

1-1-2000

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OSMANAĞAOĞLU, ÖZLEM; SAÇILIK, SEFA CAN; GÜNDÜZ, UFUK; ALTINLAR, NURTEN; and ÇÖKMÜŞ, CUMHUR (2000) "Electrophoretic Analysis of *Candida albicans* Isolates Collected From Turkish Hospitals by Native-Polyacrylamide Gel Electrophoresis (N-PAGE)," *Turkish Journal of Biology*. Vol. 24: No. 4, Article 12. Available at: <https://journals.tubitak.gov.tr/biology/vol24/iss4/12>

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## Electrophoretic Analysis of *Candida albicans* Isolates Collected From Turkish Hospitals by Native-Polyacrylamide Gel Electrophoresis (N-PAGE)

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Received: 04.08.1999

**Abstract:** Native-polyacrylamide gel electrophoresis (N-PAGE) was used to characterize *Candida albicans* strains isolated from vaginal specimens of patients suffering from vaginitis in Ankara hospitals, Turkey. Three different protein band profiles were observed among these 38 *C. albicans* isolates when whole cell protein extracts of these strains were used in N-PAGE. 34 of the 38 isolates were shown to belong to a single group or clone with a dissemination percentage of 89, while three isolates belonged to another group with a percentage of 8. Furthermore only one *C. albicans* isolate was detected with a dissemination percentage of 3 and assigned to a third group. The results presented here also suggested that N-PAGE may be an efficacious method for differentiating between *C. albicans* strains.

### Türk Hastanelerinden Toplanmış Olan *Candida albicans* İzolatlarının Native-Poliakrilamid Jel Elektroforez ile Elektroforetik Analizi

**Özet:** Ankara'daki hastanelerde vajinit problemlili hastalardan toplanmış olan 38 adet *Candida albicans* suşu native-poliakrilamid jel elektroforez (N-PAGE) ile karakterize edilmiştir. *C. albicans* izolatlarının hücre protein ekstraktları N-PAGE'de kullanıldığı zaman üç değişik protein bant patternine rastlanmıştır. Bu çalışmanın sonucunda toplam 38 *C. albicans* izolatının 34'ünün yayılım oranı %89 olarak tespit edilen tek bir gruba veya klona ait olduğu belirlenmiştir. Diğer taraftan % 3'lük bir yayılım oranına sahip olan tek bir *C. albicans* suşu tespit edilip üçüncü bir gruba veya klona ait olduğu belirlenmiştir. Bu çalışma sonucu elde edilen veriler N-PAGE'nin *C. albicans* suşları arasında ayırım sağlayacak düzeyde etkili bir metod olabileceğini önermektedir.

### Introduction

Yeasts are by far the most common fungi isolated from human patients. Their incidence has greatly increased over the past several decades with the introduction of broad-spectrum antibiotics, immunosuppressive corticosteroids, and antitumor agents as well as an increasing

number of AIDS patients (1,2). Among them, the imperfect yeast *Candida albicans* and several related *Candida* species are of foremost importance as opportunistic pathogens in immunocompromised hosts, which may cause life threatening infections. *Candida albicans* is the yeast pathogen most frequently isolated from patients with vaginitis (3-5). Recently, an increase in other species, including *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis*, which are the cause of the opportunistic infection called oropharyngeal candidiasis (OPC), has been observed (4). *C. glabrata* has become a prominent pathogen in some institutions (6). It is therefore of the utmost importance to rapidly and reliably identify *C. albicans* as well as other *Candida* species in routine clinical microbiology practice (7). Routine identification of *C. albicans* in the clinical microbiology laboratory relies on a few criteria; mainly morphology, growth characteristics and carbon source assimilation or fermentation, as well as appearance on differential isolation media (8,9). A valuable and simple test for the rapid, presumptive identification of *C. albicans* is the germ tube (GT) test (10). This method, although simple, takes several hours and requires a microscope and a skilled microscopist familiar with mycology methods (11). It has also been demonstrated that MUREX *C. albicans* (MC) (Murex Diagnostics), Albicans-Sure (AS) (Clinical Standards Laboratories) and BactiCard *Candida* (BC) (Remel) test kits can also be used for the rapid and presumptive identification of *Candida albicans* (12). In addition, many different methods have been used for typing *Candida* to date. Serotyping, antibiogram and biotyping, multilocus enzyme electrophoresis, and the analysis of whole cell protein profiles in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) are some of the (13-16).

To our knowledge, there is no report on characterization of *Candida albicans* typing using N-PAGE of whole-cell proteins. The present study was undertaken to identify and characterize *Candida albicans* species isolated from vaginal specimens of patients suffering from vaginitis in Turkey, by native-polyacrylamide gel electrophoresis (N-PAGE).

## Materials and Methods

**Collection of specimens.** A total of 38 *Candida albicans* isolates were analyzed in this study. All of the isolates were cultured from specimens received in the microbiology and bacteriology laboratories of seven different hospitals in Ankara, Turkey. Specimens were collected from patients with vaginitis by passing a sterile cotton swab several times across the vaginal surface. Immediately after sampling, each swab was replaced in its sterile tube and transported within 30 minutes to the laboratory. All test individuals were sampled within a 4-week time period. All isolates were stored at -70°C in skim milk and then subcultured and grown for 24 to 48 h on Sabouraud dextrose media before testing.

**Isolation of *Candida* spp.** The cotton end of each swab was inserted into 0.5 ml of sterile water in a microcentrifuge tube, the tube was rigorously mixed for 30 with a laboratory tabletop vortex mixer and 0.15 ml of the wash was spread onto plates containing Sabouraud dextrose agar and the plates were incubated at 37°C for 48 h.

**Fungichrom test kit for identification of *Candida* spp.** The commercially available rapid enzymatic Fungichrom test kit (International Mycoplasma, Signes, France) was used according

to the manufacturer's instructions. The Fungichrom test detects the enzymes L-prolin-aminopeptidase,  $\beta$ -galactosaminidase, ortho nitrophenyl  $\beta$ -galactosidase, glycine amidase, and phenol oxidase in yeast cells grown on culture media.

**Conventional methods for identification of *Candida* spp.** Yeastlike growing colonies on Sabouraud dextrose agar were routinely Gram-stained and if found to be yeast cells microscopically, were identified with the standard carbohydrate assimilation or fermentation tests (17) and with the API 20C system (bioMerieux Vitek, Inc., Hazelwood, Mo.) (18). The API 20C yeast identification system was inoculated with the samples, and the results were interpreted by following the manufacturer's instructions.

**Preparation of whole cell proteins (WCPs) for N-PAGE.** For each culture, a loopful of overnight growth from Sabouraud dextrose agar plate was suspended in 2 ml Sabouraud dextrose broth and incubated in a rotating incubator for 48 h (at 37°C, 150 rpm). Samples were then transferred into eppendorf tubes and centrifuged for 3 minutes at 12500 rpm, and the collected cells were washed three times with sterile distilled water. Proteins were extracted from bacterial cells by suspending cells in 75  $\mu$ l native-sample buffer (0.06 M Tris, 2.5% glycerol, % 0.001 bromophenol blue [w/v]; pH 6.8) and 4-5 times with freeze and thaw (25°C, -70°C). Supernatant was then centrifuged again for 3 minutes at 12100 rpm, collected in an eppendorf tube and kept at -50°C until the electrophoresis was carried out.

**Electrophoresis.** Whole cell proteins were analyzed by Native-PAGE according to Laemmli (19). This method used a 2 cm layer of 4% acrylamide stacking gel and a 10 cm layer of 7.5% acrylamide separating gel. Electrophoresis was performed with a discontinuous buffer system in a BRL gel apparatus model V16-2BRL Gaithersburg MD, USA. The gel was run at a constant current of 20mA through stacking gel and 30mA through separating gel, or until the bromophenol blue marker had reached the bottom. Gels were then stained with Coomassie brilliant blue (19).

## Results and Discussion

The diagnosis of disseminated candidiasis requires rapid and sensitive detection of the fungi in relevant body sites like deep tissue and blood. Because *Candida* species, especially *C. albicans*, have been isolated with increasing frequency in recent years and because they differ in their susceptibilities to antifungal agents, identification of the infecting species is important. Several typing methods are used to differentiate among strains of *Candida albicans*. In this study, a collection of *C. albicans* strains isolated from vaginal specimens of 38 patients suffering from vaginitis was analyzed by native-polyacrylamide gel electrophoresis (N-PAGE). Through the application of N-PAGE, of the 38 *C. albicans* isolates from patients suffering from vaginitis in Ankara hospitals, 34 of these were assigned to one type, three to another. Furthermore, one of these isolates was assigned to a third type, which is completely different from the others. These three different protein band patterns obtained from *C. albicans* isolates are seen in the Figure 1.

As can be clearly seen, the protein bands labelled 1 and 2 were found to be present in all of the 38 isolates analyzed. On the other hand, the protein bands labelled consecutively 3 and 4

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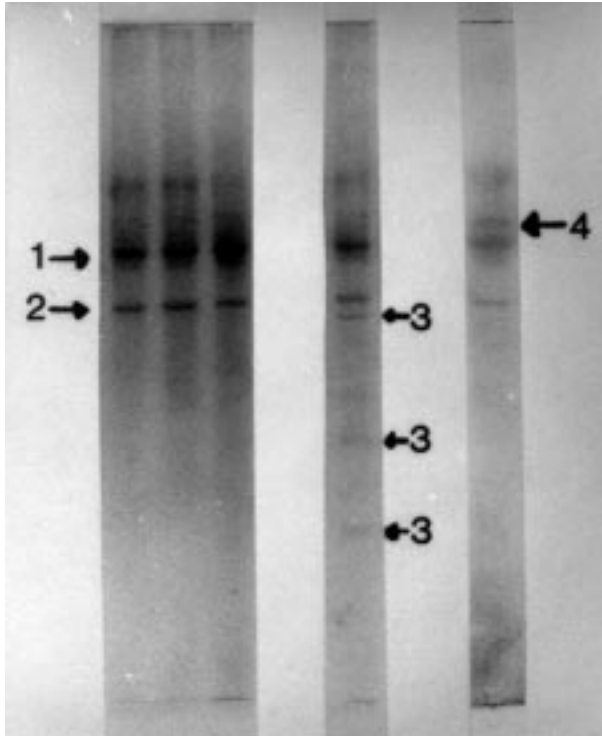


Figure 1. Protein profiles of *C. albicans* isolates by N-PAGE. Lane 1, 2 and 3 are the protein band patterns obtained from 34 of the 38 *C. albicans* isolates. Lane 4 is the protein band patterns of 3 of the 38 *C. albicans* isolates. Lane 5 is the band pattern obtained from only 1 of the 38 *C. albicans* isolates.

were found to be present only in 3 and 1 of these 38 isolates, respectively. In this study, we found an indication that because of the presence of the same protein band patterns in 34 of 38 *C. albicans* isolates, which have been isolated from people suffering from vaginitis, these isolates are very common and therefore may be responsible for dissemination. Of the 38 *C. albicans* isolates tested, it can be concluded that 34 of these belong to a single group or clone with a dissemination percentage of 89, followed by another strain with a percentage of 8. Only one of the 38 *C. albicans* isolates had different results with a completely different protein band and it shows the presence of another type of strain with a dissemination percentage of 3.

Interest in gaining a better understanding of the pathogenesis and epidemiology of *C. albicans* infections has grown in the last decade, leading to the development of a number of new methods that can be used to differentiate between strains (16). Typing systems have usually relied upon the phenotypic characteristics of the strains. Serotyping, electrophoretic karyotyping, and DNA restriction fragment length polymorphism studies have shown that isolates recovered from one or several clinical sites of the same patients are usually identical (14,20-22). Serotyping with rabbit antisera yields only two types (22,23); profiles obtained by electrophoresis of whole cell proteins differentiate only between the two serotypes (16);

biotyping and resistotyping permit the differentiation of many classes (9,15), but the reproducibility of these methods has been questioned (16,23). Methods based on genotypic variation, such as electrophoretic karyotyping (20) and restriction fragment length polymorphism (1,13,14,21,24,25) have permitted a better and more reliable means of discriminating *C. albicans* strains. Multilocus enzyme electrophoresis has been used by Caugant and Sandven (26) to assess genetic diversity, and in their work, the 98 isolates obtained from different clinical samples were assigned to 14 electrophoretic types. The clinical sources of the isolates were blood, wound, lung, urine, oropharynx, pleural fluid and bile. However, in this study, only isolates from vaginas were used and three different protein band profiles were observed among these isolates when whole cell protein extracts of these strains were used in native-polyacrylamide gel electrophoresis.

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Electrophoretic Analysis of *Candida albicans* Isolates Collected From Turkish Hospitals by Native-Polyacrylamide Gel Electrophoresis (N-PAGE)

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