Original Research
Medical Journal of Islamic Republic of Iran, Vol. 26, No. 2, May. 2012, pp. 45-49

Comparison of cell wall proteins in putative Candida albicans & Candida dubliniensis by using modified staining method & SDS-PAGE

Seyed Amir Yazdanparast[1], Seyedeh Shahrzad Mahdavi Nezarati[2]
Fariba Heshmati[3], Sepideh Hamzehlou[4]

Cellular & Molecular Research Center & School of Allied Health Sciences, Tehran University of Medical Sciences, Tehran, Iran.

Received: 26 October 2011 Revised: 21 September 2011 Accepted: 15 January 2012

Abstract

Background: *Candida* species are among the most common causes of opportunistic fungal diseases. Among *Candida* species, *Candida albicans* is responsible for most infections. Having many strains, *C. albicans* is very polymorph. *C. dubliniensis* is very similar to *albicans* species both morphologically and physiologically. For an infection to occur, cell wall proteins play an important role as they enable yeast to adhere to host cells and begin pathogenesis. Therefore, we decided to extract these proteins and examine them through common molecular methods of protein analysis including SDS-PAGE.

Methods: Initially cell wall proteins of two *C. albicans* strains (CBS 562 and PTCC6027) and one *C. dubliniensis* strain (CBS7987) were extracted by using a solution of beta-mercaptoethanol and ammonium carbonate. After dialysis against Tris-HCL buffer, SDS gel electrophoresis was performed on the proteins extract. Bands were then visualized by using three different staining methods among which one method provided improved detection.

Results: By using Coomassie Brilliant Blue staining method, proteins with molecular weight of 42, 66.2 and 200 kDa were detected. By using Silver staining method, proteins with molecular weight of 21.5, 28.5 and 37 kDa were detected. However, using combined Coomassie Brilliant Blue & Sliver staining method visualized more bands resulting in improved detection.

Conclusion: To answer many existing questions about fungal diseases, fungi cell wall proteins are necessary to be examined. To commence such examinations, a simple step may be an SDS-PAGE performance on as many strains as possible. A combined staining method can enhance bands detection.

Keywords: *Candida albicans*, *C. dubliniensis*, Protein extraction, SDS-PAGE.

Introduction

Candida species are discussed as one of the most common causes of opportunistic fungal diseases. Among *Candida* species, *Candida albicans* is responsible for most infections. *C.albicans* has many strains and therefore is a polymorph fungus. Another *Candida* species, *C.dubliniensis* which was initially isolated from oral cavity of HIV patients is very similar to *C.albicans* in respect of morphology and physiology [1]. Cell wall proteins play an important role in fungi pathogenesis as they enable fungi to

1. **(Corresponding author)** PhD, Associate Professor, Department of Medical Mycology, School of Allied Health Sciences, Tehran University of Medical Sciences, Shahid Hemmat Expressway, Shahid Chamran Crossway, Tehran, Iran. syazdanparast@tums.ac.ir
2. MSc, Department of Microbiology, Islamic Azad University, Tehran North Branch, shamim.mahdavi@yahoo.com
3. MSc, Department of Medical Microbiology, School of Allied Health Sciences, Tehran University of Medical Sciences, heshmati_99_1999@yahoo.com
4. MSc Student, Department of medical biotechnology, School of Allied Health Sciences, Tehran University of Medical Sciences, sepidy88@hotmail.com

adhere to host cells; an inevitable step in pathogenesis process. Among the fungi cell wall proteins are Vitronectin Binding Proteins, Fibronectin Binding Proteins, Laminin Binding Proteins and Entactin Binding Proteins. Heat shock protein is another example which plays an essential role in yeast survival [2, 3]. The Candida albicans cell wall proteins have been a recent matter of study in the evaluation of the yeast pathogenesis [4, 5, and 6]. Therefore, we decided to conduct a study concerning yeast cell wall proteins through common molecular analysis. Similar application of proteomic methods including different types of electrophoresis have been recently suggested for identification and diagnosis of Candida albicans [7, 8]. We used the technique SDS-PAGE which is abbreviated for Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis. SDS gel electrophoresis is a technique wildly used in molecular biology to separate proteins based on their molecular weights. This is feasible as SDS binds to proteins and gives them uniform negative charge along their lengths. Following electrophoresis, there is a staining step to visualize the bands; however, we used a modified staining method. Determination of the molecular weights is achieved by comparing the test lane and the marker lane of known sizes. Although similar studies have already been done in other parts of the globe, due to polymorphic nature of *C.albicans* more strains are needed to be examined to provide a reliable understanding.

Methods

Organism and Culture Growth

Initially yeast cells *C. albicans* strain CBS 562, *C. albicans* strain PTCC6027 and *C. dubliniensis* strain CBS 7987 were grown in Yeast Nitrogen Base (YNB) containing three amino acids of Tryptophan, Methionine and Histidine at 37 °C for 24 h. Then we subcultured the yeasts into YNB containing 0.3 M galactose, incubating overnight at 37 °C on a shaker with150 RPM. Finally yeast cells were grown in YNB containing 0.1 M glucose and 0.3 M galactose at 23-25 ° C for 24 h [9].

Protein Extraction

The culture media were centrifuged with 2000 RPM for 20 minutes. Then the supernatant of each media was discarded and the precipitation was rinsed twice with sterile distilled water. Then each precipitation was suspended in a protein extraction solution of 1.89 g/l ammonium carbonate and 1% beta-mercaptoethanol, incubated at 37 ° C for 30 minutes. After centrifugation supernatant was carefully collected using a 0.2 filter and the resulting extract was dialyzed against Tric – HCL buffer at 4° C for 24 h [10]. Quantifying the protein concentration of each sample was performed according to Bradford assay [11].

SDS-PAGE

SDS-PAGE was performed to determine molecular weight of cell wall proteins in studied *candida* species and strains. This method initially used by Laemmli in 1970[12] and secondly used by Casanova in 1989[10]. Our Separating gel was 12.5% w/v and the stacking gel was 4% w/v. Electrophoresis was done using a constant voltage of 65V.Three electrophoresis for each *candida* was done. Each lane contained 20 mg of protein extraction.

Staining

After electrophoresis, each of three series belonging to a *Candida* was stained separately according to the following methods: Silver Staining according to Chaffin et al. method [9], (Bio-Rad Laboratories, Hercules, CA), Coomassie Brilliant Blue staining according to Lee et al. method [13], (R-250, CBB; Sigma) and a combined method of Silver and Coomassie Blue staining according to Joseba and Chaffin observations [9]. The Bio-Rad Marker was used. The marker from top to bottom based on kDa, respectively included: Myosin molecular weight (200) beta-galactosidase (116), phosphorylase b (97.4), albumin bovine (66.2), ovalbumin (45), carbonic anhydrase (31) and trypsin

inhibitor (21.5).

Results

By using Coomassie Brilliant Blue staining method, the following proteins were observed: In *Candida albicans* strain PTCC6027, proteins with approximate molecular weight of 66.2kDa (Fig. 1-C), in *Candida albicans* strain 562CBS, proteins with approximate molecular weight of 42kDa (Figure 2-C) and in *Candida dubliniensis* strain CBS7987 high molecular weight proteins of 200kDa (Figure 3-B). By using Silver staining method, lower molecular weight proteins were observed: In *Candida albicans* strain 6027, proteins with molecular weight of 28.5kDa (Figure 1 - A), in *Candida albicans* strains 562, proteins with molecular weight of 37 kDa (Fig. 2-A) and in *Candida dubliniensis*, proteins with molecular weight of 21.5kDa (Fig. 3 – A/column 4, 5, 6, 7 and 8). By using combined Silver and Coomassie Brilliant Blue staining method, more bands were visible for each *Candida* including proteins with molecular weight of 80, 45 and 27 kDa in *C.albicans* strain 6027, (Fig. 1 - B), proteins with molecular weight of 35, 40 and 52 kDa in *C.albicans* strain 562 (figure 2 - B) and proteins with molecular weights of 35 and 45 and 97 kDa in *C. dubliniensis* (Fig. 3-A column, 1, 2&3). In fact, combined staining method provided a better detection of bands.

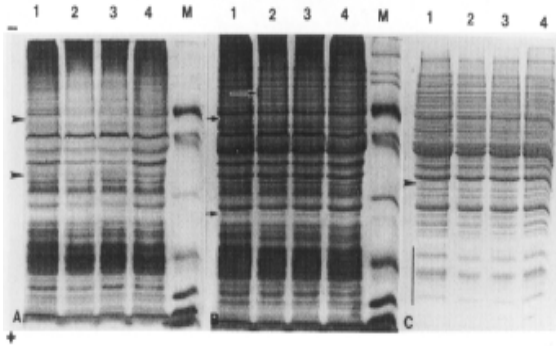

Fig. 1. Candida albicans strain PTCC 6027
(A) Silver stain, (B) Combined staining, (C) Coomassie Brilliant Blue
(A & C) The arrowheads showed weak staining bands by a single stain.
(B) The upper arrows indicate strong bands by double stain.

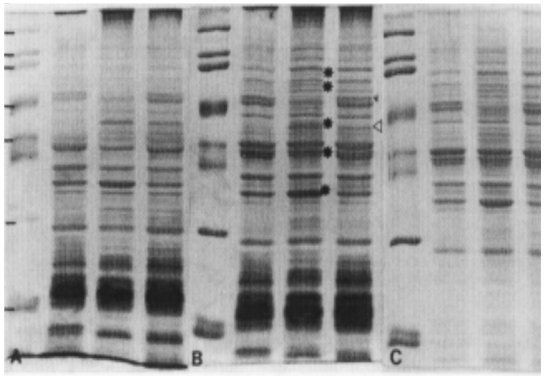

Fig. 2. *Candida albicans* strain CBS 562
(A) Silver stain, (B) Combined staining, (C) Coomassie Brilliant Blue Asterisks show improved detection of protein bands.

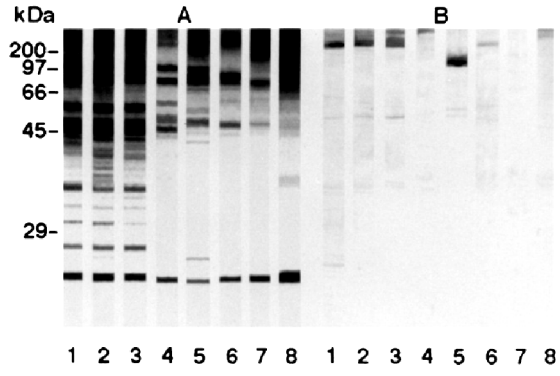

Fig. 3. *Candida Dubliniensis* strain CBS 7987
(A/column1, 2 &3) Combined staining, (A/4, 5,6, 7 & 8) Silver staining, (B) Coomassie Brilliant Blue

Discussion

According to the result of this study, by using Coomassie Brilliant Blue staining method proteins with molecular weight of 42, 66.2 & 200 kDa were displayed. This result partly resembles the result of Lee et al. work as they only visualized proteins with 42 & 43 kDa in *C.albicans* strain A3153 [13]. The two other proteins of 66.2 & 200 kDa were not observed in their result. This dissimilarity may be due to strain differences. To be clarified, examining more yeast seems to be necessary. By using Coomassie Brilliant Blue staining method, Casanova et al. observed proteins with molecular weight of 80-140 kDa in *C.albicans* strain ATCC26555 [14]. This observation resembles our result. Casanova et al. also observed that Silver staining method visual-

ized proteins with 21.5, 28.5 & 37 kDa. Chaffin et al. displayed proteins with 21.5-25 kDa in *C.albicans* strains ATCC44807 & NCPF3153 as they used Silver staining method [9]. Presence of proteins with molecular weight of 21.5 kDa is similar to the result of this study; however, proteins with 28.5 & 37 kDa were not present in Chaffin's result. These dissimilarities are probably due to strain varieties. By using combined staining method, we visualized proteins with molecular weight of 27, 35, 40, 45, 52, 80 & 97 kDa in *C.albicans* and *C.dubliniensis*. Our results mostly resemble to Joseba & Chaffin's results as they did not observe proteins of 27 & 97 kDa. Strain differences seem to be the reason. We observed that combined staining method provided better detection of proteins and more visualization of bands. Besides, this method enabled us to visualize the protein bands which stained weakly or not sharply in single stained methods [15, 16]. Clearly, such pattern of protein bands was detected as we used such combined staining method for cell wall proteins. Studying other proteins and using different staining methods might result in different patterns of bands.

In conclusion, to gain a profound understanding about fungi pathogenesis mechanism, examining their cell wall proteins is quite necessary. Examining more strains provides more reliable results. Examination method could be initially a simple gel electrophoresis and then completed with some specific immunoassays and immune-blottings. Further studies on cell wall proteins and serum of patients with candidiasis may increase our knowledge of pathogenesis mechanisms and facilitate vaccine production against fungi.

Acknowledgment

We would like to extend our appreciation to Research Vice-Chancellor of Tehran University of Medical Sciences. We would also like to thank all the colleagues in the Cellular & Molecular Research Center in School of Allied Health Sciences. Special thanks to Dr. Hamid Badali for providing us *Candida dubliniensis* strain from Amesterdam University.

References

1. James M, Toomey. Opportunistic Infections in AIDS. Jacksonville Medicine 1997: 325-328.

2. Moseley PL. Heat shock proteins and heat adaptation of the whole organism. J Apple Physiol 1997; 83:1413-17.

3. Boorstein WR, Ziegelhoffer T, Craig EA. Molecular evolution of the HSP70 multigene family. J Mol Evol 1994; 38:1-17.

4. Hashash R, Younes S, Bahnan W, El Koussa J, Maalouf K, Dimassi HI, et al.. Characterisation of Pga1, a putative Candida albicans cell wall protein necessary for proper adhesion and biofilm formation. Mycoses. 2011 Nov;54(6):491-500.

5. de Boer AD, de Groot PW, Weindl G, Schaller M, Riedel D, Diez-Orejas R, et al.. The Candida albicans cell wall protein Rhd3/Pga29 is abundant in the yeast form and contributes to virulence. Yeast. 2010 Aug;27(8):611-24.

6. Hayek P, Dib L, Yazbeck P, Beyrouthy B, Khalaf RA. Characterization of Hwp2, a Candida albicans putative GPI-anchored cell wall protein necessary for invasive growth. Microbiol Res. 2010 Mar 31;165(3):250-8.

7. Kniemeyer O, Schmidt AD, Vodisch M, Wartenberg D, Brakhage AA. Identification of virulence determinants of the human pathogenic fungi Aspergillus fumigatus and Candida albicans by proteomics. Int J Med Microbiol. 2011 Jun;301(5):368-77.

8. Koyama T, Makita M, Shibata N, Okawa Y. Influence of oxidative and osmotic stresses on the structure of the cell wall mannan of Candida albicans serotype A. Carbohydr Res. 2009 Nov 2;344(16):2195-200.

9. Chaffin WL, Lopez-Ribot JL, Casanova M, Gozalbo D, Martinez JP.Yeast cells C.albicans & YNB. Microbiol Mol Biol Rev. 1998; 62:130-180.

10. Casanova M, Chaffin WL. Protein Extraction in yeast cells. J Gen Microbiol.1991 ;137:1045-1051.

11. Bradford MM. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-254.

12. Laemmli. SDS-PAGE. Nature 1970; 227:680-685.

13. Leekl, Buckleyhr , Campbell CC . An amino acid liquid synthetic medium for development of mycelia and yeast forms of Candida albicans. Sabouraudia 1975; 13: 148-153.

14. Casanova M, Chaffin WL. Phosphate-containing proteins and glycoproteins of the cell wall of Candida albicans. Infect Immun1992; 59:808-813.

15. Dzandu JK, Deh ME, Barraatt DL, Wise GE. Combined staining method. Proceeding of the National Academy of Science 1984; USA; 1984; 81: 1733-1737.

16. De Moreno MR, Smith JF, Smith RV. Detection of proteins by staining methods. Anal Biochem1985; 151:466-470.