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ORIGINAL ARTICLE

Protein profiling for the comparison of *Candida albicans* strains isolated from different clinical condition

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ABSTRACT
A study was under taken to identify the protein marker to differentiate the virulent strains of Candida albicans isolated from different clinical condition using SDS-PAGE. Whole-cell and extracellular protein profiles of C. albicans strains were analysed in study. Totally six C. albicans strains including oral, vaginal and NCIM strains were profiled. The whole-cell protein profile of different oral isolates of Candida albicans showed similarity in some extent. Interestingly, whole cell protein profile of vaginal isolate of HIV patient varied from non-HIV patient, however it had high degree of similarity with oral isolate of non-HIV patient. The extracellular protein secretion was varied among oral isolates as well as vaginal isolates. The extracellular protein profile oral isolates also showed diversity among strains of HIV, TB and non-HIV patients. In all isolates uniformity was observed in the secretion of 37 kDa protein but difference observed among the isolates with respect to proteins with 25 kDa and 46 kDa size.
Keywords: *Candida albicans, protein profiling, strains, oral isolates, novel chromogenic media.*

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INTRODUCTION

The incidence of life-threatening fungal infections has been increasing, particularly among patients who are immunocompromised by HIV infection, who are undergoing organ transplantation or chemotherapy for cancer [19]. *Candida* species are common human commensols that can cause a wide spectrum of disease that normally colonise mucosal surfaces or skin as harmless commensals and that are triggered to cause infection by changes in the host immune system or microflora [5]. Additionally, strains of the same species can differ in key characteristics depending on the host environmental status or immune condition [11]. The host environment and status of host defense system play significant role in the development of clinical candidiasis and expression of virulence genes in *C. albicans* [14]. The advent of new antifungal drugs has improved the prospects for management of these infections. However, diagnosis remains difficult, and early initiation of antifungal therapy is critical in reducing the high mortality rate in immunocompromised patients [7]. Instead, endogenous *C. albicans* are highly adopted and have specialized mechanisms for survival in host system. In some ways, it is surprising that *C. albicans* is uniquely associated with human or animals, as it have no specific nutrient requirements that would prevent it from surviving in the outside environment. Oral and vaginal infections with *C. albicans* are extremely common in even mildly immunocompromised individuals [13]. There are multiple methods have been used to characterize *C. albicans viz.,* physiological tests, rapid amplification of polymorphic DNA, restriction endonuclease digestion. However, there no specific method available for differentiate the virulent strain of *C. albicans* in clinical condition. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is used for comparing the protein patterns of *C. albicans* [10]. The

comparison of protein profiles is an approach worthy of differentiation of *Candida* strains with would be even more advantageous, if protein-profiles could be demonstrated to be associated with virulence characteristics. Since, most of the virulent enzymes of *Candida* were secreted out and are play a significant role in the pathogenesis, it is more appropriate to study the extracellular proteins in isolates of various clinical conditions using electrophoretic separation. In the present study, a attempt was made to identify the protein markers in *C. albicans* strains isolated from various host environment and that could be play a role in diagnosis of *Candida* infections.

MATERIAL AND METHODS

C. albicans strains

A *C. albicans* NCIM 3470 from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India, three oral *C. albicans* strains isolated from HIV, tuberculosis (TB) patient and non-HIV, two vaginal isolates from HIV patient as well as non-HIV patient. All clinical strains were isolated from patients in Tiruchirappalli, India. The strains were maintained on Sabouraud agar at 37°C. Clinical isolates used were undergone 2-3 subculture on Sabouraud agar.

Proteinases and phospholipases production

Production of proteinases from *Candida* isolates were evaluated by method of Abu-Elteen *et al.* [1]. *Candida* isolates were maintained in Sabouraud's medium prior to experimentation. Cells were harvested by centrifugation (5000 rpm for 10 min) and the cell density is adjusted to ≅ 2 x 10^6 per ml. Ten µl of the cell suspension was spotted on test medium (0.1% K_2HPO_4, 0.5% $MgSO_4$, 1% glucose, 1.5% bacteriological agar and pH was adjusted to 5.0) containing 0.16% bovine serum albumin as a sole nitrogen source. After four days incubation at 37°C, the plates were fixed with 20% trichloroacetic acid and stained with amidoblack (1.25% amidoblack dissolved in methanol containing 10% acetic acid). Then plates were destained with 15% acetic acid and the appearance of clear zone around each colony was measured. Assay was performed in triplicates and results were expressed in statistical mean value.

Phospholipase activity was tested by the method of Abu-Elteen *et al.* [1]. *Candida* isolates maintained in Sabouraud's medium were harvested by centrifugation (5000 rpm for 10 minutes), and cell density is adjusted to ≅ 2 x 10^6 per ml. Ten µl of the yeast suspension was placed on plates containing egg-yolk medium (6.5% SDA, 5.84% NaCl, 0.55% $CaCl_2$ and 8% egg-yolk) and the diameter of the zone of precipitation around each colony was measured after incubation of plates at 37°C for five days. Assay was performed in triplicates and results were expressed as statistical mean value.

Preparation of whole-cell proteins

A loopful of overnight grown from Sabouraud agar plate was suspended in 3 ml Sabouraud broth and incubated for 48 h (at 37°C) at 150 rpm. Cultures were then transferred into Eppendorf tubes and centrifuged for 5 min at 8000 rpm. The cell pellets were washed three times with sterile distilled water. The washed cells were resuspended in 50 µl SDS-sample buffer (0.06 M Tris, 25% glycerol, 0.5% SDS, 1.25% β-mercaptoethanol, and 0.001% bromophenol blue; pH 6.8), and the proteins were denatured in boiling water for 5 minutes. After the centrifugation for 5 min at 10000 rpm supernatant was collected in an Eppendorf tube and kept at -20°C until electrophoresis was carried out. Protein concentrations in the samples were determined by the Bradford method.

Extracellular proteins

Each *C. albicans* strain was grown in Sabouraud broth for 48 h at 37°C. To induce the secretion of extracellular proteins, one ml of washed *C. albicans* cell suspension was added to 20 ml of medium containing 2% glucose, 0.1% KH_2PO_4, 0.5% $MgSO_4$, 1X sterile-filtered minimum essential medium vitamins and the mixture was incubated for 5 days at 37°C in a shaker at 150 rpm preparation. Culture broth was centrifuged at 8000 rpm for 15 min to remove cells. Supernatant were filter-sterilized through 0.22 mm size membrane filter (Millipore Corporation, USA). Extracellular proteins in supernatant were precipitated using equal volume of acetone and keeping at -20°C for one hour. Then a pellet was washed with 95% ethanol. Protein concentration in the samples was determined by the Bradford method. Then, proteins were denatured in boiling water for 5 minutes after adding 50 µl SDS-sample buffer (0.06 M Tris, 25% glycerol, 0.5% SDS, 1.25% β-mercaptoethanol, and 0.001% bromophenol blue; pH 6.8).

SDS-PAGE for whole cell and extracellular proteins

One-dimensional SDS-PAGE was performed using a modification of the technique of Lemmli *et al.* [12]. Samples were loaded onto a 12.5% (w/v) polyacrylamide gel in a discontinuous buffer system and subjected to electrophoresis at 50 V for 24 h using BioRad electrophoretic unit (USA). The reservoir buffer consisted of 0.25 M Tris-HCl, 1.92 M glycine and 1% (w/v) SDS (pH 8.0). The gel was silver stained by the method described by Sambrook and Rusell (2001). Protein standards used for estimation of

molecular weight are bovine albumin, 66,000 Da; egg albumin, 45,000 Da; glyceraldehyde-3-phosphate dehydrogenase, 36,000 Da; bovine carbonic anhydrase, 29,000 Da; bovine pancreas trypsinogen, 24,000 Da; soybean trypsin inhibitor, 20,000 Da and bovine milk α-lactalbumin, 14,200 Da (Sigma, USA).

Confirmation of *C. albicans* strains by PCR

Isolation of genomic DNA from *C. albicans*

Genomic DNA from the *C. albicans* strains was extracted by the modified method of Sambrok and Russell (2001). Briefly, *C. albicans* isolates were cultured on Sabouraud dextrose agar for 48 h at 30°C. Few colonies were picked from the plates and suspended in sterile water. It was washed briefly in sterile distilled water. 500 µl of cold lysis buffer [containing 2% (v/v) Triton X-100, 1% (w/v) SDS, 100 mM NaCl, 10 mM Tris–HCl pH 8.0 and 1 mM EDTA] was added to the cells collected by centrifugation at 7000 rpm for five minutes and were mixed. The cell suspension was treated with lysozyme (10 µl of 10 mg/ ml) for 10 minutes at 37°C, then with proteinase K (3 µl of 10mg/ ml) for one hour at 50 ºC. Further, 200 µl of potassium acetate and 500 µl of buffered phenol: chloroform: isoamyl alcohol (25:24:1 v/v/v) was added and vortexed for 20 sec at maximum speed. After centrifuging for 10 minutes at 9000 rpm at 4°C, the upper phase was pipetted in to one ml of ice cold isopropanol and mixed gently to precipitate the DNA. The precipitated nucleic acids were collected by centrifugation and washed with 70% ethanol to remove proteins. The pellet was redissolved in 50 µl TE buffer and 3 µl RNAse (10 µl/ml) was added and then it was incubated at 37°C for five min. The concentration of DNA was estimated using UV-visible spectrophotometer (Jasco, Japan) at wavelength of 260 nm.

Primer

The *DAP2* gene reported as key molecular marker useful for differentiation of *C. albicans* species from the other species including *C. dubliniensis* [4]. In the present study forward primer 3' GGC CAAA GGA ATT GAC TGG TA 5' and reverse primer 3' CTT TAAA CAC CGT CGG CAA AT 5' were used to amplify the *DAP2* gene. Oligonucleotide primers for polymerase chain reactions (PCR) were obtained from Alpha DNA (Guebec, USA).

Polymarase chain reaction

The DNA extracted from each *Candida* strain was subjected to PCR amplification. The PCR was performed in a reaction volume of 50 µl containing 0.2 mM of deoxynucleoside triphosphate mix (dATP, dTTP, dGTP and dCTP), buffer mix containing (50 mM KCl, 10mM $MgCl_2$, 25 mM Tris-HCl pH 9, 0.1% Triton X-100), 25 pM of each primer, 2.5 U of *Taq* DNA Polymerase (Promega, USA) and 20 ng of Candidal DNA. Reaction mixtures were subjected to 35 cycles following the denaturation at 94°C for one min, primer annealing at 58°C for one min, extension at 72°C for one min and elongation at 72°C for 10 min using automated thermal cycler (Eppedorff, Germany). The size and purity of the PCR products were tested by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide.

RESULTS

All *C. albicans* strains used in this study were initially identified by conventional tests. The proteinase and phaspholipases are key enzymes involved in pathogenesis of *C. albicans*. The activity of proteinases and phospholipases has been investigated from the six strains of *C. albicans* strains. Oral isolates isolated from HIV and tuberculosis patients showed proteinases production and activity was 22 and 19 mm (dia) respectively, one vaginal isolates of *C. albicans* from HIV patient also showed proteinases production [20 mm (dia)]. Production of phospholipases was observed in oral (11 mm dia) and vaginal (12 mm dia) isolates of HIV patients. *C. albicans* NCIM 3470 was positive for both proteinases (20 mm dia) and phospholipases (10 mm dia) All the six strains of *C. albicans* used in this study were confirmed by molecular method using *C. albicans* specific primers. The dipeptidyl aminopeptidase (*DAP2*) is key enzymes involved in brake down of peptides and play a role supply in nitrogen source to the *C. albicans*. PCR amplification of *DAP2* gene resulted in the 227-bp product in all six species (Fig. 1) suggesting the all strains belong to *C. albicans.* The whole-cell protein profiles were used to distinguish between *Candida* strains obtained from different clinical origin. Proteins were extracted as described Lemmli *et al*. [12]. SDS-PAGE revealed the clearly distinguishable protein banding patterns of the different clinical isolates (Fig. 2). Whole-cell proteins profile among oral and vaginal isolates of *C*. *albicans* shows interesting results, high degree similarity was observed between NCIM and oral isolates, but not with vaginal isolates (Fig. 3). Interestingly, all isolates of *C. albicans* isolated from oral cavity lacking the protein of 25, 26 and 27 kDa. The oral isolate of *C. albicans* from non-HIV patient showed additional 23.6 kDa and 43.5 kDa proteins, but are not present in the other oral isolates. Protein profiles of the vaginal isolates of *C. albicans* were differed from the oral isolates. There was considerable difference between the whole-cell protein profile of vaginal isolate of HIV and non-HIV patient's isolates also recorded. The HIV patient isolates possess additional 26 kDa protein, but it was lacking in isolates of non-HIV patients. Variability in

extracellular protein profile of was detected among oral isolates of HIV, TB and non-HIV patients, however there was similarity in the extracellular protein profile was recorded among the NCIM strain, oral isolates of HIV and TB patients isolates. In case of isolate of TB patient, 46 kDa protein was missing and where as in oral isolate of non-HIV patient there was 26 kDa protein found instead of 25 kDa. The vaginal *Candida* isolates were also showed difference in extracellular proteins profile. 25 kDa extracellular protein was detected in isolate of HIV patients, but it was not present in the isolate of non-HIV patient.

DISCUSSION

In the present study analysis of proteinases and phospholipase in agar-based assay indicated that oral and vaginal isolates of HIV and TB showed proteinases production, but not in isolates of non-HIV patients. The phaspholipases production was observed only in the oral as well as vaginal isolates of HIV patients. It is well known that proteinases and phaspholipases are playing significant role in virulence of *Candida* [14, 8]. dos Santos and de Araujo Soares [6] reported that *Candida guilliermondii* isolated from HIV-infected human secretes a 50 kDa serine proteinase that cleaves a broad spectrum of proteinaceous substrates. Comparison of the protein patterns of *C. albicans* strains can be done in different clinical isolates using SDS-PAGE, which can be used to identify and differentiate the *C. albicans* strains with pathogenic potentials [17]. The technique also helpful comparison of the soluble whole-cell and extracellular protein pattern of the clinical isolates and it can be used to clarify ambiguous physiologic results [10]. In the current study attempt was made evaluating the protein-profiles of six strains of *C. albicans* from various clinical origin using SDS-PAGE to find out biochemical marker differentiate the strains isolated from different clinical conditions. The protein patterns of laboratory isolates and clinical isolates of *C. albicans* showed different degree of homology in the one-dimensional polyacrylamide gel electrophoresis. When samples of different *C. albicans* were run in parallel it reveals the similarity between the strains. The banding patterns of laboratory isolate and oral isolates of HIV, TB and non-HIV patients showed high degree of similarity in whole-cell protein profile, but not in extra-cellular proteins. The vaginal isolates were differing from oral isolates both in whole-cell and extracellular protein profile. This may be due to the variations in secretion or expression of cellular or extracellular proteins with pathological significance. In turns expression of these proteins depends on the host immune status or anatomic niche. However, all six strains used in this study belongs *C. albicans* as confirmed by molecular identification with *DAP2* gene marker. The primers used for *DAP2* gene specifically amplify the *C. albicans*, but not other species including *C. dubliniensis* [4,5]. It has been reported that expression of gene involved in pathogenesis greatly differ in *Candida* isolates of HIV patients than non-HIV patients as well as among isolates of different site of infection [15, 20]. *C. albicans* can colonize or infect virtually all body sites because of its high adaptability to different host niches, which involves the activation of appropriate sets of genes in response to complex environmental signals [20, 21]. This situation makes good sense for a commensal micro-organism that is able to generate a systemic infection only in situations in which host defense mechanisms are reduced. Therefore, not only factors depending on the micro-organism are involved but also strongly dependent on the host, such as tissue-specific differences in infection susceptibility [3]. As a consequence, virulence results from a delicate equilibrium that may be unbalanced towards the fungus, thereby generating spread and disease [16]. The ability to recognize and adhere to host tissues, to respond rapidly to changes in the external environment and to secrete hydrolases are all important in virulence of *Candida albicans*. These changes are often mediated by cell surface receptors that initiate signal transduction cascades resulting in altered activity of transcription factors and thus modification of gene expression. So that there may differently level of proteins expression in whole cell protein of an *C. albicans* strain and it can analysed by protein profiling. Ability to secrete enzymes that can break down barriers to growth and polymers to provide accessible nutrients as well as inactivate host defense molecules are prime candidate virulence determinants [9]. So that detection of differently expressed extracellular proteins in *C. albicans* in clinical setting has diagnostic significance. The genomic approaches such as proteomics are identifying potentially useful diagnostic markers. However, methodological standardisation and sophisticated clinical evaluations are as important for improving molecular diagnosis [21]. This electrophoresis method enables the identification and differentiation of *C. albicans* strains from clinical samples by comparing the protein profiles with those of known reference *C. albicans* strains from culture collections. The analysis of protein profiles was useful to distinguish between strains of various clinical origins may have different protein expression pattern. Identification of pathogenic strains using biochemical protein marker or virulent enzyme is more appropriate with respect to pathogenicity than the nucleic acid based method. Further, SDS-PAGE is useful as additional

technique differentiate virulent strains along with detection of other virulent determinant such as proteinases and phospholipases.

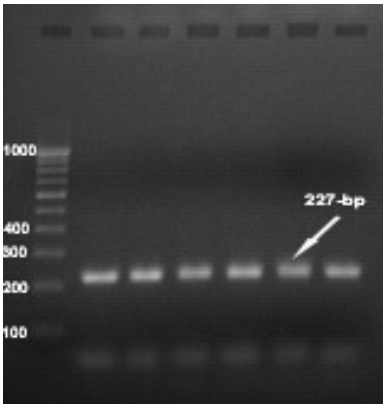

Figure 1. Gel electrophoresis of PCR products amplified with *DAP2* primers in six different *Candida* isolates. Lane M, molecular marker (100-bp DNA ladder); Lane 1 *C. albicans* NCIM 3074; Lane 2 *C. albicans* oral isolate from HIV patient; Lane 3 *C. albicans* oral isolate from tuberculosis patient (TB); Lane 4, *C. albicans* oral isolate from non-HIV and non-TB patient; Lane 5 *C. albicans* vaginal isolate from HIV patient; Lane 6 *C. albicans* vaginal isolate from non-HIV and non-TB patient.

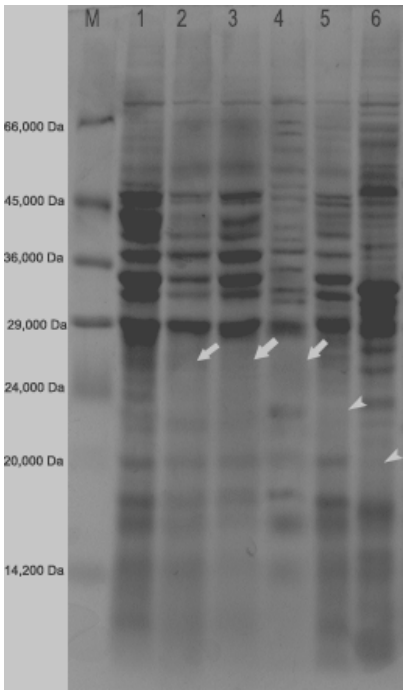

Figure 2. SDS polyacrylamide gel of whole cell protein profile of six different *C. albicans* isolates. Lane M, molecular marker (100-bp DNA ladder); Lane 1 *C. albicans* NCIM 3074; Lane 2 *C. albicans* oral isolate from HIV patient; Lane 3 *C. albicans* oral isolate from tuberculosis patient (TB); Lane 4, *C. albicans* oral isolate from non-HIV and non-TB patient; Lane 5 *C. albicans* vaginal isolate from HIV patient; Lane 6 *C. albicans* vaginal isolate from non-HIV and non-TB patient.

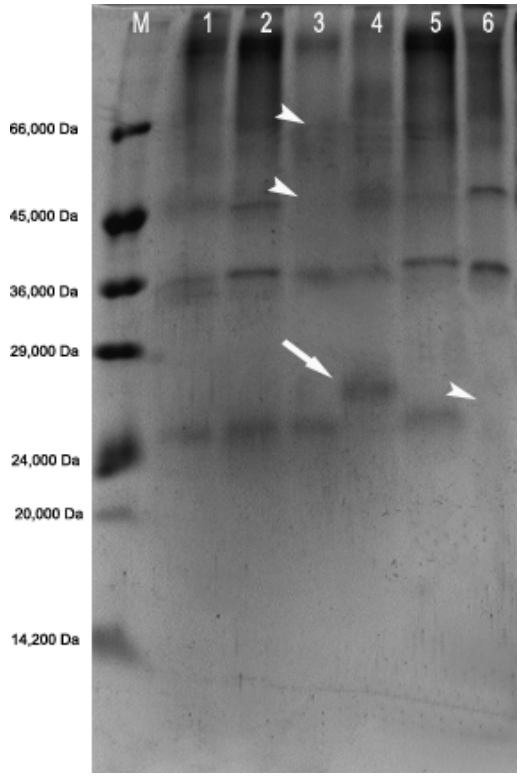

Figure 3. SDS polyacrylamide gel pattern of extracellular proteins of secreted in media. Lane M, molecular marker (100-bp DNA ladder); Lane 1 *C. albicans* NCIM 3074; Lane 2 *C. albicans* oral isolate from HIV patient; Lane 3 *C. albicans* oral isolate from tuberculosis patient (TB); Lane 4, *C. albicans* oral isolate from non-HIV and non-TB patient; Lane 5 *C. albicans* vaginal isolate from HIV patient; Lane 6 *C. albicans* vaginal isolate from non-HIV and non-TB patient.

REFERENCES

1. Al-Abeid HM, Abu-Etleen KH, Elkarmi AZ, Hamad M. (2001). Isolation and characterization of *Candida* spp. in Jordanian cancer patients: Prevalence, pathogenic determinants and antifungal sensitivity. Jpn J Infect Dis 57: 279-284.
2. Al-Rawi, N and Kavanagh K. (1998). A rapid method for the extraction of whole cell proteins from *Candida* species. Journal of Microbiological Methods 34:107–112.
3. Ashman, R.B., Fulurija, A. and Papadimitriou, J.M. (1996). Strain dependent differences in host response to Candida albicans infection in mice are related to organ susceptibility and infectious load. Infect. Immun. 64:1866-1869.
4. Bautista-Munoz C, Boldo XM, Villa-Tanaca L, Herna´ndez-Rodríguez C (2003). Identification of *Candida* spp. by randomly amplified polymorphic DNA analysis and differentiation between *Candida albicans* and *Candida dubliniensis* by direct PCR methods. *J Clin Microbiol* 41:414–420.
5. Calderone RA, (2001). In Fungal pathogenesis: principles and clinical applications. Edited by Calderone RA: Marcel Dekker Inc :3-13.
6. dos Santos A.L.S. and de Araujo Soares R.M. (2005). *Candida guilliermondii* isolated from HIV-infected human secretes a 50 kDa serine proteinase that cleaves a broad spectrum of proteinaceous substrates. FEMS Immunol Med Microbiol 43:13–20.
7. Fraser, V., M. Jones, J. Dunkel, et al. (1992). Candidemia in a tertiary care hospital: epidemiology, risk factors and predictors of mortality. Clin. Infect. Dis. 15:414–421.
8. Ghannoum, MA. (2000). Potential role of phospholipases in virulence and fungal pathogenesis. Clin Microbiol Rev 13:122–143.
9. Haynes, K. (2001). Virulence in *Candida* species. Trends in Microbiology 9:591-596.
10. Izquierdo P.M, Briones A.I. and Ubeda J.F. (1996). Relationship between the chromosomal profile and the whole-cell protein patterns of wine yeast strains. Lebensm.-Wiss. u.-Technol., 29, 738–742 .
11. Kurtzman, C. P., and C. J. Robnett. (1995). Molecular relationships among hyphal ascomycetous yeasts and yeastlike taxa. Can. J. Bot. 73:S824–S830.
12. Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-85.
13. Mendelson M. (2001). Fungal infection in immunocompromised patients. Microbiol Today. 28: 10-12.

14. Naglik J, Challacombe S. J. and Hube B. (2003). *Candida albicans* Secreted Aspartyl Proteinases in Virulence. Micro Mol Biol Rev 67: 400–428.
15. Naglik J, Albrecht A, Bader O, Hube B. (2004). *Candida albicans* proteinases and host/pathogen interactions. Cell Microbiol.6: 915-926.
16. Navarro-Garcia F, Sanchez M, Nombela C, Pla J, (2001). Virulence genes in the pathogenic yeast Candida albicans. FEMS Microbiology Reviews 25; 245-268.
17. Osmanauaoulu, Z., Altinlar N., Sailik S.C., Cokmus, C. and Akin, A. (2000). Identification of Different Candida Species Isolated in Various Hospitals in Ankara by Fungichrom Test Kit and Their differentiation by SDS-PAGE. Turk J Med Sci 30 2000 355-358.
18. Sambrook J and Russell DW. (2001). Molecular cloning: a laboratory manual. Vol. 3. Cold spring Harbor Laboratory press. New York.
19. Sobel JD. (2002). *Candida* infections in patients infected with HIV. Cur Treat Opt Infect Dis;4:101-107
20. Staib, P., Kretschmar, M., Nichterlein, T., Hof, H, and Morschha, J. (2000). Differential activation of a Candida albicans virulence gene family during infection. PNAS 97:6102–6107.
21. Weig M. and Brown, A.P.J. 2007. Genomics and the development of new diagnostics and anti-*Candida* drugs. *Trends in Microbiology* 15:310-317.
22. Yokoyama R., Kawasaki H., and Hirano H. (2006). Identification of yeast aspartyl aminopeptidase gene by purifying and characterizing its product from yeast cells. FEBS Journal 273:192–198.