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Full Length Research Paper

Discrimination and numerical analysis of human pathogenic *Candida albicans* strains based on SDS-PAGE protein profiles

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In the present study, 21 *Candida albicans* strains were investigated using the commercial kit API 20C AUX system and the numerical analysis of whole-cell protein profiles. The results of the commercial kit confirmed that the all the strains belonged to *C. albicans* species. However, the research indicated that SDS-PAGE of polypeptides of whole-cell extracts can provide more valuable taxonomic information than conventional yeast test kits at the subspecies level. Despite the fact that *C. albicans* subtypes isolated from different anatomical sites had similar protein profiles, there were some distinctive protein bands. Numerical analysis of whole-cell protein profiles of all strains revealed 2 major clusters at similarity degrees of between 46.26 and 100%. Moreover, the results of numerical analysis confirmed that each cluster had characteristic and distinctive protein profiles. The research showed that, the morphological examination of yeast isolates remains essential to obtaining a correct identification, both the commercial yeast kit system and the numerical analysis of whole-cell protein patterns can be useful for the more reliable identification of *C. albicans* strains.

Key words: *Candida albicans*, numerical analysis, SDS-PAGE and whole-cell protein patterns.

INTRODUCTION

Candida albicans is the most common fungal opportunistic pathogen in humans which causes either septicemia or mucosal infection (Odds and Bernaerts, 1994). Human pathogenic *C. albicans* strains isolated from different clinical sources are increasingly responsible for hospital outbreaks and the hands of healthcare workers may be the predominant environmental source in many countries around the world (Bikandi et al., 1998; Osmanağaoğlu et al., 2000; de Brito Costa et al., 2003; Saunte et al., 2005). Moreover, the prevalence of pathogenic *C. albican*s strains has greatly increased with the introduction of broad-spectrum antibiotics, immunosuppressive corticosteroids, and antitumor agents (Pfaller et al., 1994; de Brito Costa et al., 2003).

Strains of *C. albicans* are typically identified by their ability to form germ tubes or chlamydospores under the appropriate condition (Hilmioglu et al., 2007). The conventional methods for the characterization and discrimination of *Candida* species are based on morphological, physiological and biochemical characteristics (Odds and Bernaerts, 1994; Ruiz-Herrera et al., 2006; Hilmioglu et al., 2007). Commercial identification systems such as Albicans-sure, API ID 32C, API 20C AUX, Rap ID Yeast plus and Bichro-Dubli Fumouze® have been developed to identify *C. albicans* and other yeast species (Crist et al., 1996; Verweij et al., 1999; Fidel et al., 1999; Saunte et al., 2005; Sahand et al., 2006). However, these methods can clearly lead to misclassification particularly at the species level or lower. Due to this fact, the development and use of new molecular methods for improving the identification and detection of yeasts and other microorganisms are advisable (Monod et al., 1990; Asakura et al., 1991; Berber et al., 2003; Pryce et al., 2006; Lopes et al., 2007; Linton et al., 2007). Protein electrophoresis has been of the great value for the delineation of fungi and numerous bacterial taxa. SDS electrophoresis in a discontinuous system is by far the most widely used electrophoretic technique in fungal systematic. This tech-

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Table 1. Samples of *C. albicans* collected from various body sites.

Origin	Body site	Sample code
Faculty of Medicine, Yüzüncü Yıl University, Van-Turkey	Vaginal secretion	CA-1, CA-2, CA-3, CA-4, CA-5, CA-6, CA-7, CA-8
	Genital (male)	CA-9, CA-10, CA-11, CA-12
	Oral cavity	CA-13, CA-14, CA-15, CA-16
	Wound	CA-17, CA-18, CA-19, CA-21
American Type Culture Collection	None	*C. albicans* ATCC 27541

nique showed high specificity in addition to the significant data for classification (Höfling et al., 2001; Rodrigues et al., 2004). In several cases, one-dimensional electrophenograms of whole-cell proteins and DNA-DNA hybridization data were described as having equal discriminatory capacities (Costas et al., 1993; Bikandi et al., 1998; Osmanoğaoğlu et al., 2000). Besides, protein profiles offer considerable potential for typing strains of clinical interest and for taxonomic purposes, especially for the level of species, subspecies and biotype (Blignant and Koch, 1992). Indeed, computer-aided numerical analysis of protein patterns of the yeast provides a valuable tool for identification of such microorganisms. The aim of this present study was to analyze the similarity levels of protein profiles among *C. albicans* strains isolated from some clinical patients in Yüzüncü Yıl University, Van Turkey.

MATERIALS AND METHODS

Collection, isolation and identification of *C. albicans* strains

In this study, a total of 21 strains, one reference (*C. albicans* ATCC 27541) and 20 human pathogenic *C. albicans* isolated from different clinical patients in the Departments of Microbiology and Clinical Microbiology, Faculty of Medicine, Yüzüncü Yıl University, Van (Turkey) were analyzed. Clinical specimens were collected from various body sites of patients using a sterile cotton swab (Table 1), inoculated onto Sabouraud's Dextrose Agar (BBL-USA) plates and incubated at 37ºC for 24 - 48 h The morphological characteristics (germ tube formation) of the isolates were examined with a microscope while the biochemical properties (carbohydrate assimilation patterns) were carried out using the commercial kit API 20C AUX system (bio-Merieux-France).

Preparation of whole-cell proteins extracts

A total of 11 stains (at least 1 strain was selected from each body sites) were propagated in duplicates for the preparation of the synchronous culture. For each synchronous culture, 100 µl was inoculated into 50 ml Sabouraud's Dextrose (SD) broth and incubated in a rotary incubator for 24 h (at 37°C, 150 rpm). Each sample was centrifuged for 5 min at 12.100 rpm and the pellet collected was resuspended in 200 µl of CelLytic™ B-II Cell Lysis/Extraction Reagent (Sigma). The suspension was incubated for 30 min at room temperature. Afterwards, the sample was again centrifuged and 80 µl from each sample was transferred into a new 1.5 ml Eppendorf tube. Then, 25 µl of SDS-sample buffer (0.06 M Tris-HCl, 2.5% glycerol, 0.5% SDS, 1.25% β-mercaptoethanol) was added and the whole mixture was vortexed to ensure good homogenization. The prepared samples were kept on a boiling water bath for 5 min and denatured proteins were stored at -70°C until required.

SDS-PAGE

Solubilized proteins were subjected to SDS-PAGE in gel slabs of 1 mm thickness (3.5 cm, 4% stacking and 15.5 cm, 12% resolving gels) as described by Laemmli (1970). Electrophoresis was performed with a discontinuous buffer system in a UVP Vertical Electrophoresis Unit (Cambridge, UK). The gel was run at 30 mA until the bromophenol blue marker had reached the bottom of the gel. Protein molecular masses were calculated on the basis of a comparison with a known standard (PageRuler™ Protein Ladder SDS-PAGE Standards, Fermentas, molecular weight range 10 - 200 kDa). After electrophoresis the gels were rinsed out for 20 min in an isopropanol-acetic acid-water (1:3:6) solution, then for 5 min in methanol-acetic acid-water (3:1:6) solution. The gels were stained for 6 h in 0.01% (w/v) Coomassie brilliant blue R-250, and destained in a methanol-acetic acid-water (3:1:6) mixture until protein bands became clearly visible.

Protein profile analysis

The gels were scanned via a high resolution scanner (HP 3500 C, Hewlett Packard Co.) and the molecular weight of each band was determined by one-dimensional analysis software (Lab Image Version 2.6, Halle, Germany). Data were coded as 0 (absent) and 1 (present). A hierarchical cluster analysis was performed using the average linkage method and correlation coefficient distance. The dendrogram, based on the whole-cell protein patterns of the test strains, was constructed by the program Minitab for Windows, version 14.20 (Minitab Inc. Pennsylvania, USA).

RESULTS

Every one of the isolates exhibited characteristic oval budding yeast cells, germ tube and clusters of blastospore and terminal chlamydospore on Sabouraud's Dextrose Agar medium. Further, examination with a commercial identification kit revealed that all the isolates were members of the *C. albicans* species.

The whole-cell protein profile of 11 human pathogenic *C. albicans* strains, obtained by one-dimensional denaturing gel electrophoresis is shown in Figure 1. The protein profiles of tested *C. albicans* strains were inspected visually and compared with each others. The figure revealed that the whole-cell protein patterns of each one

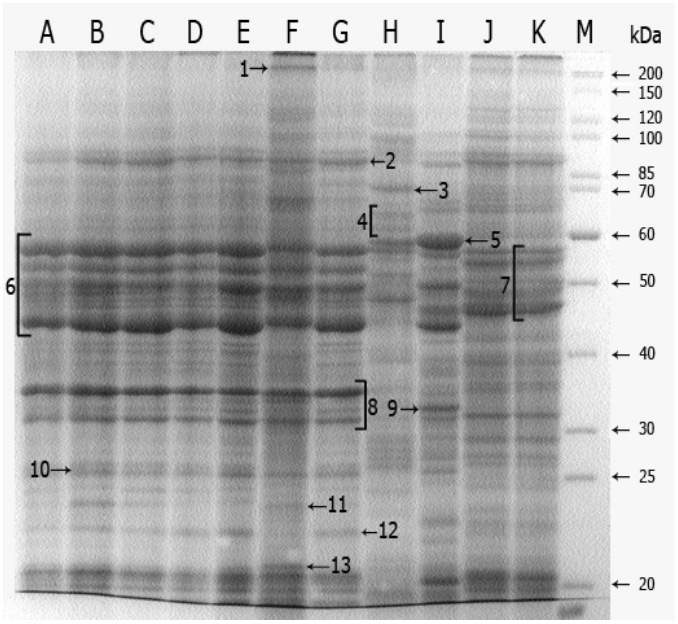

Figure 1. Coomassie brilliant blue-stained SDS-PAGE protein profiles of the following *C. albicans* strains: Lane **A**; CA-1, lane **B**, CA-2, lane **C**; CA-3, lane **D**; CA-6, lane **E**; CA-8, lane **F**; CA-10, lane **G**; CA-12, lane **H**; CA-14, lane **I**; *C. albicans* ATCC 27541, lane **J**; CA-18, lane **K**; CA-21 and lane **M**; molecular weight standards (10 - 200 kDa).

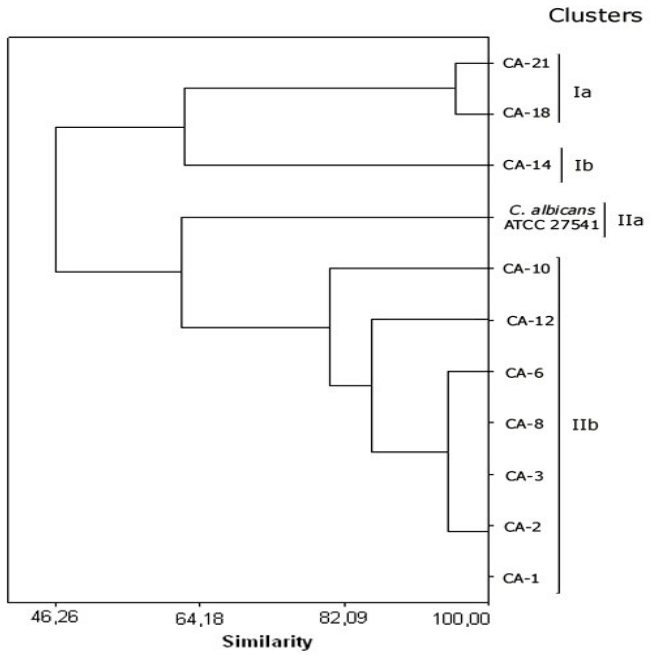

Figure 2. Grouping of *C. albicans* strains studied using hierarchical cluster analysis (average linkage and correlation coefficient distance) based on whole-cell protein profiles.

of the *C. albicans* strains had 13 major protein - bands. The tested strains had similar protein bands (molecular weights between 40 - 60 kDa). Moreover, the SDS-PAGE analyses indicated that there are major similarities between all *C. albicans* strains in their high-molecular-mass range (>40 kDa); however, the minor distinctive proteins were observed both in the low (<30 kDa) and high-molecular mass range (>60 kDa). Similarities in the profiles of all *C. albicans* strains were manifested by the existence of bands 2, 10, 12. In addition, the protein profiles of the strains of lanes A-G and I, isolated from the genitals were similar (presence of five protein bands marked 2, 6, 8, 10 and 12). But strain CA-10 (lane F) was distinguished from the other six strains by the presence of two single bands marked 11 and 13. Strain CA-14 (lane H) was particularly discernable from all the strains because of the presence of single band 3 and binary band 4. The reference strain (lane I) was very similar to strains isolated from the genitals but was discriminated by the existence of protein bands 5 and 9, respectively. Finally, strains of CA-18 (lane J) and CA-21 (lane K) isolated from wound were very similar to each other because of the presence of band 7.

The numerical analysis of the whole-cell protein profiles used for average linkage and correlation coefficient distance yielded a dendrogam, consisting of two basic clusters (I and II) at similarity levels between 46.2 and 100% (Figure 2). Cluster I divided into two subclusters (Ia and Ib) comprising of 3 *C. albicans* strains, numbered as CA-14, CA-18 and CA-21, at similarity degrees between 61.4 and 95.9%. Cluster II separated to two subclusters (IIa and IIb) including 8 strains (numbered as CA-1, CA-2, CA-3, CA-6, CA-8, CA-10, CA-12 and ATCC 27541). The intra-cluster average similarities for subclusters IIa and IIb changed between 61.3 and 100%. Subcluster IIb had seven strains, and its exhibited the highest similarity of protein profiles. The members of subcluster IIa were similar to each other, sharing many common bands as reflected in the high intra-cluster similarities (Figure 2).

DISCUSSION

In clinical microbiology laboratories yeast isolates that produce germ tube and chlamydospore are considered to be *C. albicans*, and no additional tests are performed. However, some researchers have stated that *C. dubliniensis* is difficult to distinguish from *C. albicans*, since both species produce germ tube and chlamydospore (Sullivan et al., 1995; Verweij et al., 1999; Sahand et al., 2006; Hilmioglu et al., 2007). Therefore, there is need for rapid commercially available identification kit systems for characterization of *Candida* isolates (Verweij et al., 1999; Osmanağaoğlu et al., 2000; Saunte et al., 2005; Abia-Bassey and Utsalo, 2006; Sahand et al., 2006). However, commercial identification systems fail to distinguish between germ tube positive and negative *Candida* species because of turbidity problems (Sullivan et al., 1995; Verweij et al., 1999; Saunte et al., 2005; Abia-Bassey and Utsalo, 2006). In this vein, our results con-

form to previous results and highlight the inadequacies of the kit presently used (Saunte et al., 2005; Abia-Bassey and Utsalo, 2006; Galan et al., 2006; Lopes et al., 2007; Linton et al., 2007).

In the present study, MINITAB program was used to analyze the data because of the difficulties in the visual interpretation of the bands obtained in SDS-PAGE of whole-cell proteins. The similarity values of the whole-cell protein patterns among *C. albicans* isolates in the dendrogram changed between 46 and 100%, and are in agreement with the minimum acceptable value proposed by Sneath and Johnson (1972). The results of numerical analysis confirmed that each cluster had characteristic and distinctive protein profiles. The members of subcluster IIb that were isolated from the genitals showed the highest similarity values (78.3 - 100%). Southern Blot hybridization analysis and DNA fingerprinting analysis studies also have shown that isolates recovered from one or among body site of the some patient are usually identical (Schmid et al., 1999; Rodrigues et al., 2004). Our results are therefore in agreement with previous studies (Monod et al., 1990; Osmanağaoğlu et al., 2000; Höfling et al., 2001; Rodrigues et al., 2004).

Molecular studies have demonstrated that *C. albicans* possesses a very distinct genomic organization from others emergent non-*C. albicans* species, such as *C. glabrata*, *C. krusei* and *C. dubliniensis* (Galan et al., 2006; Linton et al., 2007). Therefore, it suggested that the molecular techniques might be useful for specifically identifying pathogenic *Candida* species (Lopes et al., 2007; Linton et al., 2007). In conclusion, this study showed that the application of numerical analysis, coupled with the utilization of a standardized identification system instead of simple quantitative comparison of protein patterns, greatly enhanced the utilization of whole-cell protein profiles for identification of *C. albicans* strains.

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