Debaryomyces hansenii colonization and its protein profile in psoriasis

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Background: Psoriasis is an immune mediated skin disorder which is mainly characterized by abnormal proliferation and differentiation of keratinocytes. It is believed that *Debaryomyces hansenii (Candida famata)* can colonize skin and mucous membranes of psoriatic patients and exacerbate psoriatic lesions via toxins, antigens, and proteins. The aims of this study were to evaluate *Debaryomyces hansenii* colonization and its protein profile in psoriatic patients.

Method: Fifty-one patients with psoriasis vulgaris and 51 healthy individuals were enrolled in the study. Skin and oral specimens from all participants were cultured on the CHROMagar Candida medium. Isolated yeast like fungi were identified using the sequence of the D1/D2 domain of the 26S rRNA gene. *Debaryomyces hansenii* proteins were analyzed using Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis.

Result: *Debaryomyces hansenii* was only isolated from the oral cavity of 7.84% of the patients. The extracts obtained from various *Debaryomyces hansenii* isolates had 45 protein bands, which ranged from 18 to >180 kDa. Secretory proteins were seen only in two isolates, which ranged from 35 to100 kDa.

Conclusion: *Debaryomyces hansenii* can colonize oral cavity of patients with psoriasis. In addition, various *Debaryomyces hansenii* isolates have different somatic proteins, which may have a role in provocation and exacerbation of psoriasis.

Keywords: candida, *Debaryomyces hansenii,* protein profile, psoriasis, yeast

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INTRODUCTION

Psoriasis is a common inflammatory skin disease characterized by red thickened plaques with a silvery scales [1]. It affects 0.6-4.8% of the world's population that mostly suffer from sexual dysfunction, malnutrition, anxiety, depression, and low self-esteem [1]. Although the exact etiology of psoriasis is still unknown, the evolving evidence indicates that various microorganisms such as viruses, bacteria, and fungi (yeasts) are associated with the provocation and exacerbation of psoriasis via production of either toxins or their metabolites [2]. *Debaryomyces hansenii (Candida famata)* is an osmo-, halo- and xerotolerant hemiascomycetous yeast commonly found in the dairy products and environments and is rarely isolated from humans [3]. This fungus can cause several disease including onychomycosis, vulvovaginitis, and candidemia in immunosuppressed patients [3,4]. In addition, it is believed that *Debaryomyces hansenii* can colonize skin and mucous membranes in patients with psoriasis and exacerbate psoriatic lesions [5,6]. To our knowledge, only a few studies have investigated colonization of *Debaryomyces hansenii* in patients with psoriasis [7-9]. Therefore, the first aim of this study was to investigate the colonization of *Debaryomyces hansenii* in patients with psoriasis.

Microbial agents can exacerbate psoriatic lesions via T cell incitement by their somatic proteins and the protein metabolites. So far, somatic and secretory proteins of this fungus have not been investigated. Thus, the second aim of this study was to investigate somatic and secretory proteins of *Debaryomyces hansenii.*

PATIENTS AND METHODS

Patients

This study included fifty one patients with plaque-type psoriasis and 51 healthy individuals as the control group. It was carried out from September 2013 to March 2014 in the Department of Medical Mycology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. All participants signed informed consent forms indicating their agreement to participation in the project. The study was approved by the ethical committee of Jiroft University of Medical Sciences, Jiroft, Iran. All patients enrolled in the study were diagnosed based on clinical examinations. People who had diabetes and those who had used broad spectrum antibiotics and steroids as well as pregnant patients were excluded from the study. The Psoriasis Area and Severity Index (PASI) was used to assess the clinical severity of psoriasis [10]. Based on this formula, the clinical severity of psoriasis was categorized as mild (PASI scores of < 11), moderate (PASI scores of 11–49), and severe (PASI scores of > 50).

Yeasts isolation

All samples were collected from the lesions of the oral cavity and skin by the swab and scalpel, respectively. All of the samples were cultured on the CHROMagar 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 the procedure of Kurtzman et al [11].

Identification of somatic and secretory proteins of *Debaryomyces hansenii*

Each *Debaryomyces hansenii* isolate was cultured in Sabouraud's dextrose broth containing 5g of glucose and 5g malt extract per liter and then incubated at 25ºC for 7 days and for 30 days for somatic and secretory proteins extraction respectively on a gyratory shaker (100 rpm). In order for somatic proteins extraction, the pellets were washed 3 times with 10 mM phosphate buffered saline (PBS), pH 7.5 by centrifugation at 3000 rpm for 5 min. The yeasts were disrupted in PBS containing glass beads (diameter; 1 mm) for 10 min. After cell disruption, the crude extracts were separated from other cell components by centrifugation at 13000 rpm for 90 min at 4 ºC. The supernatants were collected and stored at –20ºC until required. In order to collect secretory proteins, the supernatants were collected from 30-day cultures of yeasts by centrifuging at 4000 rpm for 5 min. Freeze drying was applied to concentrate the proteins. The extracted proteins were analysed using Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis according to the procedure of Laemmli [12].

RESULTS

In this study, 51 patients (19 males and 32 females; mean age 37.2 ± 13.79 years) and 51 controls (22 males and 29 females; mean age 38.9 ± 12.45 years) were examined. *Debaryomyces hansenii* only was isolated from the oral cavity of 4 (7.84%) patients but not in healthy people. PASI scores < 11 were found in 68.63% of the patients, PASI scores of 11-49 were reported in 19.61% of the patients,

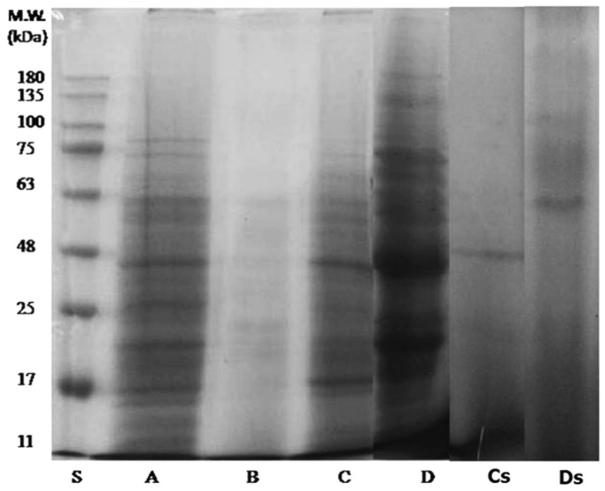

Figure 1. Electrophoretic patterns of somatic proteins of *Debaryomyces hansenii* on Sodium Dodecyl Sulphate-Polyacrylamide gel. Lane S: Protein Ladder; lanes A-D: somatic protein profile of *Debaryomyces hansenii* isolates; Cs, Ds: secretory protein profile of C and D isolates respectively.

Table 1. Protein profile of *Debaryomyces hansenii* isolated from patients with psoriasis

Isolate A	Somatic proteins	75.8, 70.7, 66, 63, 57.5, 53.7, 51.2, 46.7, 44.6, 38.9, 35.4, 33.8, 30.1, 28.1, 24.5, 22.9, 19.9, 18.6 kDa
	Secretory proteins	-
Isolate B	Somatic proteins	57.5, 44.6, 37.1, 32.3, 30.1, 28.1, 24.5, 22.9 kDa
	Secretory proteins	-
Isolate C	Somatic proteins	75.8, 70.7, 66, 57.5, 53.7, 51.2, 46.7, 44.6, 38.9, 37.1, 35.4, 33.1, 30.1, 28.1, 24.5, 22.9, 19.4, 18.1 kDa
	Secretory proteins	35 kDa
Isolate D	Somatic proteins	>180, 180, 151.3, 135, 125.8, 120.2, 109.6, 70.7, 64.5, 58.8, 53.7, 48.9, 43.6, 32.3, 29.5, 26.3, 23.4, 20.8, 19.9 kDa
	Secretory proteins	100, 75, 63 kDa

and PASI scores > 50 were seen in 11.76% of the patients. There was no significant relationship between the PASI score and *Debaryomyces hansenii* colonization (P = 0.16)

In the current study, results from the SDS-PAGE method indicated that extracts obtained from various *Debaryomyces hansenii* isolates had 45 protein bands, which ranged from 18 to >180 kDa (Figure 1 and Table 1). Secretory proteins were seen only in isolate C (35 kDa) and isolate D (63 kDa, 75kDa, 100 kDa). Isolate D had the maximum number of somatic protein bands (22 bands) ranging from 19.9 to >180 kDa and isolate D had the minimum somatic protein bands (5 bands) ranging from 30 to 120 kDa. Somatic protein bands with a molecular weight of >180 kDa were present only in isolate D. Common somatic protein bands including 70.7, 53.7, 44.6, 30.1, 28.1, 24.5 and 22.9 kDa were detected with a frequency of 75% in *Debaryomyces hansenii* isolates.

DISCUSSION

Debaryomyces hansenii is a hemiascomycetous yeast capable of secreting antigens and different metabolites including protease B, arginyl aminopeptidase, and toxin, which cause various systemic and skin diseases [13]. In the present study, 7.84% of the patients were colonized by *Debaryomyces hansenii.* This finding is incompatible with the results of studies by Leibovici et al [7] and Waldman et al [8] but was in accordance with repors of the studies by Bedair et al [6] and Taheri Sarvtin et al [10]. The differences in results of various studies might depend on the severity and duration of the disease. *Debaryomyces hansenii* can adapt itself to different pH and salinity of the environment through changes in the sterol to phospholipid ratio of the plasma membrane [13]. So, it seems that patients with psoriasis are more susceptible to colonization with *Debaryomyces hansenii* due to the abnormal conditions of the mucous. In addition, this fungus is able to form a biofilm on the oral mucosa tissue which contributes to the damage to the structure of the mucosal tissue [14]. Protein antigens of *Debaryomyces hansenii* can exacerbate psoriasis via the secretion of several proinflammatory cytokines (IL-1β, TNFα) and infiltration of leukocytes into both the dermis and the epidermis [14]. The results from the SDS-PAGE analysis indicated that extracts obtained from various *Debaryomyces hansenii* isolates contained 45 somatic protein bands with molecular weights between 18 and >180 kDa. *Debaryomyces hansenii* isolates had different somatic protein bands in number and molecular weights. Thus, various *Debaryomyces hansenii* strains can have different virulence in provocation and exacerbation of psoriatic lesions. In the present study, only 4 secretory protein bands were seen. Therefore, it does not seems that the secretory proteins of *Debaryomyces hansenii* have an important role in provocation and exacerbation of psoriasis. Additional research is also required to shed light on the role of each of the proteins in proinflammatory cytokines secretion, stimulation, and aggravation of psoriasis.

In conclusion, *Debaryomyces hansenii* can colonize the oral cavity of the patients with psoriasis. In addition, various *Debaryomyces hansenii* isolates have different somatic proteins, which may have a role in the provocation and exacerbation of psoriasis.

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