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Morphotyping: A Simple Method for Differentiation of Candida Albicans

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Abstract

Fifty strains of *C.albicans* were isolated from specimens obtained from 3 various anatomical sites: vagina (20 strains), fingernails (10 strains) and oral cavity (20 strains). All of them caused clinical illness except for 10 vaginal strains, which were isolated from clinically free females. The identification of *C.albicans* was done by the germ tube test. Each strain was cultured on malt extract agar plates in the form of streaks for strain differentiation by morphotyping using the modified simplified scheme of Hunter et al . This coding system depends mainly on the fringe characters which are more conspicuous, more consistent and more readily coded than those associated with the streak surface. Fifteen different morphotypes were described. The morphotypes most frequently isolated were those showing discontinuous narrow fine fringes (26%), those lacking any fringes (24%) and those giving discontinuous narrow coarse fringes (18%). There was a significant difference in the distribution of fringe characters between the 3 categories of superficial infections. The main differences were the predominance of discontinuous fringes among oral isolates and narrow coarse fringes among vaginal cases. The combination of simplicity and reproducibility of morphotyping make it an ideal typing method for first line use.

Introduction

DURING the last years several attempts have been made for the subspecies differentiation of C.albicans. Biotyping [1], resistotyping [2], sensitivity to "killer yeasts" [3], extracellular enzyme production [4], immunoblotting [5] and DNA fingerprinting [6] are some examples of these attempts.

In 1987 Phongpaichit et al [7]. developed a new typing scheme, morphotyping, for the differentiation of *C.albicans* below

species level. This typing scheme was based on the original observation made by Brown-Thomsen [8] that different strains of C.albicans produce different morphologies when streaked on malt agar. In order to simplify the analysis of the morphotyping scheme of Phongpaichit et al [7], Hunter et al [9] developed a modified typing scheme in which a limited code was used. Fringe characters were recorded in full, whereas only the topography of the streak surface was taken into consideration (Table 1).

In this study a collection of clinical isolates of *C.albicans* are subjected to determine the variety and distribution of the morphotypes isolated from different superficial sites.

Material and Methods

Candida Strains :

Forty strains of *C.albicans* were isolated from cases suffering from monilial infections: 20 oral moniliasis, 10 vaginal moniliasis and 10 monilial infection of the fingernails. In addition, 10 strains of *C. albicans* were obtained from vaginal specimens of clinically free women. The strains examined were grouped according to site of isolation as oral, vaginal and fingernail strains. Swabs from these sites were first inoculated on Sabaroud dextrose agar with antibiotics. Identification of the strains as *C.albicans* was confirmed by the germ tube test using human serum.

Morphotyping:

Plates of malt agar (Oxoid) were inoculated in duplicate by a small part of *C.albicans* colony in the form of a single diametric streak. This was best done by drawing a sterile swab containing the test organism lightly across the agar surface without scratching to avoid alteration of colonial appearance by minor irregularities of inoculation. Cultures were incubated at 30°C for 48 hours, after which visual comparison using the hand lens was done to demonstrate any differences in colonial morphology between streaks prepared from different isolates. Each strain examined was assigned a morphotype code. The code of Hunter et al. [9] was used in this study, in which fringe characters were recorded in full, but only the topography of the streak surface was analysed. This abbreviated code is shown in table (1). The first digit of the code refers to the proportion of the margin which is fringed with mycelial growth. The other digits were assigned on the basis of the width of the fringes and their degree of coarseness (texture). The surface topography of the streak was coded according to its predominant characteristic, e.g. smooth, nodular, pitted.... etc. To simplify the analysis of the streak surface topography, streaks with a smooth surface were classified as featureless, whereas all others were classified as featured.

Results

The distribution of the different morphotypes of *C.albicans* amongst strains isolated from the 3 various anatomical sites (vagina, fingernails, oral cavity) is

presented in table (2): 23 different morphotypes were identified. Most of the strains (66%) had featureless streak surface topography. If streak surface topography was not taken into consideration, and only fringe characters were analysed, the number of the different morphotypes described in this study was reduced to 15. Table (3) shows the number and percentage of the different morphotypes based on the fringe characters only, as well as their distribution among strains of various sites. The morphotypes most commonly isolated were those showing discontinuous narrow fine fringes e.g. morphotype code 124, 324, 234 (26%), followed by morphotypes lacking any fringes 000 (24%) and those giving discontinuous narrow coarse fringes e.g.

Table (1): Coding of Morphological Features.

Feature	Code	Description
Fringe:	0	Absent
Distribution	1	Discontinuous : < 20% of margin
	2	Discontinuous; 20-50% of margin
	3	Discontinuous; 60-90% of margin
	5	Continuous at periphery only or strands conspicuously fan shaped
	7	Continuous; filamentous outgrowths parallel.
Width	0	Absent
	2	≤ 2 mm
	3	3-5 mm
	5	≥ 6 mm
Texture	0	Absent
	1	Very coarse
	2	Coarse
	3	Intermediate
	4	Fine
Streak surface:		
Topography	0	Smooth
	1	Nodular
	2	Pitted
	4	Crateriform
	5	Crateriform plus wrinkles or folds
	6	Wrinkles or folds
	8	Hairy

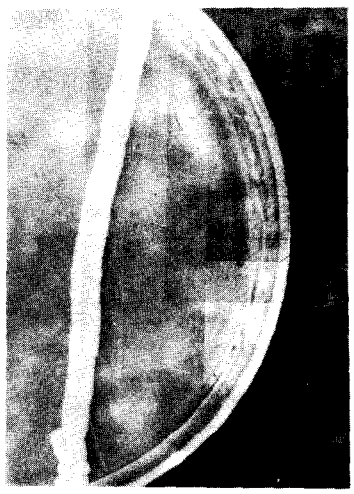
Table (2): Distribution of Morphotypes of C.Albicans between Strains Idsolated from Different Sites.

Morphotype Code		No. of Strains Isolated from Vagina (asymptomatic)	Vagina (Symptomatic)	Fingernail	Oral Cavity
000	0	2	1	1	4
000	2	0	0	1	0
000	5	1	0	1	0
000	6	1	0	0	
124	0	0	0	0	1
222	0	1	0	0	0
223	2	0	0	0	1
223	6	0	0	0	2
224	0	0	0	2	0
234	0	0	0	0	1
321	0	1	0	1	1
321	5	0	1	0	0
322	0	0	2	0	1
332	6	0	0	0	0
323	0	0	1	0	0
323	5	0	0	0	0
324	0	0	0	0	3
324	2	1	0	1	1
722	0	1	1	1	0
722	2	0	1	0	0
722	5	0	0	0	1
724	0	0	1	0	1
724	5	1	0	0	1
724	6	0	0	0	1
732	0	1	0	0	0
732	5	0	1	2	0
734	0	0	1	0	

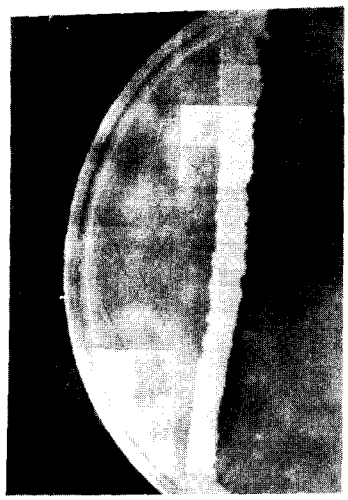
Table (3): Distribution of Fringe Characters between Strains Isolated fromVarious

Fringe Characters	No. (%) of Isolated from				
	Vagina (asymptomatic) (n =10)	Vagina (Symptomatic) (n =10)	Fingernails (n =10)	Oral Cavity (n =20)	Total (n =50)
NoFringes	4(40)	1(10)	3(30)	4(20)	12(24)
Discontinuous narrow coarse	3(30)	3(30)	1(10)	2(10)	9(18)
Discontinuous narrow fine	0(0)	1(0)	3(30)	9(45)	13(26)
Discontinuous wide coarse	0(0)	0(0)	0(0)	0(0)	0(0)
Discontinuous wide fine	0(0)	0(0)	0(0)	0(0)	0(0)
Discontinuous narrow coarse	2(20)	3(30)	1(10)	2(10)	8(16)
Discontinuous wide fine	1(10)	2(20)	2(20)	3(15)	8(16)
Discontinuous wide coarse	0(0)	0(0)	0(0)	0(0)	0(0)
Discontinuous wide fine	0(0)	0(0)	0(0)	0(0)	0(0)

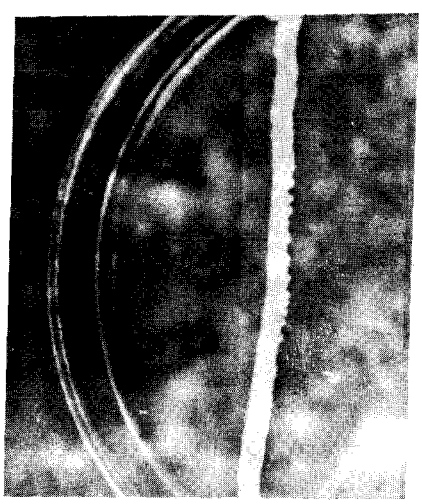
n = Number of isolates.



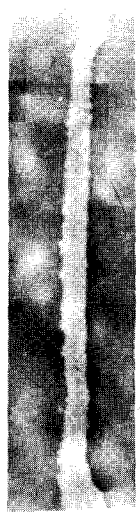
A: 000
(no fringes)



B: 732
(Continuous narrow coarse fringes)



C: 222
(discontinuous narrow coarse fringes)



D: 323
(discontinuous narrow fringes)

Fig. (1) Examples of *C.albicans* morphotypes with their abbreviated

322, 321, 222 (18%). Continuous fringe formation was observed in 32% of cases, whether these were narrow coarse e.g. 722, 732 or narrow fine e.g. 724, 734. No wide fringes were recorded.

Some differences could be observed in the distribution of superficial infections (Table 3). The main differences were the preponderance of the discontinuous narrow fine fringes among oral isolates and the narrow coarse fringes among vaginal strains, whether these were causing clinical disease or not. Fig. (1) shows some examples of *C.albicans* morphotypes with their abbreviated code.

Discussion

In 1987, Phongpaichit et al [7] described a morphotyping scheme in order to differentiate *Candida albicans* below species level. This morphotyping scheme was based on colonial morphology, which is a feature liable to undergo phenotypic variation. This "phenotypic switching" occurs at frequencies of C. 1.4×10^{-4}, although high frequency switching has also been reported at frequencies up to 2×10^{-2} [1]. However, phenotypic switching mainly affects the colony surface [8]. Therefore, Hunter et al [9] developed a modified morphotyping scheme, which was mainly based on the fringe characters that were constant and more stable than the surface features. They classified the streak surface topography into a simpler form: featureless and featured, referring to those having a smooth surface and those with a non-smooth surface respectively. This simplification was also important for statistical analysis. This modified scheme of Hunter et al [9] was chosen for this study.

The reproducibility of the morphotyping scheme has been investigated by many workers. Phongpaichit et al [7] found that the in vitro reproducibility over 2 years for the morphotyping scheme relying mainly on fringe features is 84% for those giving an identical morphotype, and 96% for those giving a morphotype differing by one or less adjacent characters. Hunter and Fraser [11] found 100% reproducibility in a study involving 15 cases of candida vaginitis.

It was noted by Phongpaichit et al [7] that the features of the streaks are affected by any alteration in the medium or condition of inoculation; e.g. over-heating of the medium during its preparation abolishes fringe formation and this may explain the large number of 000 coded strains described by Brown-Thomsen [8]. Also, the incubation of plates within plastic bags to minimize drying out elevates the relative humidity and this encourages spread of superficial layer of yeast cells from the edge of the streak, which can obscure features, of the fringes. Depth and uniformity of thickness of the agar medium and smoothness of inoculation can also influence the colonial development (and hence the coding). In this study inoculation was done by drawing a sterile swab containing a small portion of *C.albicans* colony lightly

across the agar surface without scratching. The use of a straight needle or loop was avoided as these cause minor irregularities of inoculation and affect colonial appearance. In addition, all the other factors mentioned that may lead to alteration in colonial morphology, were avoided.

In the present work, morphotyping was done relying only on the fringe characters, that are known to be more stable and constant than the topography of the streak surface, that was found to be featureless (smooth) in most of the cases (66%). Using this simplified scheme, the 50 isolated strains of *C.albicans* were analysed into 15 different morphotypes. Most of them showed discontinuous narrow fine fringes, no fringes and discontinuous narrow coarse fringes with percentages of 26, 24 and 18 respectively. Wide fringe formation was not noticed among all the 50 isolates. This may be because of the relatively limited number of strains examined, or there may have been some unidentified factor in the preparation of the medium or manipulation of the strains that disencouraged wide fringe formation

Phongpaichit et al [7] described 29 morphotypes obtained from 91 isolates, whereas Hunter et al [9] described 50 morphotypes of *C. albicans* obtained from 446 isolates.

No precise estimate can be made of the number of morphotypes which can be distinguished by this scheme. Using DAN finger printing as a typing method ,10 different types were described, 16 types by immunoblotting and 4 types by enzyme biotyping. Morphotyping, compared with other systems for strain differentiation, is technically simple and requires no specialized equipment and no special expertise.

A considerable association of morphotype and anatomical source of the isolate was noted. This was particularly marked in case of vaginal strains, among which narrow coarse fringe formation was predominant (55% of isolates), whether these were causing clinical manifestations or not. The mechanism for this association is not known; but it is well known that some surface molecules are essential for adherence of *C.albicans* to mucosal surfaces [12,13]. They may as well affect the colonial morphology [14,15]. These molecules allow the organisms to colonize the vaginal wall, whether as normal commensals or-if there is any interference with the normal balance of vaginal microbial flora or with normal host defenses-as pathogenic organisms. The narrow coarse fringe formation among these vaginal isolates was probable because of the presence of these adhesion molecules on the surface of both commensal as well as pathogenic organisms adhering to the epithelial lining.

Another observation regarding morphotyping and anatomical site was the association between discontinuous fringe formation and organisms isolated from the buccal cavity, where 55% of oral isolates showed discontinuous fringes, most of them of the narrow fine type.

In their study, Hunter et al [9], also isolated a higher percentage of narrow coarse-fringed strains from the vagina and discontinuous-fringed strains from the oral cavity. Their explanation for the predominance of the discontinuous fringed strains in the oral cavity was based on the fact that the oral cavity is an anatomical site known to contain a variety of niches, and therefore a variety of ecological environment. The discontinuous fringed strains may be more capable of adapting themselves to the different niches and different environments within the buccal cavity and may, therefore, predominate at such sites.

As regard *C.albicans* recovered from infections of the fingernails, no specific morphotype was predominating and different morphotypes were identified with more or less the same percentages. Maybe the very superficial nature of this infection allows different strains from various sources to settle easily at such site starting the infectious process.

Whatever the mechanism of association between fringe formation and anatomical site, morphotyping may offer a valuable tool for epidemiological studies of candida and candidiasis.

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