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DNA FINGERPRINTING AND KARYOTYPE OF *CANDIDA ALBICANS* ISOLATED FROM VAGINAL INFECTIONS

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ABSTTRACT

The vaginopathic potential and the intravaginal morphology of different *Candida albicans* strains isolated from 25 symptomatic cases suffering from vaginitis and from 25 asymptomatic cases were studied. During infection of sypmtomatic patients, the *Candida albicans* cells did not maintain their yeast morphology but gave rise to hyphal filaments and germ tube formation as well as protease production. Vaginal *Candida albicans* isolates taken from the asypmtomatic patients were found to have inability of protease production and germ tube formation. *Candida albicans* cells forming germ tube demonstrated the highest percentage of adherence to epithelial cells. DNA figerprinting and electrophoretic karyotype analysis of *Candida albicans* strains of different morphology and pathology proved to have different DNA bands pattern of fingerprint. The results suggest that protease production, germ tube formation and adherence to epithelial cells play an important role in virulence of *Candida albicans*.

INTRODUCTION

Yeast are commonly encountered in the genital tract and nearly all are of *Candida species*, about 95% of all Candida species found are *Candida albicans*. Approximately 20% of asymptomatic sexually active women are found to have yeast forms responsible for about 25% of all cases of vaginitis (1). *Candida albicans* is considered the most virulent yeast in the genus candida and cause a variety of infections in human (2).

Vulvovaginitis is one of the most frequent diseases caused by *Candida albicans* (3). Although some authors claim that candidal colonization is usually associated with vaginal symptoms (4), it is widely held that *Candida albicans* may persist in the vagina without causing overt disease(5). Thus, virulence factors of the fungus are likely to play a role in the inflammatory process, and it is possible that they are dynamically expressed in the vaginal environment as a consequence of antigenic variations and phenotypic switching (6).

Among the recognized virulence factors of *Candida albicans*, adherence to mucosal cells, secretion of proteinase enzyme (s), and germ tube formation, factors which are possibly interrelated(7& 8), are candidates to favor the vaginal implant of the fungus. Germ- tube formation has been particularly associated with virulence expression, since a variant of *Candida albicans* unable to germ tubes at 37°C was less able than germ- tube forming strains to give an experimental vaginal infection in pseudoestrus rats (9).

Adhesion of the pathogenic fungus *Candida albicans* to epithelial cells has been the subject of a number of studies (10 & 11). A major focus of interest in these studies has been the identification of the fungal cell wall components involved in attachment process. Continuous series of studies found that chitin which is a macromolecule present in the cell walls of almost all fungi was involved in the adhesion of the fungus to mammalian tissues (12 & 13). In *Candida albicans*, chitin is essential cell wall component involved in the germ tube and budding process (14).

Fingerprint analysis of amplified DNA has been shown to be of value in epidemiological typing of microorganisms (15). This technique allows quick and objective identification of isolates, accommodates new variants and does not require culturing microorganisms. The aim of the present work was to study morphology, pathology, virulence factors and genetic characterization of *Candida albicans* in vaginal infections.

MATERIAL AND METHODS

Yeast isolation:

Twenty-five samples obtained from vagina from asymptomatic women and twenty-five samples were collected from cases of human vaginitis through 6 months. *Candida albicans* isolated on Sabouraud glucose agar (Difco, USA) for 24 h at 37 °C.

Morphological and biochemical characterization :

All strains were subjected to morphological and biochemical tests for *Candida albicans* identification using API 20 C system and for formation of germ tube(16).

Protease assay :

Protease secretion was tested using basic medium consisted of 2 g saccharose, 0.3 g sodium nitrate, 0.1 g magnesium phosphate, 0.05 g potassium chloride, 0.001 g ferrous sulphate , 0.1 g dipotassium phosphate and made up to 100 ml with water.

Cell free supernatant fluids were obtained by centrifugation of 5 ml at 12000 g for 10 min at 4°C, casein was dissolved at concentration of 20 g/L, in 0.1 M sodium phosphate buffer at pH 6 containing 5 mM $CaCl_2$. 2 ml of this substrate solution was mixed with 1

ml of cell-free supernatant fluid and incubated at 37°C for 30 min. 6 ml of 5 g/L trichloroacetic acid (0.3 N) was added and the tubes were left to stand for 30 min. The precipitated protein was separated by centrifugation at 5000 g for 15 min. The quantity of soluble peptide in supernatant was estimated according to [17] at wavelength of 500 nm. Units of enzyme activity (U) were calculated from a standard curve and expressed as μg of tyrosine released from casein per h at 37°C. Specific activity was expressed as units of protease activity per mg of cell dry mass.

Epithelial cells :

The urine samples were obtained in the morning from healthy women and centrifuged at 350 rpm for 10 min to harvest the epithelial cells. The sediment was washed twice in phosphate buffered saline (PBS) pH 7.2 and the number of cells were estimated microscopically with a counting chamber, then standerdized to 10^5 cell /ml in PBS buffer.

Adhesion assay :

A mixture of equal volumes of epithelial cells (10^5 cell/ml) and *Candida albicans* (10^8 cell/ml) was incubated in plastic tubes on a rotator at 37°C for 2 h. The epithelial-yeast mixture was passed through polycarbonate filters (12 μm pore size) to remove non-adhering yeast. Adhesion was evaluated using spectrophotometer by measuring the optical density of epithelial cells with adherent yeasts according to [13].

Molecular biotyping techniques :

The methods for biotyping *Candida albicans* isolates have been described[18] using polymerase chain reaction (PCR). *Candida albicans* cells were grown in yeast peptone dextrose medium (YPD), 1% (W/V) yeast extract, 2% (W/V) peptone, 2% dextrose at 30°C with good aeriation to the stationary phase (optical density 0f 1.5 at 600 nm. The cells were suspended in SE medium (1.2 M sorbitol, 0.1 M EDTA at pH 7.5 and treated with Lyticase (Sigma, 100 μl of stock solution at 1mg/ml) for 1 h at 37°C.

For DNA extraction, the cells were centrifuged (1,800 g, 5 min) and incubated in a lysine buffer (50 mM Tris HCL, 20 mM EDTA, 1% (W/V) sodium dodecyl sulfate at pH 7.5), in a final volume of 4 ml, and centrifuged at 12,000 x g for 15 min. The pellet was discarded, and the supernatant was treated for DNA purification. For restriction endonuclease analysis, the total DNA was incubated with restriction endonucleases (EcoRI, HindIII) obtained from Boehringer (Mannheim, Germany). Twenty units of each enzyme were used and the electrophoretic karyotype of each isolate was determined on agarose gel stained with ethidium bromide and photographed.

The electrophoretic karotype analysis :

The electrophoretic karotype of each isolate was determined according to **Bernardis, et al.,** . The DNA samples in agarose immersed in running buffer (24.2 g of tris, 2.9 g of EDTA, 5 ml of glacial acetic acid). The parameters of each run were: time 24 h; voltage, 70 mA; time interval, 120, 240, 420 and 600 s for the first, second, third and fourth runs respectively. After electrophoresis, the gel was stained with ethidium bromide and photographed.

RESULTS

Morphology of *Candida albicans* strains isolated from vagina of asymptomatic and symptomatic patients:

The results demonstrated nongerminative nonvirulent strains of *Candida albicans* with typical yeast-like form similar to the morphology in their respective in vitro cultures isolated from the vagina of 25 asymptomatic patients (Figure 1). The morphology of *Candida albicans* strains isolated from the vagina of 25 symptomatic patients showed long filaments tube of pseudomycelial (Figure 2) and germ tube forming cells (Figures 3 and 4).

Protease production :

Most *Candida albicans* strains isolated from the asymptomatic patients showed no proteolytic activity. However, *Candida albicans* s trains isolated from the symptomatic patients revealed proteolytic activity especially these strains of the highest germ tube formation.

Adhesion of *Candida albicans* strains :

Figure 5 showed adhesion of three strains of *Candida albicans* (yeast like cells, filamentous cells and germ tube forming cells). Germ tube forming *Candida albicans* strain revealed the highest percentage of adherence to epithelial cells (highest optical density). In comparison to germ tube forming *Candida albicans* strain, filamentous *Candida albicans* strain showed 60% adhesion and avirulent yeast like *Candida albicans* strain reported 10 % adhesion.

DNA figerprinting and electrophoretic karyotype of vaginal isolates of *Candida albicans* strains

Yeast like cells of *Candida albicans* strain isolated from asymptomatic case, filamentous and germ tube forming cells isolated from symptomatic vaginal infections were taken and subjected to DNA fingerprinting PCR analysis. *Candida albicans* strains of different morphology proved to have different fingerprinting and electrophoretic karyotype analysis. In DNA fingerprinting PCR analysis (Figure 6), germ tube forming cells showed 9 bands with sizes 20, 10, 8, 4, 2, 1, 0.5, 0.2, and 0.1 kb. Filamentous cells revealed 7 DNA bands with sizes 20, 10, 8, 4, 0.5, 0.1 and 0.05 kb.

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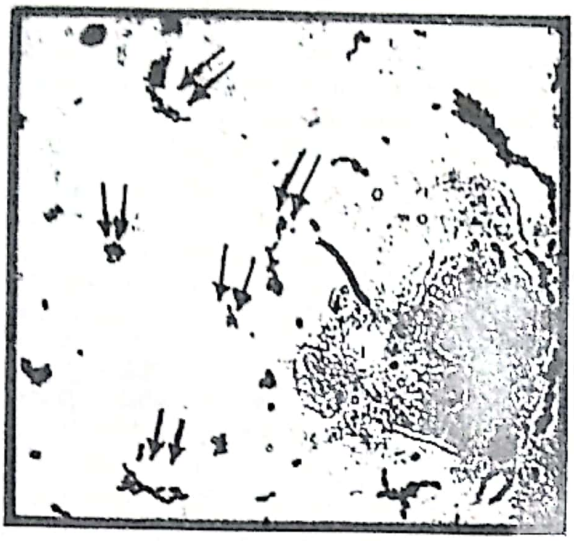

Figure 1 : Morphology pattern of non-virulent yeast like *Candida albicans* cells isolated from asymptomatic patient. Yeast like cell is indicated by double arrows.

Figure 2: Morphology pattern of virulent filamentous *Candida albicans* cells isolated from asymptomatic patient.

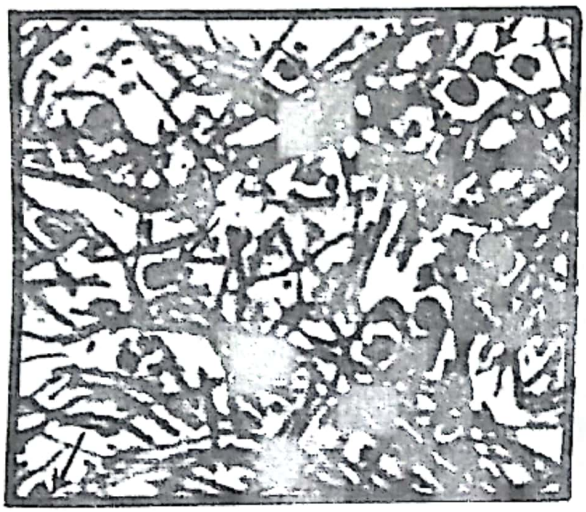

Figure 3: Morphology pattern of virulent germ tube forming cells *Candida albicans* isolated from asymptomatic patient. Each germ tube forming cell is indicated by a single arrow.

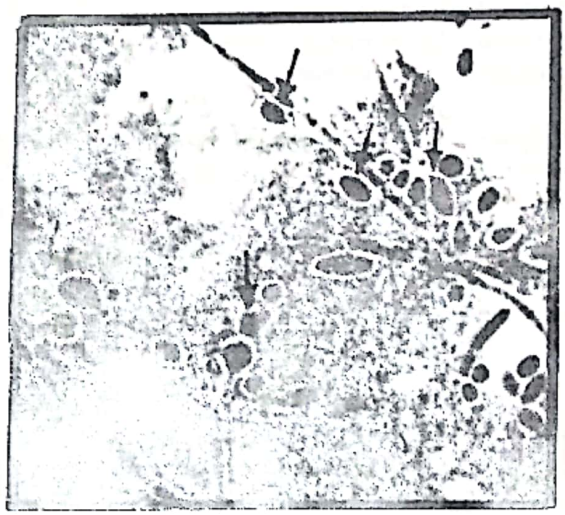

Figure 4: Morphology pattern of virulent germ tube forming cells *Candida albicans* isolated from asymptomatic patient. Each germ tube forming cell is indicated by a single arrow.

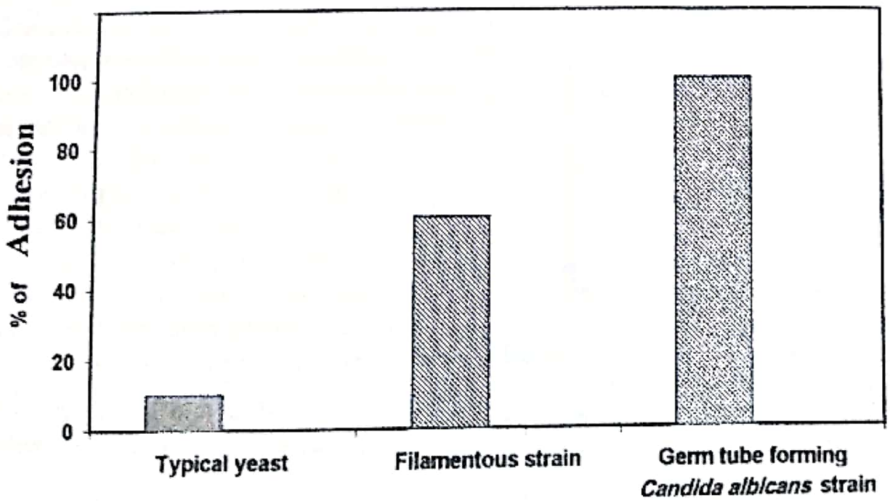

Figure 5: Adhesion of *Candida albicans* to epithelial cells

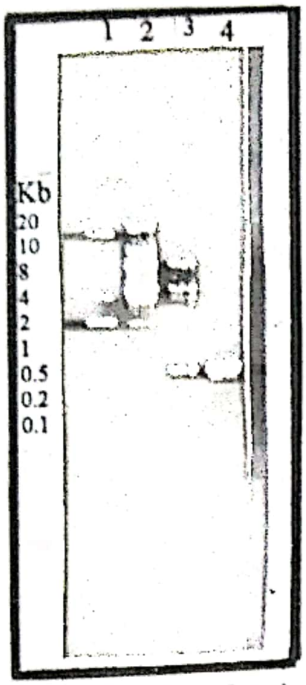

Figure 6: DNA fingerprinting of vaginal isolates of *Candida albicans* strains.1)DNA ladder, 2)germ tube forming cells,3) filamentous cells, 4) yeast like cells .

Only three DNA bands with sizes 0.5, 0.1 and 0.05 kb were observed for yeast like cells of *Candida albicans*.

Electrophoretic karyotype analysis of *Candida albicans* strains (Figure 7) revealed 5 bands with sizes 10, 8, 5, 2 and 1 kb for germ tube forming cells, 3 bands with sizes 10, 5, and 2 kb for filamentous cells and 2 bands with sizes 10 and 8 kb for yeast like cells.

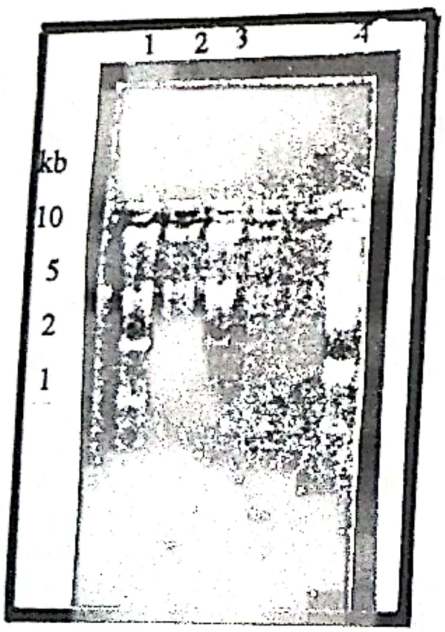

Figure 7 : Electrophoretic karyotype of vaginal isolates of *Candida albicans* strains. 1)DNA ladder, 2) yeast like -cells, 3) filamentous cells, 4) germ tube forming cells.

DISCUSSION

Germ tube and hyphal development of *Candida albicans* is likely to play a role in pathogenesis of acute candidal vaginitis. A widely accepted suggestion holds that the yeast form of this fungus colonize the healthy vagina asyptomatically, whereas hyphal or pseudohyphal development is associated with the onset of disease [19]. Mechanism by which hyphal growth can favor fungal infection, such as the increase of fungal adherence to vaginal epithelial cells, have been also postulated [20].

From *in vitro* and *in vivo* studies, it is clear that hyphal development is accompanied by remarkable changes in the antigens expressed in the cell surface [21]. These antigenic variations may have particular relevance in consideration of the recognized role of T-cell mediated immune responses in the control of mucosal candidiasis [22, 23 & 24] and of the fact that the modulated antigens in vitro induce some which are the main targets of T-cell responses in humans [25 & 26]. Production of the enzyme aspartyl proteinases is also advocated as a characteristic of the most vaginopathic *Candida* strains [27], but the relationship between proteinase production and germ tube formation is not clear.

The modes by which *Candida albicans* yeast cells elaborated the filamentous growth were different from those of typical germ tube formation. Initially the growth pattern was rather polymorphic and coarsely resembled a pseudomycelium-like, cell elongation development. However, the final net result was a filamentous pattern not distinct from the hyphal mass formed by the parental, germ tube forming strain. This mode of growth of *Candida albicans* cells in the vaginal environment highlights an unusual, alternative way of making hyphal filaments, by passing the more classic germ tube formation. This alternative pathway could be an extreme characteristic of the nongerminative strains of *Candida albicans* under those *in vivo* situations, with strong selective pressure for hyphal filaments.

The vaginal cavity may be such an environment, since this peculair morphological development of *Candida albicans* cells was not found in internal organs of chronically infected mice and was totally absent in any simple or complex medium including serum or organ extract- enriched medium [28]. Although the morphogenic factors in the vagina remain to be identified, the peculair hyphal development of *Candida albicans* cells intravaginally is likely to bear relevance to high vaginopathic potential since the non-germ- tube forming strains were reported to maintain typical yeast form in the vagina [29].

Candida parapsilosis gives the same pattern of vaginitis as *Candida albicans*, although it maintains a

pure yeast form growth in the vagina [27]. **Agatensi et al.,** [30] demonstrated another germ-tube negative species, *Torulopsis glabrata*, was totally unable to produce vaginitis. Therefore, hyphal development, whatever the type of transitional morphological stage as germ tube formation is not an absolute requirement for successful vaginal infection. Its apparent relevance as a vaginopathic factor for *Candida albicans* can be possibly replaced by the expression of other virulence factors such as secretion of aspartyl proteinase in a species like *Candida parapsilosis* which is unable of forming hyphal filaments [8].

Ross, et al [31] have suggested that proteinase secretion of *Candida albicans* is likely to play a role in human vaginitis. The proteinase negative but fully germinative strains of *Candida albicans* were less pathogenic than the highly proteolytic strains. The virulence of *Candida albicans* in vaginitis seems to be pleiotropically determined, and it is possible that a single virulence determinant like germ tube formation or proteins secretion, because of its elevated penetrance, accounts for most of the virulence in a particular strain.

Molecular biotyping was used for identification of vaginal isolates of *Candida albicans* during infection. All of the data indicate that the filamentous and germ tube formig fungus isolated from symptomatic cases was indeed the progeny of that of nongerminative yeast like fungus of asymptomatic cases. It would seem that *Candida albicans* isolates of different morphology and pathology could not combine the same DNA fingerprinting and electrophoretic karyotyping.

Stephen et al., [32] have suggested that filamentous and germ tube formation of *Candida albicans* exhibited fungal adhesion to human vagina. **Kennedy and Sandin** [33] found that differing growth conditions, altered cell surface of *Candida albicans* resulting in modified adhesion ability of the fungus to epithelial cells. It can be assumed that under specific growth conditions, components of more inert cell wall layers may be exposed and act as possible mediators of adhesion. Similar observations were made [34].

The adhesion assay revealed that the germ tube forming of *Candida albicans* exhibited higher attachment to epithelial cells than non-germinated yeast like cells. Under changed conditions, different cell wall component may exhibit adhesion of *Candida albicans* isolates to epithelial cells as can be deduced from previous studies [35].

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التحليل الجيني للبصمة الوراثية لبكتيريا السيكاتز المعزولة من الأمراض المهملة

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