

ORIGINAL ARTICLE

Study on Somatic Protein Profile of Six Various Yeasts Isolated from Patients with Psoriasis

Mohadeseh Kamali¹, Mehdi Taheri Sarvtin*²

¹Dolatabad Hospital, Jiroft University of Medical Sciences, Jiroft, Iran

²Department of Medical Mycology and Parasitology, School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran.

Assistant professor of Medical mycology, Department of Medical Mycology and Parasitology, School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran

E-mail: mehditaheri.mt@gmail.com

ABSTRACT

Yeasts are microscopic, one-celled organism belonging to the fungi family. These fungi can exacerbate psoriasis via somatic protein antigens. Most studies have addressed only the role of somatic proteins of *Candida albicans* in psoriasis triggering. The aim of this study was evaluation of somatic protein profiles of six various yeasts isolated from psoriatic patients. Two isolates of *Candida albicans*, two isolates of *Candida parapsilosis*, two isolates of *Candida guilliermondii*, one strain of *Candida lipolytica*, one strain of *Candida tropicalis*, and as well as one strain of *Saccharomyces cerevisiae* were isolated from patients with psoriasis. These yeasts fungi were identified by using the sequence of the D1/D2 domain of the 26S rRNA gene. Somatic proteins of isolated yeasts were analysed using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis. Isolated yeasts had 56 different somatic protein bands, which ranged from 11 to >180 kDa. *Candida albicans*, *Candida parapsilosis*, *Candida guilliermondii* and *Candida lipolytica* had 12 protein bands, and *Candida tropicalis* and *Saccharomyces cerevisiae* had 7 protein bands and 8 protein bands, respectively. There was no significant difference in the number of somatic protein bands between *Candida albicans* and other yeasts ($P= 0.391$). The results of this study showed that different strains of the *Candida* genus have the same electrophoretic pattern of somatic proteins.

Keywords; *Candida albicans*, *Saccharomyces cerevisiae*, Somatic protein, Yeast

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INTRODUCTION

Psoriasis is an immune-mediated skin disorder that seems to be increasing in prevalence throughout the world [1]. This disease characterized by erythematous lesions covered with silvery scales and infiltration of leukocytes into both the dermis and the epidermis [2]. Although the exact etiology of psoriasis is still unknown, it is known that yeasts can play a significant role in exacerbation of psoriasis via stimulation of immune responses by their protein antigens [3]. Yeasts are eucaryotic single celled fungi which occur in two divisions of fungi [Ascomycotina and Basidiomycotina], 100 genera and 1500 species [4,5]. Among the yeast *Candida* spp., and *Saccharomyces cerevisiae*, that are found in human and environment, are more important in stimulating the immune system [4]. Somatic proteins are the most important antigens and allergens of yeasts that can provoke and exacerbate psoriasis by stimulating the inflammatory response of the immune system [3]. Most studies have examined only somatic proteins of *Candida albicans* and not other species [4-7]. The genus *Candida* comprised more than 150 species but only five species are regarded as frequent colonizer of skin and mucosal surfaces of patients with psoriasis [4,8]. So this study aimed to assess somatic proteins of five various species of *Candida* genus including: *Candida albicans*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida lipolytica*, *Candida tropicalis*, and as well as *Saccharomyces cerevisiae* isolated from patients with psoriasis.

MATERIAL AND METHODS

Yeasts isolation:

All yeasts were recovered from oral cavity of patients with psoriasis by swab and cultured on CHOROMagar Candida medium and incubated at 27°C for 4 days. Isolated yeasts were identified to the species level using sequence analysis of the D1/D2 domain of the 26S ribosomal RNA gene according to a previous study [9].

Somatic proteins extraction:

In this study two isolates of *Candida albicans*, two isolates of *Candida parapsilosis*, two isolates of *Candida guilliermondii*, one strain of *Candida lipolytica*, one strain of *Candida tropicalis*, and as well as one strain of *Saccharomyces cerevisiae* were examined. In order to extraction of somatic proteins, each of yeast was cultured on Sabouraud's dextrose agar containing chloramphenicol separately and then incubated for 72 h at 30°C. Yeasts grown were washed three times with 10 mM Phosphate buffered saline (PBS), pH 7.5 by centrifugation at 3000 rpm for 4 min. The yeasts were disrupted in PBS containing glass beads (diameter; 1 mm) for 5 min. After cell disruption, the crude extracts were separated from other cell components by centrifugation at 12000 rpm for 90 min at 4°C. Freeze drying was applied to concentrate the proteins. 40 ul of each extract was mixed with 10ul of 6x TDG buffer (0.2 M Tris-HCl [pH 6.8], 6% sodium dodecyl sulfate, 38% glycerol, 0.006% bromophenol blue) and 5 ul of 2-mercaptoethanol. The mixture was heated at 100°C for 6 min and used as sample for Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis technique.

Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis:

Extracted proteins were analysed using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to the procedure described in previous study [3]. briefly, 20 ul of each of samples was loaded on gel (12.5% separation gel and 4% stacking gel). Prestained Protein Ladder (CinnaGen Co, Iran) was used as a standard marker. Electrophoresis was performed at 150 V for 1 h. In the end of electrophoresis, the gels was stained with 0.1% coomassie brilliant blue R-250 (Sigma). The molecular weights of somatic proteins were estimated by logarithmic plot of migration of the standard marker.

RESULTS

In this study somatic proteins of two isolates of *Candida albicans*, two isolates of *Candida parapsilosis*, two isolates of *Candida guilliermondii*, one strain of *Candida lipolytica*, one strain of *Candida tropicalis*, and as well as one strain of *Saccharomyces cerevisiae* were assessed. The results from the Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis method showed that extracts obtained from various isolated yeasts had 56 different protein bands, which ranged from 11 to >180 kDa (Figure 1 and Table 1). Somatic protein electrophoretic pattern was identical in the similar isolates. *Candida albicans*, *Candida parapsilosis*, *Candida guilliermondii* and *Candida lipolytica* had 12 protein bands, and *Candida tropicalis* and *Saccharomyces cerevisiae* had 7 protein bands and 8 protein bands, respectively (P= 0.181). Somatic protein band with a molecular weight of >180 kDa was present only in *Candida lipolytica*. *Candida lipolytica* had the highest number of strong bands (7 strong bands). *Saccharomyces cerevisiae* had the highest number of weak bands (5 weak bands). Somatic protein band with a molecular weight of 11 kDa was detected in all yeasts.

Table 1. Somatic protein profile of yeasts isolated from patients with psoriasis

<i>Candida albicans</i>	Isolate A	93.3, 75.8, 70.8, 58.9, 44.4, 31.6, 20.8, 19.9, 18.2, 17.4, 12.6, 11 kDa
	Isolate B	93.3, 75.8, 70.8, 58.9, 44.4, 31.6, 20.8, 19.9, 18.2, 17.4, 12.6, 11 kDa
<i>Candida parapsilosis</i>	Isolate C	109.6, 63, 50.1, 44.6, 36.3, 31.6, 19, 16.6, 15.8, 14.8, 12.9, 11 kDa
	Isolate D	109.6, 63, 50.1, 44.6, 36.3, 31.6, 19, 16.6, 15.8, 14.8, 12.9, 11 kDa
<i>Candida guilliermondii</i>	Isolate E	74.1, 63, 50.1, 37.1, 26.3, 24.5, 20.8, 18.6, 18, 17.4, 12.6, 11 kDa
	Isolate F	74.1, 63, 50.1, 37.1, 26.3, 24.5, 20.8, 18.6, 18, 17.4, 12.6, 11 kDa
<i>Candida lipolytica</i>	Isolate G	>180, 79.4, 60.2, 53.7, 43.6, 30.9, 24.5, 23.9, 19.7, 15.1, 14.1, 11 kDa
<i>Candida tropicalis</i>	Isolate H	63, 39.8, 28.1, 20.4, 19, 17.8, 11 kDa
<i>Saccharomyces cerevisiae</i>	Isolate I	60.2, 53, 28.1, 19.9, 18, 16.5, 14.8, 11 kDa

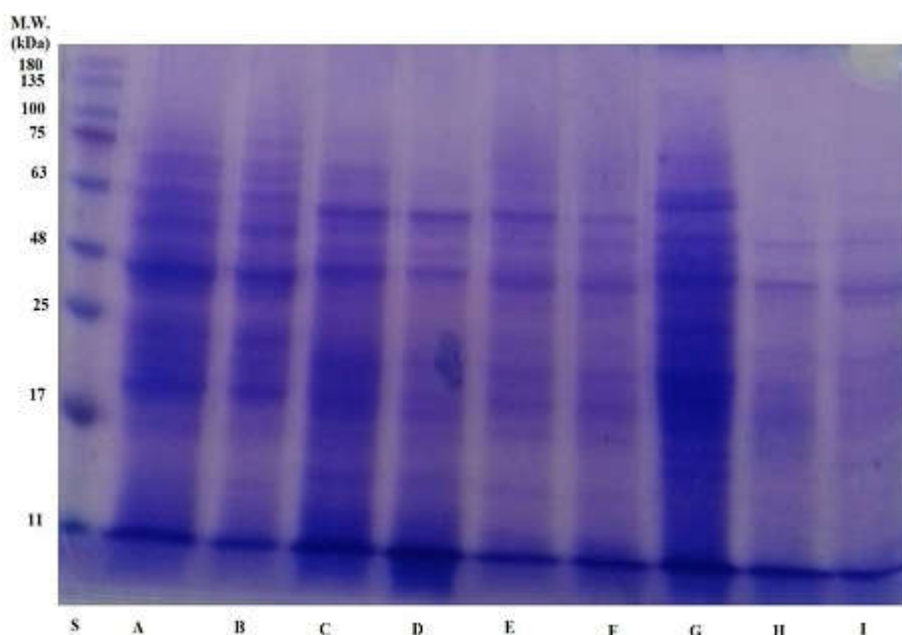


FIG.1. Electrophoretic patterns of somatic proteins of yeast on Sodium Dodecyl Sulphate-Polyacrylamide gel. Lane (s): Protein Ladder; lanes (A,B): *Candida albicans*; Lane (C,D): *Candida parapsilosis*; Lane (E,F): *Candida guilliermondii*; Lane (G):*Candida lipolytica*; Lane (H): *Candida tropicalis*; Lane (I): *Saccharomyces cerevisiae*.

DISCUSSION AND CONCLUSION

Candida species and *Saccharomyces cerevisiae* are the most important yeasts that can stimulate the immune system in patients with psoriasis by somatic protein antigens [4]. In this study, a total of 12 somatic protein bands within range of 11 to 93.3 kDa were detected in extract obtained from *Candida albicans*. In Ishiguro et al. [6] study, the crude extract obtained from *Candida albicans* showed 24 somatic protein bands with molecular masses ranging from 14 to 85 kDa. In Nissen et al. [7] study, a total of 11 protein bands within range of 16 to >200 kDa were identified in extract of *Candida albicans*. In Athenstaedt *et al.* [10] study, the extract of *Saccharomyces cerevisiae* showed 19 protein bands with molecular masses ranging from 33.9 to 71.7 kDa. In the current study, 8 protein bands with molecular weight range between 11 and 60.2 kDa were identified in extract of *Saccharomyces cerevisiae*. The differences in results of various studies might depend on differences in the calculation of the molecular weight and presence of several antigens with the same molecular weight. Most studies have addressed the role of *Candida albicans* in psoriasis triggering [4-7]. In this study, there was no significant difference in the number of somatic protein bands between *Candida albicans* and other yeasts ($P=0.391$). On the other hand, the number of strong bands in *Candida lipolytica* was more than *Candida albicans*. So, in psoriasis triggering, *Candida albicans* cannot be more important than other species. There are some ideas that somatic proteins are different in various strain of a species of the *Candida* genus [6,7,9]. In the current study, we investigated somatic proteins two isolates of *Candida albicans*, two isolates of *Candida parapsilosis* and two isolates of *Candida guilliermondii*. Protein electrophoretic pattern was identical in similar isolates. These results indicate that similar strains of the *Candida* genus have the same somatic proteins.

In conclusion, *Candida* species and *Saccharomyces cerevisiae* have various somatic proteins. In addition, different strains of the *Candida* genus have the same electrophoretic pattern of somatic proteins.

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