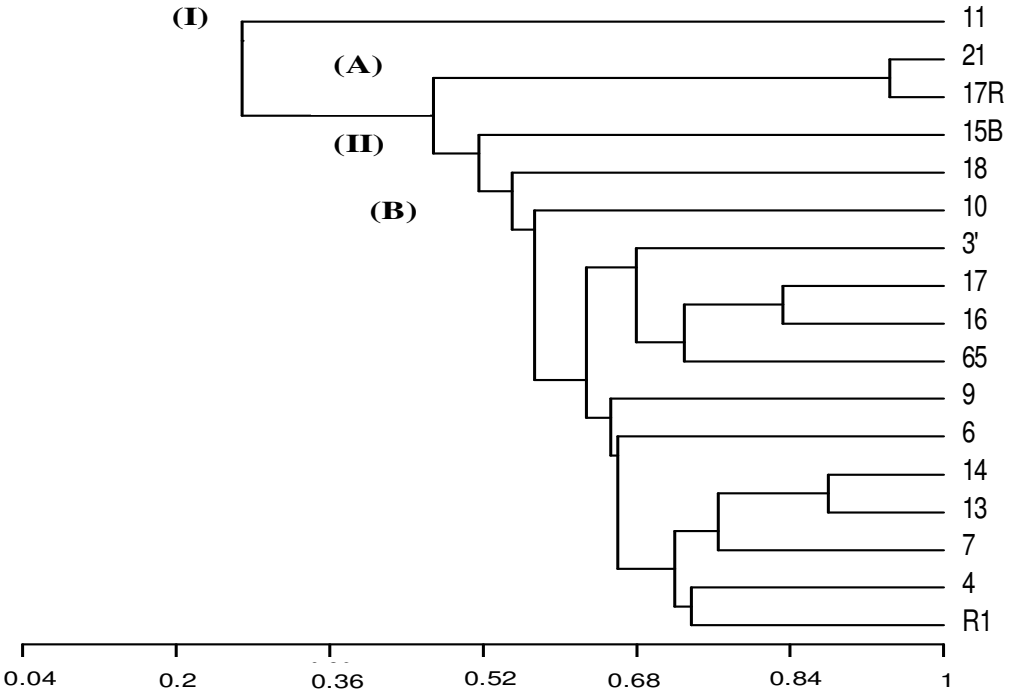

Figure 2. Dendrogram of genetic relatedness of all *C. albicans* isolates. The dendrogram was generated after RAPD amplification with CA1 and CA2 primers.

in Caco-2 cells of four and three *C. albicans* isolates, respectively. Six (6) strains (35%) were unable to adhere to Hep-2 cells and two (11%) to Caco-2 cells. Eleven (11) strains (64%) were able to adhere strongly to Hep-2 cells and eight (8) (47%) to Caco-2 cells. Seven *C. albicans* strains (strains 3^1, 10, 14, 16, 17, 21 and 65) isolated from oral cavity of patients suffering from denture stomatitis were adhered strongly to the two cell lines used in the present work (Figure 3). All these data are summarized in Table 1.

DISCUSSION

This study represents the first attempt to study the phenotypic and genetic diversity of oral *C. albicans* strains

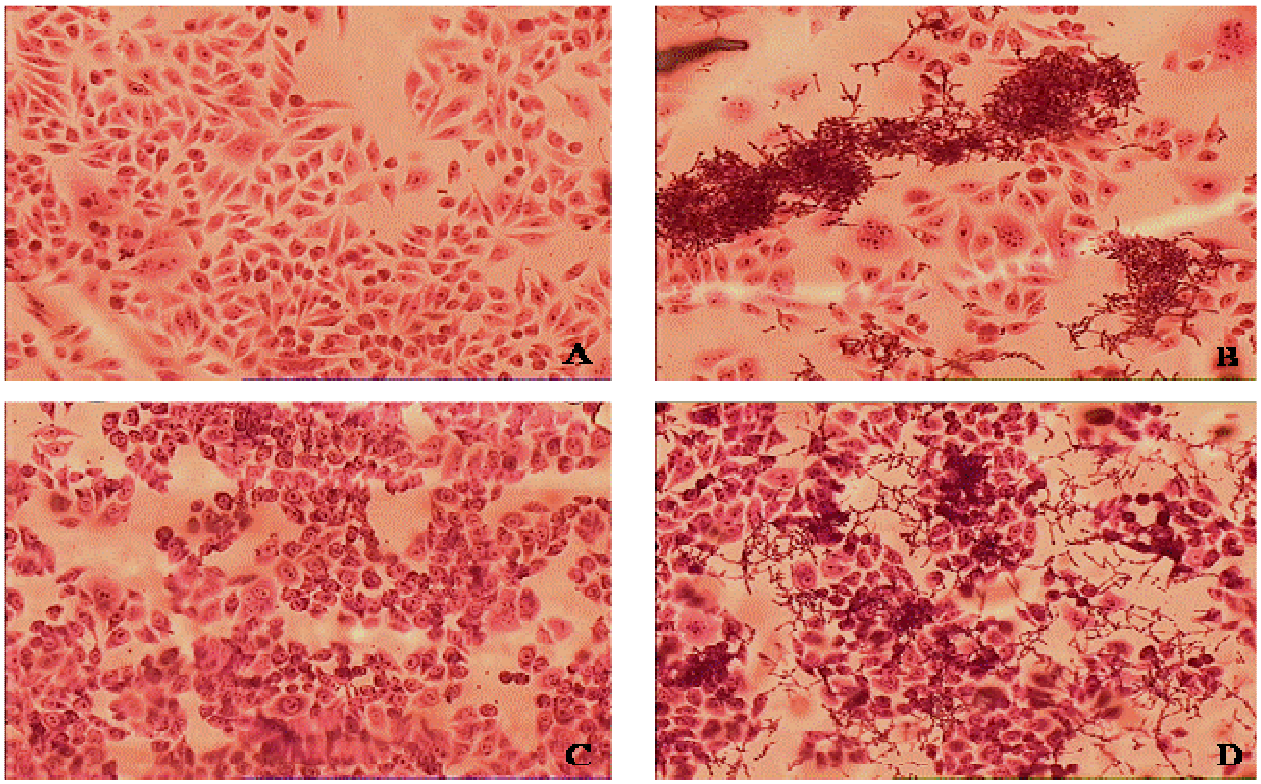

Figure 3. Optic microscopy of *C. albicans* (strain n° 16) showing a strong adherence to Hep-2 and Caco-2 epithelial cells. A and C: Negative controls, B and D: adhesion to Hep-2 and Caco-2 epithelial cells, respectively. The adherence assay was performed as described in the text. Giemsa stain: magnification ×10.

associated with oral candidiasis and isolated from patients frequenting the Dental Hospital in Monastir (Tunisia) between 2006 and 2007. All the patients involved in this study demonstrated symptoms (erythematous, pseudo-membranous, and denture stomatitis) that are characteristics of oral candidiasis. This oral candidiasis was often associated with diabetes, smoking, prosthesis and poor oral hygiene.

C. albicans is still the most commonly involved species. On the other hand, *C. albicans* is frequently isolated from stomatitis in denture wearing; phenotypic and DNA fingerprinting methods can be applied for epidemiological studies (Soll, 2000).

In this study, the phenotypic, genetic and epide-miological relationships among the *C. albicans* isolates were investigated. Biochemical charac-terization of the isolates using ID 32 C strips showed that there were biochemically hetero-geneous. All *C. albicans* strains were genetically confirmed at the species level as they amplify a 310-pb fragment (Baquero et al., 2002).

Fifty two percent (52%) of *C. albicans* isolates (9 isolates) in this study demonstrated phosph-olipase activity. Oliveira et al. (1998) and Samaranayake et al. (1984) also reported similarly high percentage (100 and 79%, respectively) of phospholipase production among oral *C. albicans* isolates at pH ranges of 3.6 and 4.7. It was suggested that phospholipases produced by *Candida* spp. mediate adhesion to host cells Kuhn et al., (2002).

In this study, 35% of all the *C. albicans* isolates obtained were proteinase producers, whereas, Candido et al. (2000) reported that 86.7% of yeasts isolated from oral lesions were protease producers. These proteinases are only active at low pH of 3.0 - 5.5 (Cassone et al., 1987).

Our results showed that all *C. albicans* strains produced haemolysins which are thought to play a key role in virulence of pathogens that enables their survival and infection within the human host (Manns et al., 1994; Stehr et al., 2003).

In this study, the genotypic variability of the isolates was determined in the present study using RAPD molecular system. Over the last decade, molecular biology-based methods for DNA typing have become routinely used because of the relative ease and rapidity of performing the analyses. Other molecular methods that can be used include; electrophoretic karyotyping, sout-hern blot hybridization, restriction fragment length polymorphism (RFLP) analysis and RAPD (Bostock et al., 1993; Del Castillo et al., 1997; Pujol et al., 1997). Besides, molecular techniques such as RAPD analysis are well recognized as tools that are useful in tracing the routes of transmission of different microorganisms,

including *Candida* species (Gupta et al., 2004). RAPD is less time consuming and easy to apply, and the results have demonstrated the high discriminatory power and typing efficacy of this molecular technique (Robert et al., 1995; Soll, 2000).

Thus, analyses of RAPD profiles of 17 *C. albicans* isolates obtained with primers CA1 and CA2, which were analysed by convenient statistical tools (Figure 1), permit a correct discrimination between the different isolates and allowed us to establish a possible epidemiologic relationship. Adhesive potency of *C. albicans* isolates to epithetlial cells was determined because adhesion of *Candida* cells is an initial event in the development of candidiasis and pathogenesis (Cannon and Chaffin, 1999; Bulad et al., 2004; Biswas and Chaffin, 2005).

C. albicans adheres to several host cell types, such as oral epithelial cells and cultured epithelial cell lines (Bulad et al., 2004; Cannon et al., 1995). Previous studies using cell lines have mostly used cells (such as HeLa, Hep-2 and Caco-2) that are not of oral origin. In this study, *C. albicans* isolates were able to adhere to the two epithelial cell lines with varying degrees (Caco-2 cells, 88%; Hep-2 cells, 64%). Holmes et al. (2002) in their studies on the effects of saliva on human epithelial cell adherence also reported that *C. albicans* adhered to monolayers of all three epithelial cell lines (A549, Hep-2, and HET-1A).

Conclusion

In this study oral *C. albicans* strains exhibited several virulence related properties, and RAPD-PCR as a molecular tool was used to differentiate the isolates into various genotypes.

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REFERENCES

Aoki S, Ito-Kuwa S, Nakamura Y (1990). Comparative pathogenicity of wild-type strains and respiratory mutants of *Candida albicans* in mice. Zol. Bakt. 273: 332-343.

Ausubel FM, Brent R, Kingston RE (1996). Current protocols in molecular biology. New York, John Wiley and sons' 2: 13.11.1-13.13.8.

Baquero C, Montero M, Sentandreu R, Valentin E (2002). Identification of *Candida albicans* by polymerase chain reaction amplification of a *CaYST1* gene intron fragment. Rev. Iberoam. Micol. 19: 80-83.

Bektic J, Lell CP, Fuchs A (2001). HIV protease inhibitors attenuate adherence of *Candida albicans* to epithelial cells *in vitro*. FEMS Immunol. Med. Microbiol. 31: 65-71.

Bello MD, Gonzalez A, Barnabé C, Larrouy G (2002). First Characterization of *Candida albicans* by Random Amplified Polymorphic DNA Method in Nicaragua and Comparison of the Diagnosis Methods for Vaginal Candidiasis in Nicaraguan Women. Mem. Inst. Oswaldo. Cruz. 97(7): 985-989.

Biswas SK, Chaffin WL (2005). Anaerobic growth of *Candida albicans* does not support biofilm formation under similar conditions used for aerobic biofilm. Curr. Microbiol. 51:100-104.

Bostock A, Khattak MN, Matthews R (1993). Comparison of PCR fingerprinting, by random amplification of polymorphic DNA, with other molecular typing methods for *Candida albicans*. J. Gen. Microbiol. 139(9): 2179-2184.

Budtz-Jorgensen E, Stenderup A, Grabowski M (1975). An epidemiologic study of yeasts in elderly denture wearers. Community. Dent. Oral. Epidemiol. 3:115-9.

Bulad K, Taylor RL, Verran J (2004). Colonization and penetration of denture soft lining materials by *C. albicans*. Dent. Mat. 20: 167-175.

Calderone RA, Fonzi WA (2001). Virulence factors of *Candida albicans*. Trends. Microbiol. 9: 327-350.

Candido RC, Azevedo RVP, Komesu MC (2000). Enzimotipagem de espécies de *Candida* isoladas da cavidade bucal. Rev. Soc. Bras. Med. Trop. 33: 437-342.

Cannon RD, Chaffin WL (1999). Oral colonization by *Candida albicans*. Crit. Rev. Oral. Biol. Med. 10: 359-383.

Cannon RD, Holmes AR, Mason AB (1995). Oral *Candida*: clearance, colonization, or candidiasis? J. Dent. Res. 74: 1152-1161.

Cassone A, De Bernardis F, Mondello F (1987). Evidence for a correlation between proteinase secretion and vulvovaginal candidosis. J. Infect. Dis. 156: 777-783.

Chen KW, Lo HJ, Lin YH (2005). Comparison of four molecular typing methods to assess genetic relatedness of *Candida albicans* clinical isolates in Taiwan. J. Med. Microbiol. 54: 249-258.

Cotter G, Kavanagh K (2000). Adherence mechanisms of *Candida albicans*. Br. J. Biomed. Sci. 57: 241-249.

Del Castillo L, Bikandib J, Nieto A (1997). Comparison of morphotypic and genotypic methods for strain delination in *Candida*. Mycoses, 40: 445-450.

Del Castillo-Agudo L, Gavidia I, Pérez-Bermúdez P (1995). PEG Precipitation, a Required Step for PCR Amplification of DNA from Wild Plants of *Digitalis obscura* L. BioTechniques. 18(5): 766-768.

Felsenstein J (1995). PHYLIP (Phylogeny Inference Package) version 3.5. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.

Foulet F, Nicolas N, Eloy O (2005). Microsatellite marker analysis as a typing system for *Candida glabrata*. J. Clin. Microbiol. 43: 4574-4579.

Ghannoum M (2000). Potential role of phospholipases in virulence and fungal pathogenesis. Clin. Microbiol. Rev. 13: 122-143.

Gupta N, Haque A, Latif AA (2004). Epidemiology and molecular typing of *Candida* isolates from burn patients. Mycopathologia, 158: 397-405.

Holmes AR, Bandara BMK, Cannon RD (2002). Saliva promotes *Candida albicans* adherence to human epithelial cells. J. Dent. Res. 81(1): 28-32.

Hunhreys H (2004). Does molecular typing make any contribution to the care of patients with infection? Clin. Microbiol. Infect. 10: 269-271.

Ibrahim AS, Mirbod F, Filler SG (1995). Evidence implicating phospholipase as a virulence factor of *Candida albicans*. Infect. Immun. 63: 1993-1998.

Kuhn DM, Chandra J, Mukherjee PK, Ghannoum MA (2002). Comparison of biofilms formed by *Candida albicans* and *Candida parapsilosis* on bioprosthetic surfaces. Infection and immunity,;70(2):878-888.

Luo G, Samaranayake LP, Yau JY (2001). *Candida* species exhibit differential *in vitro* hemolytic activities. J. Clin. Microbiol. 39: 2971-2974.

Manns JM, Mosser DM, Buckley HR (1994). Production of a hemolytic factor by *Candida albicans*. Infect. Immun. 62: 5154-5156.

Neugnot V, Moulin G, Dubreucq E (2002). The lipase/acyltransferase from *Candida parapsilosis*: molecular cloning and characterization of purified recombinant enzymes. Eur. J. Biochem. 269: 1734-1745.

Nikawa H, Hamada T, Yamamoto T (1998). Denture plaque-past and recent concerns. J. Dent. 26: 299-304.

Oliveira EE, Silva SC, Soares AJ (1998). Killer toxin and enzyme production by *Candida albicans* isolated from buccal mucosa in-patients with cancer. Rev. Soc. Bras. Med. Trop. 31: 523-527.

Pei PC, Siti Rohani AH, Yee LL (2007). Genotyping and drug resistance

profile of *Candida* spp. In recurrent and one-off vaginitis, and high association of non-*albicans* species with non-pregnant status. Infect. Gen. Evol. 7: 449-456.
Price MF, Wilkinson ID, Gentry LO (1982). Plate method for detection of phospholipase activity in *Candida albicans*. Sabouraudia, 20: 7-14.
Pujol C, Joly S, Lockhart SR (1997). Parity among the random amplified polymorphic DNA method, multilocus enzyme electrophoresis and Southern blot hybridization with the moderately repetitive DNA probe Ca3 for fingerprinting *Candida albicans*. J. Clin. Microbiol. 35: 2348-2358.
Robert F, Lebreton F, Bougnoux ME (1995). Use of random amplified polymorphic DNA as a typing method for *Candida albicans* in epidemiological surveillance of a burn unit. J. Clin. Microbiol. 33: 2366-2371.
Samaranayake LP, Reaside JM, Macfarlane TW (1984). Factors affecting the phospholipase activity of *Candida* species *in vitro*. J. Med. Veter. Mycol. 22: 201-207.
Sneath PHA, Sokal RR (1973). Numerical Taxonomy, Freeman, San Francisco, CA.
Soll DR (2000). The ins and outs of DNA fingerprinting the infectious fungi. Clin. Microbiol. Rev. 13: 332-370.
Stehr F, Kretschmar M, Kroger C (2003). Microbial lipases as virulence factor. J. Mol. Catal. 22: 347-355.
Webb BC, Thomas CJ, Willcox MD (1998). *Candida*-associated denture stomatitis. Aetiology and management: a review. Part 2. Oral diseases caused by *Candida* species. Aust. Dent. J. 43: 160-166.