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Enzyme Production and Biotypes of Vaginal *Candida albicans*

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Abstract

Candidial vulvovaginitis is one of the most common forms of vaginal infection. However, the origin of the infecting organism is sometimes doubtful. Therefore, epidemiological investigation can help to recognize routes of infection spreading. The aim of the present study was to determine the ability to produce esterases by clinical isolates of *C. albicans* and to find the relationship between their serotypes. Also, it was intended to determine the ability of these strains to produce proteases and lipases as well as the ability of the strains to assimilate carbohydrates. 46 strains of *C. albicans* isolates from the vagina of women suffering from vulvovaginitis were examined. Three main kinds of esterases were distinquished by their spectra of hydrolytic activity toward α-naphthyl acetate, β-naphthyl propionate and indoxyl acetate. The strains were grouped into four categories: three categories in which esterase patterns were observed and one category in which esterase bands were not observed. On the basis of the 20 carbon sources assimilated, the *C. albicans* strains were categorized into 11 biotypes with the major biotype accounting for 21 (45.7%) strains. The examination of proteolytic activity using casein and albumin enabled to divide the strains into four groups. All of the examined strains belonged to serotype A and all of them expressed lipolytic activity. Esterase electrophoretic patterns and biotypes based on proteolytic activities were compared with the ability to assimilate carbon from various sources.

Key words: *C. albicans*, vulvovaginitis, biotypes, esterases

Introduction

The use of epidemiological markers to type *C. albicans* in communicable diseases is recognized as being very important for the following purposes: detection of sources and routes of infections; distinction between recurrent and chronic infection; identification of endemic strains; selection of specific immunoprophylaxis and immunotherapy (al Rawi and Kavanagh, 1999; Kubota, 1998; Magliani *et al.*, 2002; Mendling *et al.*, 2000; Moraes *et al.*, 2000). In the case of *Candida* vulvovaginitis, the origin of the infecting organism is unclear. It has been suggested that there are many women who carry *C. albicans* in the vagina without subjective symptomatology, often with low *Candida* concentration. These observations are in agreement with the view that *C. albicans* may be either human commensal or a pathogen (Kubota, 1998; Marai, 2001). *C. albicans* is a common opportunistic pathogen in HIV-infected patients. Diabetes mellitus increases the rate of vaginal colonization and infection with *C. albicans* spp. (Goswami *et al.*, 2000; Haberland-Carrodeguas *et al.*, 2002; Taylor *et al.*, 2000). However, *C. albicans* is a potent allergen in some situations and it has been suggested that local hypersensitivity to these fungi is a factor which prolongs recurrent vaginal candidiasis (Moraes *et al.*, 2000).

Various methods have been used to type *Candida* isolates, with the objective of developing epidemiological tools. The most widely used methods include biotyping, serological typing, comparison of the susceptibility or resistance response to antifungal chemicals, and the ability of the strains to produce hydrolytic enzymes (Kantarcioglu 2002; Kurnatowska, 1998; Mendling *et al.*, 2000; Mercure *et al.*, 1996; Quindos *et al.*, 1996).

Hydrolytic enzymes of *C. albicans* have been implicated as virulence factors, and particular emphasis has been given to the extracellular protease, esterase and lipase (De Bernardis *et al.*, 1999; Kantarcioglu 2002; Kurnatowska, 1998). The goals of the present study were: firstly, to determine the patterns of esterase

electrophoretic types (zymotypes) among clinical isolates of vaginal *C. albicans,* secondly, to find out whether the zymotypes could be used to define strains of *C. albicans* for epidemiological purposes, thirdly, to determine the serotype of the strains. Moreover, the aim was to compare *C. albicans* strains with respect to their esterase patterns, their capability of secreting protease and lipase and their ability to assimilate carbohydrates.

Experimental

Materials and Methods

46 strains of *Candida albicans* were isolated from vagina of women suffering from vulvovaginitis. The age of patients ranged from 17 to 67 years. Samples were taken from the wall or fluid (pool) of the vaginal canal with a sterile cotton swab and immediately streaked on Sabouraud glucose plates agar. Isolates were found to be *C. albicans* if they showed germ tube production in rabbit serum and if they were able to produce chlamydospores on Nickerson-Mankowski medium. Apart from that, strains of *Candida albicans* were typed using API 20C AUX (bioMerieux). Proteolytic activities of the strains examined were determined using human albumin and bovine casein as substrates according to the procedure described by Staib (1965). Lipolytic activities were determined using the medium containing Tween 80 according to Werner (1966). API 20 C AUX test was performed according to the manufacturer's instruction. For each of the isolates the serotype was determined by slide agglutination test according to Hannula *et al.* (2001). The determination of the esterase patterns was performed using the techniques described by Goullet and Picard (1991) and Branger *et al.* (1990) which were adapted in order to examine strains of *C. albicans.* The single colony of each examined strain of *C. albicans* growing on Sabouraud agar was inoculated into Sabouraud broth then used to determine the esterase pattern.

C. albicans was grown overnight at 37°C in 40 ml of Sabouraud broth in Erlenmayer flasks being vigorously shaken. After centrifugation the yeasts were washed in 0.075 M Tris-0.06 M glycine buffer (pH 8,7), resuspended in 1.5 ml of the same buffer and then disrupted 10 times by refrigeration and thawing. Debris was removed at 10.000 × g for 15 min. at 4°C. The supernatants containing at least 6 mg of protein per 1 ml were stored at –20°C until used for electrophoresis. Each strain was cultured and extracted at least twice. Horizontal slab polyacrylamide-agarose gel electrophoresis and an estimation of electrophoretic mobility were performed as described by Goullet and Picard, 1991 and Uriel 1966. The detection of hydrolytic activity to a naphthyl acetate, b naphthyl propionate and indoxyl acetate was carried out by staining with the dye Fast Blue.

Results

All of the examined strains belonged to serotype A and all of them showed lipolytic activity. The examination of esterase electrophoretic motility allowed to differentiate the examined strains into four groups of which one group was constituted by strains not producing esterases detected in the present study. All of the three substrates used: α-naphthyl acetate, β-naphthyl propionate and indoxyl acetate, were hydrolysed by fungal esterases. 15 of the *C. albicans* strains exhibited an esterase pattern consisting of two distinctly stained bands. 7 strains showed a simple esterase pattern consisting of a single band and the pattern of 5 strains consisted of three bands (zymotypes A, B, C, respectively). The mobility of these enzymes and esterase patterns of the examined strains are shown in Fig. 1. 19 strains of *C. albicans* did not exhibit esterase activity detected in this examination.

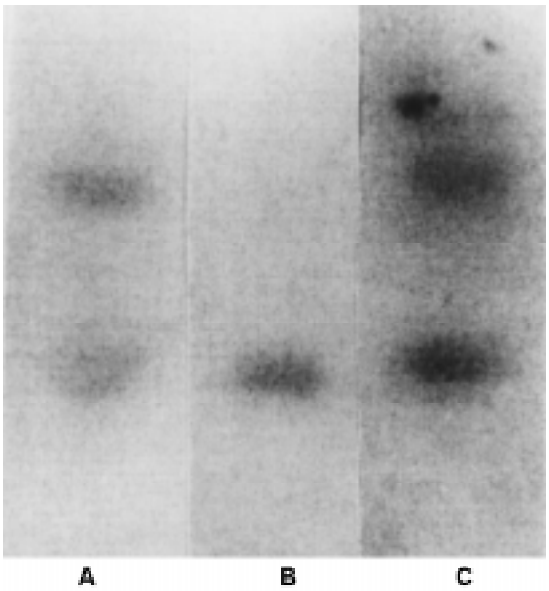

Fig. 1. Esterase patterns of *C. albicans*

Electrophoretic mobility of *Candida albicans* esterases in horizontal slab polyacrylamide – agarose gel electrophoresis. Three esterase patterns of *C. albicans* are presented. All patterns show esterases which hydrolysed a-naphthyl acetate, b-naphthyl propionate and indoxyl acetate. 27 of 46 examined *C. albicans* strains exhibited esterase activity detectable in used method.

Basing on proteolytic activities, the strains were divided into four groups. 23 of 46 examined strains exhibited proteolytic activity to human albumin and bovine casein, 10 strains were active with respect to only bovine casein and 11 strains were active only with respect to human albumin. 2 strains did not exibit proteolytic activity with respect to both substrates used (Table I).

On the basis of 20 carbon sources assimilated, the *C. albicans* isolates were categorized into 11 biotypes with the major biotype accounting for 21 (45.7%) strains of numerical profile 2 576 174. 13 of them hydrolyzed both substrates: human albumin and bovine casein. 8 of the strains from these 21 strains belonged to pattern A of esterase production. The relationship between esterase

Table I
Biotypes according to proteolytic activity of *C. albicans*

Biotype	Proteolytic activity		Number of strains
	H.A.[a]	B.C.[b]	
I	+	+	23
I	–	+	10
III	+	–	11
IV	–	–	2

H.A.[a] – human albumin, B.C.[b] – bovine casein
(+) – presence of proteolysis
(–) – absence of proteolysis

Table II
The comparison of 21 strains of *C. albicans* exhibiting the same biochemical properties

Numerical profile (2576174) of 21 strains examined	Proteolytic activity with respect to			Lack of proteolysis	Esterase pattern			
	HA[a]	BC[b]	HA&BC		A	B	C	0
No of strains	3	4	13	1	8	2	2	9

HA[a] human albumin, BC[b] bovine casein
A, B, C – zymogram characterized by two, one and three esterase bands, respectively
0 – lack of esterase pattern.

patterns and proteolytic activity of the strains exhibiting the most common biotype is shown in Table II. The remaining 25 strains belonging to ten different biotypes showed a variety of esterase paterns and they differed in their proteolytic activities to the substrates used, making any comparison impossible.

Discusion

Candida albicans is an important cause of a wide spectrum of diseases including vulvovaginitis (Farina *et al*., 2000; Linhares *et al*., 2001; Marai, 2001; Mendling *et al.*, 2000). The presence of *Candida* vulvovaginitis cannot be definitively identified by clinical criteria (Linhares *et al.*, 2001; Novikova *et al.*, 2002). Among methods used in yeast infections diagnosis, the most important are cultures of these microorganisms (Linhares *et al.*, 2001). The identification of *C. albicans* is based on a number of phenotypic characteristics, of which carbohydrate assimilation is of primary importance. For these purposes we used the commercial API 20 C AUX system, which is commonly used for diagnosis of *Candida* spp. (Ellabib *et al.*, 2001; Mercure *et al.*, 1996; Smith *et al.*, 1999; Wadlin *et al.*, 1999).

When microbial strain typing methods are compared, the most important characteristics are typeability, reproducibility and discriminatory power. The results of the present study confirm those of other authors, and namely that the API system shows good reproducibility, sensitivity and specificity (Wadlin *et al.*, 1999). However, the discriminatory power of API system is not high, and, if epidemiological investigation has to be performed, results of examination with the use of Api system should be supplemented with other methods. For example electrophoretic typing of esterases and biotyping based on proteolytic activity can be used as additional methods for differentiating strains. Other authors have already indicated the value of using a combined typing system (Quindos *et al.*, 1996).

We tentatively grouped the *C. albicans* strains into four categories, three with esterase patterns, and one without an esterase band. Because the *C. albicans* isolates show serological differences according to their geographical origin (Hannula *et al.*, 2001; Mercure *et al.*, 1996), serological examination of *C. albicans* seems to be very important from the epidemiological point of view. In our investigation, all examined strains belonged to the A serotype.

It must be pointed out that the application of esterase zymogram in bacterial taxonomy and epidemiology is often used (Branger *et al.*, 2003; Chetoui *et al.*, 1998; Gilot and Andre, 1995 and 1996; Giver *et al.*, 1998). The examination of properties of *C. albicans* enzymes is widely applied (De Bernardis *et al.*, 1990; Pichova *et al.*, 2001; Tsuboi *et al.*, 1996; Vazquez-Reyna *et. al.*, 1999) and may be useful for epidemiological purposes. Tsuboi *et al* 1996 showed that the induction level of *C. albicans* extracellular esterase was found to be correlated with fungal growth. Protease production is one of the most relevant factors of *Candida* spp. virulence in mucosal diseases, including vaginitis (De Bernardis *et al.*, 1990 and 1999; Kantarcioglu and Yucel, 2002; Pichova *et al.*, 2001; Rodrigues *et al.*, 1999; Smolenski *et al.*, 1997).

C. albicans is significantly more proteolytic than the non-albicans yeasts (Wu and Samaranayake, 1999). Vaginal candidiasis may be observed as an acute disease which is characterized by a presence of inflamation symptoms. The cause of development of vaginal candidiasis may be reinfection from a gastrointestinal reservoir, sexual transmission, or impaired host defence mechanisms and enhanced *Candida* virulence (Kubota, 1998). There is clear evidence that proteolytic *C. albicans* strains were more virulent than the non proteolytic ones (De Bernardis *et al.*, 1999). In our study, 95.7% of the investigated strains exhibited proteolysis, when albumin, albumin and casein or casein were used as substrates (Table I). Similar results of

proteolytic activity are reported by Kantarcioglu *et al.*, 2002, who showed that 95% of clinical *C. albicans* isolates exhibited proteolysis on media with bovine serum albumin. On the other hand, Odds *et al*. (1983) reported that only one-third of *C. albicans* strains isolated from the female genital tract produced proteinase against human albumin. In the present study, the analysis of albumin and casein proteolysis is used in biotyping of *C. albicans* isolates. We found that 23 (50%) of the strains exihibited lysis of both these proteins and only two strains did not reveal proteolytic activity. It seems probable, then that the examination of lipolytic activity are of minor importance in epidemiological investigation of vaginal *C. albicans.*

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