
BIOTYPES OF *Candida albicans* ISOLATES FROM AIDS PATIENTS

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SUMMARY

The phenotypic features of *C. albicans* oral isolates recovered from AIDS patients were determined by serotyping, morphotyping and biotyping in nine media with different biochemical characteristics. The patients were divided into: Group 1, comprising subjects with detectable lesions of the oral mucosa and Group 2, corresponding to carrier patients. Despite observing a greater frequency of serotype B isolates among subjects with symptomatic oral candidosis as compared to the other group of patients, these results were not statistically significant. When correlating the presence of serotypes A and B with T lymphocyte counts, we verified that occurrence of serotype B was more prevalent ($p < 0.05$) than serotype A in individuals with $CD4^+ T < 200$ cells/mm³. The occurrence of fringes greater than 3 mm in length was a typical feature of the oral isolates from our AIDS patients, though no differences in this respect were detected between the two groups of subjects. A lack of ability to assimilate urea and sorbose and variation in sensibility to 5-fluorocytosine were also features expressed by the majority of the isolates, with a predominance of the biotype 347 in 51.9% of all the oral isolates studied.

KEYWORDS: Biotypes. Morphotypes. Oral candidosis. *Candida* serotypes.

INTRODUCTION

Individuals with acquired immunodeficiency syndrome (AIDS) present an impairment of cell-mediated immunity that strongly correlates with dysfunction of the lymphocyte-macrophage populations, making these

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subjects highly susceptible to opportunistic infections (4, 5, 6). Oral candidosis develops in 75% to 95% of patients with AIDS (24).

The characterization of *C. albicans* is of great significance to the epidemiology of candidosis as this species is responsible for the majority of diseases caused by *Candida spp.* (2, 10). Epidemiological investigations to differentiate and classify pathogenic yeasts into subgroups or biotypes have been proposed (13, 19, 22). Based on the agglutination activity of antisera to various isolates, *C. albicans* was subdivided into serotypes A and B (7). Other procedures for *C. albicans* biotyping relying on the yeast's growth patterns in different media and features of colony morphology have also been used for strain differentiation (11, 14, 16).

The aim of the present work was to analyze the phenotypic characteristics of 52 isolates of *C. albicans* recovered from patients with AIDS.

MATERIAL AND METHODS

Patients and *C. albicans* isolates

A total of 52 isolates of *C. albicans* were recovered from the oral mucosa of AIDS patients under care at the Hospital for Tropical Diseases of the City of Goiânia, GO, Brazil. The subjects were diagnosed as group IV according to the criteria defined by the CDC (Center for Disease Control and Prevention, Atlanta, USA).

Material from mouth swabs was cultured on Sabouraud dextrose agar. Yeast isolates were identified as *C. albicans* following the production of characteristic germ tubs, development of chlamydospores in corn meal agar containing tween 80 and assimilation and fermentation of carbon sources, as recommended by Kurtzman & Fell (9). Patients CD4⁺-lymphocyte counts were determined at the time of specimen collection.

The subjects were divided into two categories:

Group I: comprised 27 patients with clinical symptoms typical of oral candidosis; in these cases, specimens were collected by swabbing of the lesion area. Within this group, 65% patients presented with pseudomembranous lesions, 25% with erythematous lesions and 10% with an association of cheilitis and rhomboid glossitis.

Group II: comprised 25 patients with no symptomatic lesions of candidosis. Specimens were obtained by swabbing different areas of the oral mucosa.

Serotyping of *C. albicans*

A monospecific antiserum to *C. albicans* serotype A was prepared following the method recommended by Hanseclever and Mitchel (7).

Standard agglutination procedures described by the authors were used to establish the serotypes of all the isolates. Suspensions of *C. albicans* strains were used as antigens. Agglutination activity with the monospecific serotype A antiserum was determined in duplicate. Positive agglutination reactions indicated a serotype A strain and no evidence of agglutination characterized a serotype B strain.

Morphotyping of *C. albicans*

Differentiation of the *C. albicans* isolates based on morphological characteristics of the colonies was done according to the method of Phongpaichit et al., 1987 (20). Briefly, strains of *C. albicans* were suspended in 0.85% saline and the turbidity adjusted to 3 McFarland standard. Yeast cell suspension was used to inoculate malt agar plates with approximately 5mm streaks, using a sterile cotton swab stick. The plates were incubated at 25°C for 10 days, in the dark, and the cultures morphotyped according to fringe distribution, width and texture as well as the topography of the streak surface.

Biotyping of *C. albicans*

The 52 isolates of *C. albicans* were grown in nine different test media as described by Odds and Abbot (14). To codify the biotype, the test media were divided into 3 groups, as follows: Group 1: tolerance to pH 1.4 (code 1), production of proteinase (code 2) and resistance to fluorocytosine (code 4). Group 2: urea assimilation (code 1), sorbose assimilation (code 2), and tolerance to salinity (code 4). Group 3: citrate assimilation (code 1), resistance to boric acid (code 2), and resistance to safranin (code 4). For each group, growth in the first medium was coded as + 1, in the second as + 2 and in the third as + 4. A single number of three was established per group by adding the corresponding positive codes. In each, 3 µL of strain suspension giving a McFarland 3 turbidity index was used as inoculum. Cultures were checked for colony growth after incubation at 37°C during 4 days for the safranin, citrate, urea, tolerance to salinity, and boric acid tests and during 7 days for the pH 1.4, sorbose, proteinase and 5-FC tests.

Statistics

A chi-square was performed and the Fisher exact test was used when appropriate. ($p < 0.05$).

RESULTS

Serotyping of the *C. albicans* oral isolates from AIDS patients showed that 41 (78.8%) were serotype A and 11 (21.2%) were serotype B.

Among the strains recovered from symptomatic individuals, 20/27 (74.1%) were serotype A and 7/27 (25.9%) were serotype B. Within the group of patients with no detectable oral lesions, serotype A also prevailed over serotype B, the frequencies being 21/25 (84%) for the former and 4/25 (16%) for the latter. The differences were not found to be statistically significant ($X^2=0.77$; $p>0.005$).

Evaluation of CD4⁺ lymphocyte numbers showed that 90.4% (47/52) of the AIDS patients had less than 400 cells/mm³. Correlating the data on T cells with serotype frequencies of the *C. albicans* oral isolates, it was found that 31.7% of serotype A (13/41) and 72.7% (8/11) of serotype B occurred when CD4⁺ count was less than 200 mm³. It was statistically significant.

The analysis of colony morphology in yeast extract agar revealed 10 distinct *C. albicans* morphotypes with predominance of type 5330 (fringe continuous at periphery, 3-5 mm of width, fine texture and smooth), representing 34 (65.4%) of the oral isolates. Sixteen of these strains were recovered from symptomatic patients and 18 from patients with no observable oral lesions due to *Candida spp* (Table 1).

Table 1. Morphotypes according to the method of Phongpaichit *et al.*, 1987, of the 52 *C. albicans* isolates recovered from oral mucosa of AIDS patients

Code	Symptomatic patients n=27 (%)	Asymptomatic patients n=25 (%)	Total n=52 (%)
5330	16 (59.2)	18 (72.0)	34 (65.4)
5330	03 (11.1)	04 (16.0)	07 (13.5)
5230	03 (11.1)	--	03 (5.8)
7330	01 (3.7)	--	01 (1.9)
5320	01 (3.7)	--	01 (1.9)
5220	01 (3.7)	01 (4.0)	02 (3.8)
7320	01 (3.7)	--	01 (1.9)
5240	--	01 (4.0)	01 (1.9)
7530	--	01 (4.0)	01 (1.9)
0000	01 (3.7)	--	01 (1.9)

Fifty one (98.1%) of the oral isolates had fringes, with sizes varying from 3 to 5 mm in 71.2% of the cases. Intermediate textures were observed in 90.4% of the fringed isolates. All the strains analyzed had smooth streak surfaces.

Biotyping in nine different media showed a prevalence of biotype 347, which is unable to assimilate urea and sorbose and corresponded to 51.9% of the oral isolates. The data are presented in Table 2.

Table 2. Growth behavior of *C. albicans* oral isolates from AIDS patients in nine different typing media

Strain characteristic detected	Symptomatic patients n=27 (%)	Asymptomatic patients n=25 (%)	Total n=52 (%)
Tolerance to pH 1,4	27 (100)	25 (100)	52 (100)
Production of proteinase	27 (100)	25 (100)	52 (100)
Resistance to 5-FC	05 (18.5)	07 (28)	12 (23.1)
Urea assimilation	05 (18.5)	08 (32)	13 (25.0)
Sorbose assimilation	05 (18.5)	09 (36)	14 (26.9)
Tolerance to NaCl	26 (96.3)	25 (100)	51 (98.1)
Citrate assimilation	26 (96.3)	25 (100)	51 (98.1)
Resistance to boric acid	27 (100)	25 (100)	52 (100)
Resistance to safranin	27 (100)	25 (100)	52 (100)

DISCUSSION

The observation of clinical signs of oral candidosis in 51.9% (27/52) of our patients agrees with the study of MacCullough et al (11) who reported the development of infection in 51.1% (23/45) of their AIDS patients. However, a higher prevalence of 70% was found by Myasaki et al (12). This discrepancy might be linked with the immunodeficiency status of patients at the time of specimen collection. Rodero et al (24) noted that oral candidosis affects 75 to 95% of HIV seropositive individuals, may cause more than one episode of overt morbidity and facilitates the obtainment of a greater number of yeast cells in more advanced stages of AIDS.

Our data indicates that serotype A prevailed among the AIDS patients. The occurrence of 21.2% (11/52) oral isolates of serotype B found in this study is in agreement with results from other research groups. Pires et al. (21), Ricci et al (23) and Drouhet (3) detected respectively, 30%, 34.9% and 31.2% of serotype B among *C. albicans* strains recovered from the oral mucosa of subjects diagnosed with AIDS.

The association between yeast pathogenicity and *C. albicans* serotypes has been extensively discussed. In this respect, the fact that serotype B prevailed among our AIDS patients with lesions of the oral mucosa is worth noting, although the association was not statistically significant. However, independently of patient symptomatology, 72.3% of our isolates belonging to serotype B (8/11) and 31.7% of those belonging to serotype A (13/41) were recovered from individuals with CD4⁺ counts below 200 mm⁻³, a correlation that was significant. Brawner and Cutler (1) observed that immunocompetent individuals colonized by oral *C. albicans* were almost equally likely to carry serotype A as serotype B cells, while immunocompro-

mixed individuals were at least twice as likely to be infected by serotype B than by serotype A strains.

The various morphotypes observed show that *C. albicans* isolates present a great morphological variability. The recurrent observation of continuous fringes in 98.1% (51/52) of the cases, however, appears to be a significant feature if we considered that Oliver and Reade (16) and Pires et al (21) found this same characteristic in 52% and 68% of *C. albicans* isolates, respectively, while studying patients with AIDS.

Fringed morphotypes have been correlated with virulence of *C. albicans* isolates (8,2,17). A relationship between production of discontinuous fringes and systemic infections has been verified by Hunter et al (8). Additionally, Oliveira (12) found a greater percentage of fringed *C. albicans* isolates from the oral mucosa of patients with cancer or AIDS when compared to strains from healthy individuals, lending support to the association of such characteristics with strain virulence. However, we observed no differences between isolates from patients with symptomatic oral candidosis and those from asymptomatic patients.

According to Otero et al. (18), biotyping is the best system for strain phenotyping since it accounts for a great number of characteristics, such as tolerance, assimilation, susceptibility to compounds and enzyme production. This author found a better discrimination index with respect to biotyping when comparing the technique to morphotyping, auxotyping and resistotyping of *C. albicans* isolates from vaginal secretions. Odds and Abbott (14) demonstrated this discriminatory power in 85 *C. albicans* oral and vaginal strains, detecting 45 different biotypes. Our results showed a total of 10 biotypes among the 52 oral isolates obtained from AIDS patients, with predominance of biotype 347.

The biotype features expressed by *C. albicans* isolates may correlate with strain virulence. Biotyping -57, which is resistant to safranin and to boric acid, it is tolerant to Na Cl, assimilates urea and citrate, it does not assimilate sorbose and varies with respect to proteinase production and resistance to 5-FC, was found to be associated with *C. albicans* vaginal isolates from patients with severe clinical manifestations, as reported by Odds et al. (15). Such features were also expressed by most of our oral isolates, differing only with respect to assimilation of urea. The lack of ability to assimilate urea or sorbose found in 75% (39/52) and 73.1% (38/52) of our *C. albicans* isolates, respectively, may be a characteristic of strains from the oral mucosa of individuals with AIDS. Such features should not be exploited as possible marker of strain virulence, however, since the frequencies of biotypes bearing these characteristics did not differ among our groups of patients classified as symptomatic or asymptomatic for oral candidosis.

RESUMO

Biotipos de isolados de *Candida albicans* retirados de pacientes com Aids

Características fenotípicas de isolados de *C. albicans* da mucosa bucal de pacientes com Aids foram determinadas quanto ao sorotipo, aspecto morfológico de colônias e comportamento perante nove testes bioquímicos. Os pacientes foram classificados em dois grupos. O grupo 1 foi constituído de pacientes que apresentavam lesões detectáveis na mucosa bucal, e o grupo 2 de portadores de *C. albicans*. Apesar de se observar um grande número de isolados do sorotipo B em pacientes com quadro clínico de candidose, a comparação entre os grupos não foi estatisticamente significativa; no entanto verificou-se que o número de linfócitos CD4+ inferior a 200 células/mm³ em pacientes cujos isolados pertenceram ao sorotipo B foi estatisticamente superior aos com sorotipo A. A presença constante de franjas maiores de 3 mm foi uma peculiaridade dos isolados de *C. albicans* em pacientes com Aids, não se observando entretanto diferença de comportamento quanto à morfologia de colônia quando se comparavam os dois grupos. A carência de assimilação de uréia e de sorbose e diferença no comportamento quanto à sensibilidade à 5-fluorocitosina também foi uma característica expressa pela maioria dos isolados, verificando-se um predomínio do biotipo 347 em 51,9% do total das leveduras analisadas.

DESCRITORES: Biotipos. Morfotipos. Candidose oral. Sorotipos de *Candida*.

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