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ORIGINAL ARTICLE

Cell Morphology Variations and Budding Patterns in *Candida* Isolates

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ABSTRACT

Genitourinary *Candida* spp isolates (n = 595) represented by *C. glabrata*, *C. tropicalis*, *C. guilliermondii*, *C. albicans*, *C. pseudotropicalis*, *C. parapsilosis* and *C. stellatoidea* were microscopically examined to determine their cell shapes, budding types and morphological responses to different pH values. Four cell shapes (spherical, oval, oval/spherical and elongated) were observed, with the oval/spherical type having the highest occurrence of 48.7% while elongated cells were 9.0% of the total isolates. The cell shapes were variously distributed among the different *Candida* species. Three distinct budding types observed were terminal, subterminal and bipolar/multipolar/random, with the bipolar/multipolar/random budding type having the highest occurrence (70.8%), while the subterminal had the lowest (1.8%). The three budding types were present in all cell shapes except spherical which exhibited only terminal budding. There was no relationship between species and cell shape or between species and budding type, but the pattern of budding seemed to be dependent on cell shape ($p<0.05$).

Key words: *Candida*, budding, cell shape, genital specimens.

Introduction

Candida species are yeast and members of the Fungi Imperfecti. They are also widely distributed and often considered to be unicellular organisms, even though they are, in fact polymorphic and can form variety of additional morphotypes, (Grigoriu *et al.*, 1987; Abaitua *et al.*, 1999). *Candida* occurs as a harmless endosaprophyte or commensal in man but can become pathogenic when the microflora balance in the body is disrupted, causing a disease called candidiasis Genitourinary *Candida* infections have become increasingly common in clinical practice and are having a major impact on public health causing life-threatening disorders (Stafford, 2000; Carlsen, 2001). Also, *Candida* infections are becoming difficult to treat due to the organism's resistance to commonly used antimycotic drugs (Ako-Nai *et al.*, 1993).

Figuring out exactly how the transformation of *Candida* from a non-pathogenic to a pathogenic state and how it can be interrupted is a challenge for mycologists, if this pathogen must be brought under control. Budding (reproduction) is an essential part of the origin and spread of a disease. *Candida* species are diverse (Okungbowa *et al.*, 2003; Vivier *et al.*, 1997). Therefore, a study to investigate cell properties is appropriate. The aim of this study was, therefore, to find out and document information on cell morphology variations and budding patterns, in C*andida* species, which will bring to light the diversity in *Candida* species and pave way for future research especially in the understanding of its morphogenesis, pathogenicity and search for new drugs.

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Materials and Methods

Candida-positive genital specimens comprising high vaginal swabs (480), endocervical swabs (22) and urine (15) were collected from symptomatic female patients who reported to hospitals and medical diagnostic laboratories in seven cities located between latitude 5-7.5^{O}N and longitude 2.5-7^{o}N in the southern rainforest and middle-belt region of Nigeria, from March 2000-August 2001. Each sample was cultured on Sabouraud Glucose Agar, SGA (SIFIN, Berlin) at 37^{0}C for 48 hours. Wet preparations of pure cultures (without staining) were observed under the X40 power of an optical microscope.

Subcultures were made on SGA slants, in McCartney bottles, and incubated for 24 hours at 37 ^{0}C. Each stored culture was revived on SGA every two weeks.

Candida species were identified by the CHROM-Agar and API 20C System (Analytab Products, Plainview, USA) as used by Houang *et al.* (1997) and Rex *et al.* (1995), respectively.

Results and Discussion

Four categories of cell shapes were observed, namely, spherical, oval, oval/spherical and elongated (Tab. 1; Fig. 1). Where both oval and spherical cells were present in about the same number such cells were described as oval/spherical; these cells had the highest frequency of 48.7 % while elongated cells were few (9.0 %). The cell shapes were variously distributed among the different *Candida* species. About 98.4 % of *C. guilliermondii* and 1.7 % *C. glabrata* were spherical while 77.1% *C. albicans* and 33.3 % *C. pseudotropicalis* were oval. Oval/spherical cells were present in all species except *C. albicans and C. parapsilosis*. Elongate cells were found in *C. parapsilosis* (100 %) and *C. albicans* (22.9 %).There were three distinct budding types (Tab. 2 and Fig. 2). Bipolar/Multipolar/Random budding was most frequent being present in all species (Tab. 3). The three budding types were present in all cell shapes, except spherical which exhibited only terminal budding (Tab.4).

Table 1: Percentage distribution of cell shapes among *Candida* species.

Cell shape	Distribution among *Candida* species (%)							
	C. glab.	*C. trop.*	*C. guill.*	*C. alb.*	*C. pseud.*	*C. para.*	*C. stell.*	% Total
Spherical	1.7	21.3	98.4	-	-	-	-	27.1
Oval	-	-	-	77.1	33.3	-	-	14.8
Oval/Spher	98.3	78.7	1.5	-	-	-	100	48.7
Elongate	-	-	-	22.9	66.7	100	-	9.4

* (-) = Absent.

Table 2: Description of budding in *Candida.*

Budding type	Position of bud
Terminal	Any of the two polar ends
Subterminal	Skewed away from polar end
Bipolar/Multipolar/ Random	Both polar ends plus any other position

Table 3: Distribution of budding types among *Candida* species

Candida species	Distribution (%) of budding types		
	Terminal	Subterminal	Bipolar/ Multipolar /Random
C. glab.	2.8	-	97.2
C. trop.	21.3	-	78.7
C.guill.	98.5		1.5
C.alb.	-	-	100
C. pseu.	-	-	100
C. para.	-	61.1	38.9
C. stell.	-	-	100
% Total	27.4	1.8	70.8

* (-) = Absent.

Table 4: Relationship between cell shape and budding type in *Candida* species

Cell shape	Type of budding associated with it
Spherical	Terminal
Oval/Spherical	Terminal, Subterminal, Bipolar/Multipolar/Random
Oval	Terminal, Subterminal, Bipolar/MultipolarRandom
Elongated	Terminal, Subterminal, Bipolar/Multipolar/Random

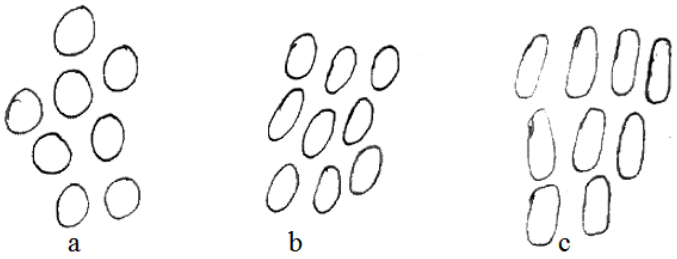

Fig. 1: Diagrammatic representation of different cell shapes in Candida as seen under a light microscope (a = spherical, b = oval, c = cylindrical/elongated). Magnification = x400.

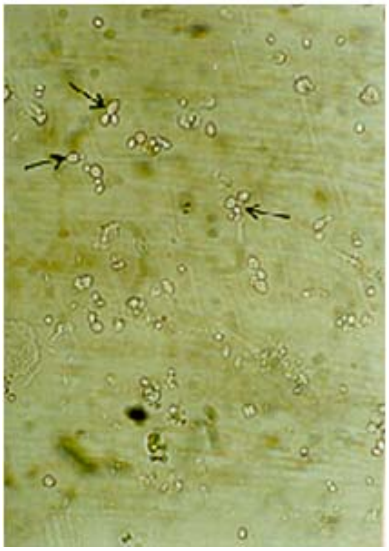

Fig. 2: Photomicrograph of budding types in a 48 hr old SDA culture of Candida grown at 37oC.Top arrow = subterminal, left = terminal, right = multipolar/random budding. Bar = 16.?8m.

Discussion:

Candida albicans and *C. parapsilosis* had no oval/spherical cells at all. This shape may be inherently absent in these species (strains in the case of *C. albicans*). Stafford (2000) described *C. albicans* cells as oval or spherical. Although all the *C. stellatoidea* cells were oval/spherical, the small number was a hindrance in determining if this was the only shape characteristic of this species. Several factors, such as cultural and environmental factors, could affect cell shape (Abaitua *et al*, 1999; Rycovska *et al.*, 2000; Szabo 1999). There was no correlation between cell shape and species type. There is no documented report of any correlation between cell shape and virulence but it would be of interest to investigate if there is a link between cell shape and ability of *Candida* cell to attach itself to host tissue.

The site for bud formation is determined in a genetically programmed manner that depends on cell type (Gausmann *et al.*, 1999; Park *et al.*, 1999). These reports and others (Chant, 1994; Lew and Reed 1995; Yang *et al.* 1997) all point to the distribution of the substance, actin, and bud site proteins as the determinants of bud site. According to these workers, when a cell reaches the threshold size there is redistribution of the actin cytoskeleton with a high concentration at the selected site, followed by cytokinesis. The localization of actin and bud site proteins at more than one site leads to bipolar and multipolar budding. Budding type may not be directly related to pathogen virulence but multipolar budding may lead to faster proliferation of pathogen since the buds (which later form daughter cells) appear at various points on the reproducing parent cell. Table 4 shows the relationship between cell shape and budding type. Regression ANOVA showed that budding type was determined by cell shape ($p \leq 0.05$). The implication of this is that in the light of pathogen control, the multipolar type of budding, and the spherical shape (in which only terminal budding was observed), might be important for consideration as the former will lead to faster, and the latter, slower reproduction. Two pertinent questions to be addressed in future research are the factors that determine the shape of a cell, and how cell shape determines pattern of budding, in *Candida* species.

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