

Variability in expression of antigens responsible for serotype specificity in *Candida albicans*

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The monoclonal antibody (mAb) B9E, which reacts with a cell wall surface determinant of *Candida albicans* serotype A, and a polyclonal monospecific antiserum against the antigen 6 (IF6) were used to investigate the expression of the antigens responsible for the serotype specificity in *C. albicans* under different growth conditions. By indirect immunofluorescence, both antibodies reacted with the cell wall surface of serotype A yeast cells and germ tubes grown *in vitro* but no reactivity was observed with serotype B yeast cells. In some cases, only a weak reactivity restricted to a zone close to the parent yeast cell was observed in serotype B germ tubes stained with mAb B9E. Both antibodies reacted strongly with yeast cells and germ tubes present in kidney abscesses from rabbits infected with both serotypes, but only serotype A yeast cells and germ tubes present in smears from patients with vulvovaginal candidiasis reacted with B9E and IF6 antibodies. The expression of antigens reactive with both antibodies was modulated by the pH of the environment in which the fungus was grown. Both antibodies showed a similar pattern of reactivity when studied with a spectrofluorometer. Serotype A yeast cells showed maximum reactivity when cells were grown on Sabouraud dextrose broth supplemented with yeast extract at pH 4.6. The lowest reactivity was observed in cells grown at pH 2.0. Conversely, the reactivity of serotype B yeast cells increased at alkaline pH values, the highest being in cells grown at pH values of 7.2 and 9.5. A precise use of the methods employed in studies on *C. albicans* serotype prevalence will be important to avoid the influence of pH on the expression of antigens conferring serotype specificity.

Keywords: *Candida albicans*, serotypes, pH, monoclonal antibodies

INTRODUCTION

Candida albicans is the leading fungal pathogen in immunocompromised patients, causing life-threatening infections of difficult diagnosis. The organism presents two serotypes, A and B, which differ in antigenic composition (Guinet & Gabriel, 1980), susceptibility to some antifungals (Drohuert *et al.*, 1975), geographic distribution (Brawner *et al.*, 1992), ability to adhere to

epithelial cells (Miyakawa *et al.*, 1992) and ability to infect immunocompetent patients (Brawner & Cutler, 1989). Serotyping, usually in combination with other typing systems, is currently used in epidemiological studies (Kennedy *et al.*, 1992; Neely *et al.*, 1988).

Since their discovery in 1961 (Hasenclever & Mitchell, 1961) much knowledge has been gained about the antigens responsible for the serotype specificity. Initially, serotype A was antigenically related to *Candida tropicalis* and serotype B to *Candida stellatoidea* (Hasenclever *et al.*, 1961). Summers *et al.* (1964) demonstrated that the antigen giving the specificity for the serotype was the mannan and Kobayashi *et al.* (1992) have recently shown that the

Abbreviations: mAb, monoclonal antibody; IF6, polyclonal monospecific antiserum against the antigen 6; PBSET, PBS supplemented with Evans blue and Tween 20.

oligosaccharides $\text{Man}\beta\text{1-2Man}\alpha\text{1-2Man}\alpha\text{1-2Man}\alpha\text{1-2Man}$ and $\text{Man}\beta\text{1-2Man}\beta\text{1-2Man}\alpha\text{1-2Man}\alpha\text{1-2Man}\alpha\text{1-2Man}$ are major determinants in serotype A specificity.

From the initial studies it was concluded that serotype A strains possessed antigens not found in serotype B strains (Hasenclever, 1965), and Tsuchiya *et al.* (1984), using monospecific antisera, identified the antigen 6, which was present in serotype A strains but not in serotype B strains. However, there is evidence to suggest that serotypes are determined phenotypically rather than genotypically. Poulain *et al.* (1983) showed that serotype B yeast cells and germ tubes expressed the antigen 6 in infected tissues and that serotype B germ tubes but not the parent yeast cells expressed the antigen *in vitro*. On the other hand, Kobayashi *et al.* (1991) have shown that at pH 2.0 serotype A strains behave as serotype B since they do not express antigen 6. In the present study, a monoclonal antibody (mAb) which reacts with a cell wall surface determinant of *C. albicans* serotype A strains and a polyclonal monospecific antiserum against the antigen 6 (IF6) were used to investigate the expression of the antigens in both serotypes under different growth conditions.

METHODS

Fungal strains and culture conditions. *Candida albicans* serotype A (NCPF 3153), *C. albicans* serotype B (NCPF 3156), *C. tropicalis* (NCPF 3111), *C. krusei* (NCPF 3100), *C. parapsilosis* (NCPF 3104), *C. guilliermondii* (NCPF 3099), *C. glabrata* (NCPF 3203), *C. kefyr* (NCPF 3106) and *Saccharomyces cerevisiae* (NCPF 3178) were obtained from the National Collection of Pathogenic Fungi (London, UK). *C. stellatoidea* (ATCC 20408) was obtained from the American Type Culture Collection. The rest of the species used in some experiments, including *C. albicans* serotype A (six strains), *C. albicans* serotype B (four strains), *C. tropicalis* (four strains), *C. krusei* (seven strains), *C. parapsilosis* (eleven strains), *C. guilliermondii* (twelve strains), *C. glabrata* (fourteen strains), *C. famata* (five strains), *C. humicola* (one strain), *C. kefyr* (two strains), and *S. cerevisiae* (two strains), were isolated from clinical specimens and they were identified by the ID 32C identification gallery (BioMérieux), as well as by germ tube and chlamyospore production. The strains were maintained at 4 °C on slants containing 20 g glucose, 10 g yeast extract and 20 g agar per litre. For serotyping, *C. albicans* strains were grown on slants containing 20 g glucose, 5 g yeast extract, 10 g peptone and 15 g agar per litre, pH 6.5 ± 0.1, at 25 °C for 48 h. For some experiments, *C. albicans* serotype A and B yeast cells and germ tubes were grown in medium 199 (Sigma) as previously described (Pontón & Jones, 1986).

In some of the immunofluorescence studies, both *C. albicans* serotypes were grown on Sabouraud dextrose broth supplemented with 0.5% (w/v) yeast extract, or Brain Heart Infusion (BHI) (Difco) at different pH values as described by Kobayashi *et al.* (1991). Briefly, three serotype A and three serotype B strains were inoculated at a concentration of 1×10^5 cells ml⁻¹ (as determined by counting the cells in a haemocytometer) into tubes containing 3 ml medium at pH 9.5 ± 0.1, 7.2 ± 0.1, 5.9 ± 0.1, 4.6 ± 0.1, 3.3 ± 0.1 and 2.0 ± 0.1. The pH was adjusted by adding 10 M HCl or 10 M NaOH to the medium. After incubation on a reciprocal shaker at 27 °C for 72 h, the microorganisms were harvested by centrifugation, washed three times with saline and used for the immunofluorescence studies. The pH of the supernatant was measured with a Crison pH meter.

Antibodies. The mAb B9E is an IgM produced following standard methods. Briefly, BALB/c mice were immunized by subcutaneous injections of a partially purified antigen of 260 kDa from a germ tube cell wall extract eluted from SDS-PAGE gels (Pontón *et al.*, 1993). Fusion of splenocytes from the high-responding mouse with the SP₂O cell line was performed using polyethylene glycol 4000 (Merck). The culture supernatants were assayed for antibodies against a DTT germ tube extract by ELISA (Voller *et al.*, 1980) and against whole *C. albicans* germ tubes and yeast cells by indirect immunofluorescence. Positive hybrids were subcloned twice by limiting dilution. Antibodies used in this study were contained in cell supernatant fluid harvested from 10–15 d-old cultures of the respective hybridoma cells grown in Iscove's medium (Flow Laboratories) supplemented with 10% (v/v) foetal bovine serum (Flow).

The rabbit polyclonal *Candida* Check factor 6 (IF6) antiserum was purchased from Iatron Laboratories. Serotyping of the *C. albicans* clinical isolates was performed according to the manufacturer's instructions.

Immunofluorescence. Indirect immunofluorescence assays were carried out as previously described (Quindós *et al.*, 1987). Briefly, 10 µl aliquots of each antibody (B9E undiluted and IF6 diluted 1:50) were applied to the wells of teflon-coated microscope slides to which *C. albicans* yeast cells and germ tubes or other *Candida* species had been fixed and incubated for 30 min at 37 °C. PBS (0.01 M sodium phosphate, 0.137 M NaCl, 0.0027 M KCl) (10 µl) was added to the control wells. After being washed, the slides were incubated with fluorescein-isothiocyanate (FITC)-conjugated goat anti-mouse IgM or goat anti-rabbit IgG (Sigma) diluted 1:100 in PBS supplemented with Evans blue (0.001%, w/v) and Tween 20 (0.001%, v/v) (PBSET) for another 30 min under the same conditions. The slides were washed again, mounted with carbonate-glycerol mounting fluid, and examined with a microscope equipped to detect reflected fluorescence.

In some experiments, the fluorescence was quantified using the CytoFluor 2350 fluorescent plate reader (Millipore). Briefly, yeast cells grown at different pH values were harvested, washed twice with PBS and 200 µl of a suspension of 3.7×10^8 yeast cells ml⁻¹ in PBS was added to 96-well round-bottom microtitre plates (Costar). The plates were centrifuged at 400 g for 5 min and supernatants were discarded. They were then incubated with mAb B9E diluted 1:4 or IF6 antiserum diluted 1:50 in PBS (100 µl per well) for 1 h at 37 °C. Plates were washed and incubated with FITC-conjugated goat anti-mouse IgM or goat anti-rabbit IgG (Sigma) diluted 1:150 in PBS for 30 min at 37 °C. Plates were washed again and the fluorescence was read in a spectrofluorometer with an excitation filter of 495 nm, an emission filter of 530 nm and a sensitivity of 5. Controls were used throughout these experiments to examine possible interference from autofluorescence and nonspecific binding. For each pH value, the first antibody (mAb B9E or IF6) was replaced by 100 µl PBS in the control wells and its reactivity was subtracted from that shown by the test wells.

Characterization of antigens. Live *C. albicans* serotype A yeast cells at a concentration of 1×10^7 organisms ml⁻¹ were subjected to different treatments essentially as described by Sundstrom & Kenny (1984). Briefly, organisms were: (i) boiled for 10 min, (ii) incubated in 2.5 mg pronase ml⁻¹ (Sigma) in PBS at 37 °C for 24 h, (iii) incubated in 2.5 mg trypsin ml⁻¹ (Sigma) in PBS at 37 °C for 24 h, (iv) reduced with 10 mM DTT (Sigma) in 0.1 M Tris/HCl buffer (pH 8.1) overnight at 37 °C, and (v) oxidized with 0.05 M sodium metaperiodate (Sigma) in 0.05 M sodium acetate buffer (pH 4.5) overnight at 4 °C. After the treatments,

organisms were washed twice in PBS and fixed to the wells of teflon-coated microscope slides.

Expression of the serotype *in vivo*. The expression of the serotype *in vivo* was studied in *C. albicans* cells present in vaginal specimens from patients with vulvovaginal candidiasis and in an animal model of systemic infection. Vaginal specimens were obtained by instillation and subsequent aspiration of 10 ml sterile isotonic saline into the posterior vaginal fornix. They were centrifuged at 400 *g* and the sediment was resuspended in 1 ml PBS. The suspensions (10 μ l) were applied to the wells of teflon-coated microscope slides and studied by indirect immunofluorescence with mAb B9E and anti-factor 6 antiserum as described above. In the controls, the antibodies were replaced by PBSET. A systemic infection was established in two New Zealand White rabbits, one of them injected intravenously with 1×10^7 yeast cells of *C. albicans* NCPF 3153 (serotype A) and the other with the same inoculum of *C. albicans* NCPF 3156 (serotype B). When the animals showed signs of acute infection, they were sacrificed and their kidneys were removed. The portions of the kidneys showing microabscesses were disaggregated using a stainless steel mesh and 10 μ l of the cellular suspension was placed in the wells of immunofluorescence slides, incubated with the antibodies and serotyped by indirect immunofluorescence as described above. Controls consisted of smears in which mAb B9E and IF6 antibodies were replaced by PBSET. Cellular suspension (10 μ l) was also plated onto Sabouraud dextrose agar plates containing chloramphenicol and the yeasts isolated were identified and serotyped as described above.

Antigenic extraction, SDS-PAGE and Western blotting. Cell walls of *C. albicans* blastospores and germ tubes were extracted in the presence of DTT as described by Smail & Jones (1984). SDS-PAGE was performed by the method of Laemmli (1970) in a minigel system (Bio-Rad). The total amount of protein loaded per lane was 5 μ g for each extract. Electrophoresis was carried out in 13% (w/v) or 5–14% (w/v) gradient gels at 200 V for 45 min. Molecular mass standards were: myosin (200 kDa), *Escherichia coli* β -galactosidase (116.25 kDa), rabbit muscle phosphorylase *b* (97.4 kDa), BSA (66.2 kDa), hen egg white ovalbumin (45 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa) and hen egg white lysozyme (14.4 kDa). Subsequently, the gels were either stained with Coomassie blue or were electrophoretically transferred to a nitrocellulose membrane (Bio-Rad) for 30 min at 60 V, 10 W and 5 mA cm⁻² according to Towbin *et al.* (1979). After the transfer, the nitrocellulose membranes were blocked in 10% (w/v) nonfat dry milk in Tris-buffered saline, washed in Tris-buffered saline and incubated with 1:1 and 1:20 dilutions of the mAb and anti-factor 6, respectively, washed, and incubated with peroxidase-labelled, affinity-purified goat anti-mouse IgM or goat anti-rabbit IgG (Sigma). Immunoreactive bands were visualized after staining for 30 min with a substrate solution [0.05%, w/v, 4-chloro-1-naphthol (Sigma) and 0.015%, v/v, H₂O₂ in Tris-buffered saline].

Statistics. Student's *t* test was used to assess the significance of differences between means in immunofluorescence assays. Data were considered significant at $P < 0.05$.

RESULTS

Antigenic expression *in vitro* and *in vivo*

When studied by indirect immunofluorescence, mAb B9E reacted with the cell wall surface of both serotype A yeast cells and germ tubes grown in medium 199. Yeast cells

showed stronger reactivity than germ tubes (Fig. 1a). No reactivity was observed with serotype B yeast cells but in some cases (about 10%), a weak reactivity restricted to a ring-shape zone close to the parent yeast cell was observed in serotype B germ tubes (Fig. 1c). Factor 6 antiserum showed a reactivity similar to that shown by mAb B9E with serotype A yeast cells and germ tubes but it differed from mAb B9E in that, again in few cases, it weakly stained the whole surface of the serotype B germ tubes. Nonspecific fluorescence was not observed in any of the controls.

Despite the differences in reactivity shown by both antibodies between serotype A and B yeast cells and germ tubes grown *in vitro*, no differences in reactivity were observed when serotype A and B yeast cells and germ tubes present in kidney abscesses from infected rabbits were studied by indirect immunofluorescence. In all cases, yeast cells and germ tubes from both serotypes showed fluorescence. Particularly remarkable was the reactivity shown by serotype B cells (Fig. 1b). The reactivity of serotype B cells with both antibodies suggested that serotype B germ tubes and yeast cells were able to express *in vivo* antigens usually present on serotype A cells. However, this expression was not permanent, since when the yeast cells from the kidney abscesses from rabbits infected with the serotype B were isolated on Sabouraud dextrose agar and serotyped, they did not react with both antibodies and therefore they behaved again as serotype B. Conversely, serotype A cells present in the kidney abscesses and the yeast cells isolated on Sabouraud dextrose agar from that organ were always reactive with both antibodies.

In an attempt to investigate if the expression by serotype B cells of antigens usually expressed on serotype A cells observed in the rabbit infections was also possible during human infections, the reactivity of both antibodies with *C. albicans* cells present in smears from 10 patients with vulvovaginal candidiasis was also studied by indirect immunofluorescence. Six of the specimens showed fluorescence on both yeast cells and germ tubes and the rest were negative. The isolation of the *C. albicans* strains on Sabouraud dextrose agar allowed us to serotype the isolates. In this case, the reactivity of the cells grown *in vitro* was the same as that shown by the cells present in the clinical specimens, and therefore six serotype A and four serotype B strains were found.

Characterization of the antigens

Serotype A yeast cells were subjected to different treatments to denature components in their cell wall, and the reactivity of the cells after the treatment was determined by indirect immunofluorescence. Antigens reacting with mAb B9E and IF6 were unaffected by the treatment of the cells with heat, 10 mM DTT and proteolytic enzymes such as trypsin and pronase. However, they were sensitive to the treatment with 0.05 M sodium metaperiodate. By immunoblotting, both the mAb B9E and IF6 antiserum reacted with polydispersed materials from serotype A

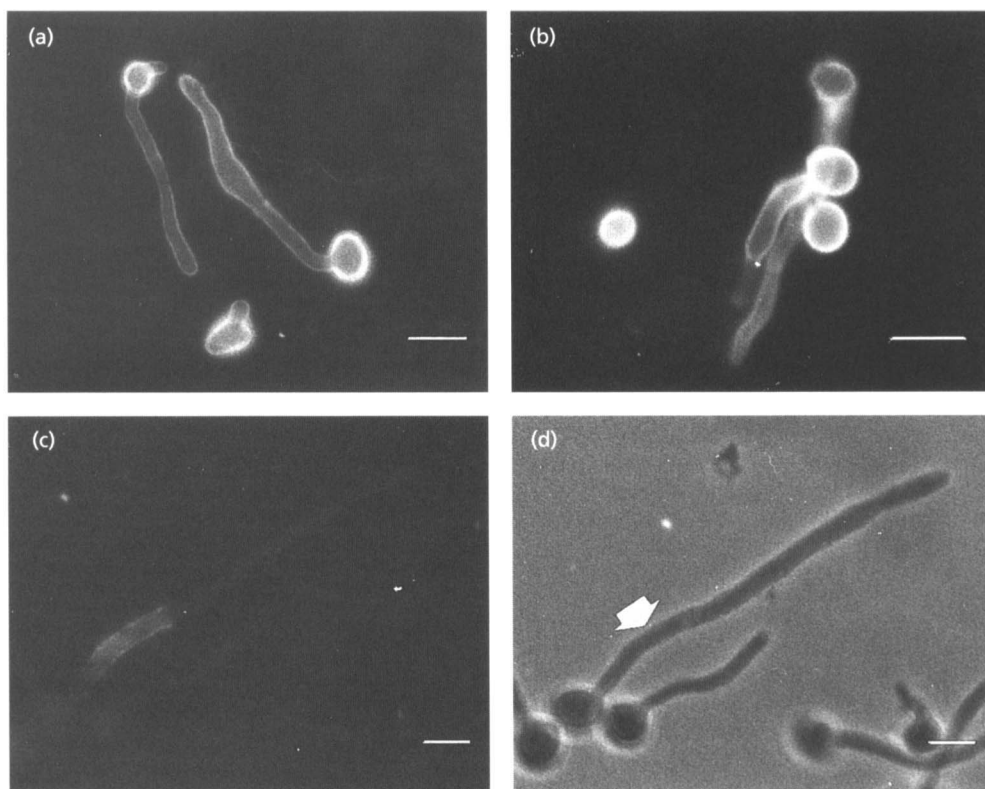


Fig. 1. Immunofluorescence photographs of *C. albicans* serotype A yeast cells and germ tubes grown *in vitro* (a) and *C. albicans* cells extracted from the abscesses of kidneys taken from a rabbit infected with a serotype B strain (b), stained with mAb B9E. (c) Immunofluorescence and (d) phase-contrast photographs, of the same microscopic field, of serotype B yeast cells and germ tubes grown *in vitro* stained with mAb B9E. The arrowhead points to the part of the germ tube cell wall that is reactive with the mAb. Bars, 10 μ m.

yeast cells and germ tubes of molecular masses > 66 kDa. No reactivity was observed in serotype B extracts (Fig. 2). The same results were observed in different clinical isolates of *C. albicans* (data not shown).

The distribution of the antigen in yeast cells of several *Candida* species was studied by indirect immunofluorescence (see Table 1). mAb B9E showed a strong reactivity with *C. albicans* serotype A, *C. stellatoidea*, *C. tropicalis*, *C. famata*, *C. guilliermondii* and *C. humicola*. Ten out of the fifteen *C. glabrata* strains tested reacted with the B9E antibody. Factor 6 antiserum showed similar results with most of the species studied. However, discrepancies were observed for *C. famata*, *C. guilliermondii* and *C. humicola*. All the *C. glabrata* strains studied reacted with the IF6 antiserum.

Influence of pH and growth medium on the antigenic expression

The reactivity of serotype B cells growing *in vivo* was surprising since reactivity occurred with both antisera when the cells were extracted from the abscesses of kidneys taken from infected rabbits but not when the cells were obtained from vaginal infections. A possible ex-

planation for this observation is that the expression of antigens reacting with both antibodies is modulated, among other factors, by the pH of the environment in which the fungus is growing. In an attempt to demonstrate this possibility both serotypes were grown *in vitro* on media at different pH values and the reactivity with both antibodies was quantified with a spectrofluorometer. The growth of *C. albicans* NCPF 3153, serotype A, and NCPF 3156, serotype B, strains on Sabouraud dextrose broth supplemented with yeast extract for 72 h produced a statistically significant decrease in the initial pH of the culture medium in most experiments, the most important being in media at pH 9.5 (Table 2). In contrast, the growth on BHI lowered the initial pH only in the media at pH 9.5 and increased the pH in the media at initial pH values of 4.6, 5.9 and 7.2 (Table 2).

The pH of the culture medium had a marked influence on the reactivity of both serotypes with both antibodies. Serotype A yeast cells showed maximum reactivity with IF6 antiserum in cells grown on Sabouraud dextrose broth supplemented with yeast extract at pH 4.6. The growth of serotype A cells at pH values lower than 4.6 showed a statistically significant decrease in reactivity with the IF6 antibody, the lowest observed in cells grown

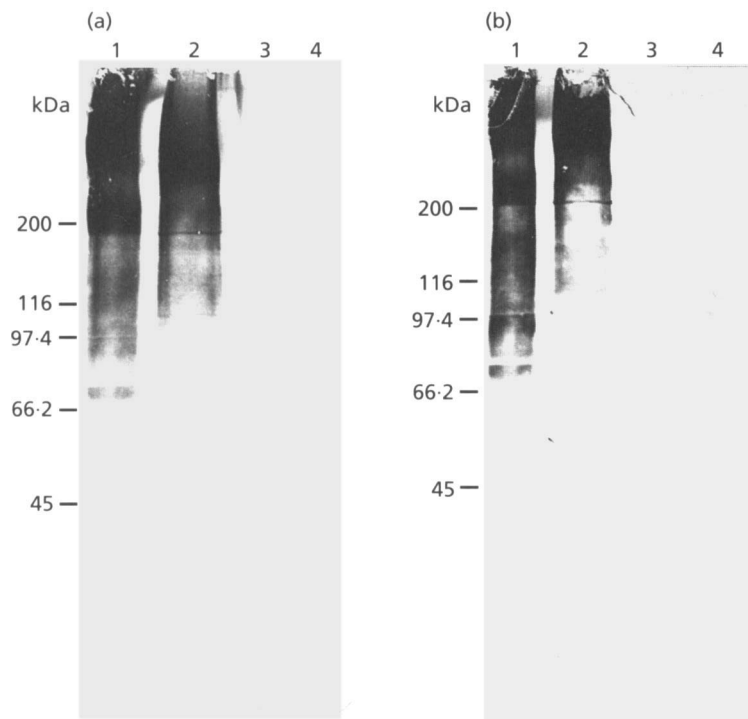


Fig. 2. Western blots of 5–14% (w/v) slab gels loaded with DTT extracts from serotype A (lanes 1 and 2) and serotype B (lanes 3 and 4) strains. (a) Reactivity of germ tube (lanes 1 and 3) and yeast cell (lanes 2 and 4) extracts with mAb B9E; (b) reactivity of germ tube (lanes 1 and 3) and yeast cells (lanes 2 and 4) extracts with IF6 antiserum. Molecular mass standards are given to the left of the gel.

Table 1. Reactivity of mAb B9E and IF6 antiserum by indirect immunofluorescence

Species	No. of reactive strains/no. of strains tested	
	B9E	IF6
<i>Candida glabrata</i>	10/15	15/15
<i>Candida guilliermondii</i>	13/13	0/13
<i>Candida parapsilosis</i>	12/12	0/12
<i>Candida krusei</i>	0/8	0/8
<i>Candida albicans</i> serotype A	7/7	7/7
<i>Candida albicans</i> serotype B	0/5	0/5
<i>Candida tropicalis</i>	5/5	5/5
<i>Candida famata</i>	5/5	5/5
<i>Candida kefyr</i>	0/3	0/3
<i>Candida buncicola</i>	1/1	1/1
<i>Candida stellatoidea</i>	1/1	1/1
<i>Saccharomyces cerevisiae</i>	0/3	0/3

at pH 2.0 (Fig. 3a). Similar results were observed with the mAb B9E (Fig. 3b). The reactivity of serotype B yeast cells grown on Sabouraud dextrose broth supplemented with yeast extract increased from acidic to alkaline pH values with both antibodies. The effect was more clear with mAb B9E (Fig. 3b) than with IF6 antiserum (Fig. 3a) in the serotype B strain used throughout the study (NCPF 3156). However, other serotype B strains tested showed a more marked increase in reactivity with IF6 antiserum (data not shown).

Table 2. Changes in pH during the growth of *C. albicans* serotype A and B strains

Strains were grown in Sabouraud dextrose broth supplemented with yeast extract and in Brain Heart Infusion for 72 h at 27 °C. Results representing a typical experiment are expressed as the mean \pm SEM of four determinations.

Initial pH	Final pH			
	Serotype A strain		Serotype B strain	
	Sabouraud	BHI	Sabouraud	BHI
2.02	1.92 \pm 0.03*	2.08 \pm 0.00†	1.98 \pm 0.00	2.07 \pm 0.00†
3.32	2.93 \pm 0.02†	3.74 \pm 0.03†	2.99 \pm 0.03‡	3.32 \pm 0.00
4.63	4.01 \pm 0.00†	6.93 \pm 0.25†	4.10 \pm 0.01†	5.29 \pm 0.41
5.92	4.50 \pm 0.00†	7.56 \pm 0.11†	4.52 \pm 0.01†	6.78 \pm 0.25*
7.20	4.70 \pm 0.01†	8.11 \pm 0.02†	4.87 \pm 0.02†	7.74 \pm 0.17*
9.50	6.33 \pm 0.26†	8.66 \pm 0.02†	6.22 \pm 0.02†	8.80 \pm 0.00†

* $P < 0.05$

† $P < 0.0001$

‡ $P < 0.001$

The low buffering capacity of the Sabouraud dextrose broth supplemented with yeast extract allowed the decrease in the pH of the medium during the growth of both *C. albicans* serotypes. However, in most cases the growth of both serotypes on BHI maintained or increased the pH of the medium. Therefore, the growth in this medium was used to assess the influence of both the

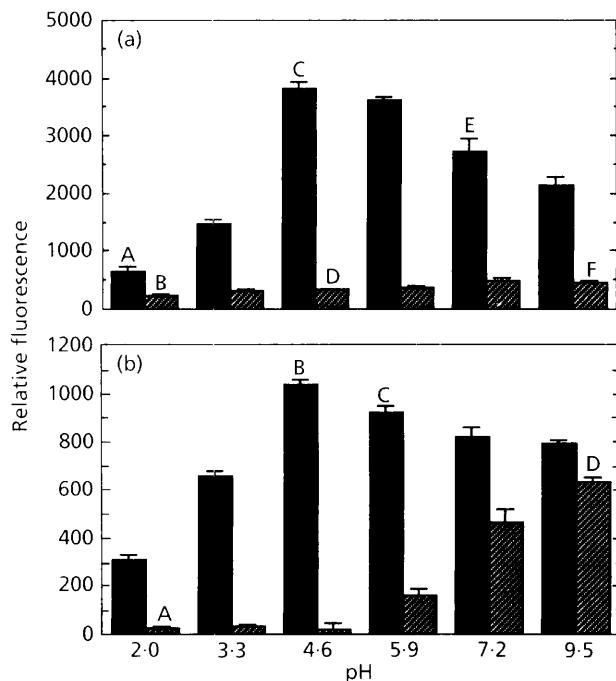


Fig. 3. Reactivity of IF6 antiserum (a) and mAb B9E (b) with serotype A (■) and serotype B (▨) yeast cells grown in Sabouraud dextrose broth supplemented with yeast extract at different pH values, measured with a spectrofluorometer. Data representing a typical experiment are expressed as the mean \pm SEM of four determinations. (a) For comparison: C versus A and B versus F, $P < 0.001$; C versus E, $P < 0.05$; B versus D, $P < 0.01$. (b) A versus D, $P < 0.001$; B versus C, $P < 0.05$.

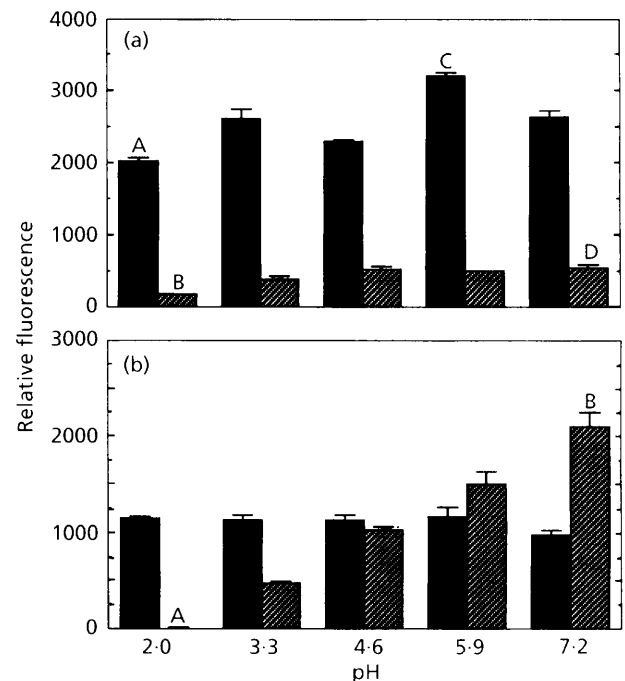


Fig. 4. Reactivity of IF6 antiserum (a) and mAb B9E (b) with serotype A (■) and serotype B (▨) yeast cells grown in BHI at different pH values, measured with a spectrofluorometer. Data representing a typical experiment are expressed as the mean \pm SEM of four determinations. (a) For comparison: C versus A, $P < 0.001$; B versus D, $P < 0.01$. (b) A versus B, $P < 0.001$.

culture medium and higher and maintained pH values on the reactivity of both serotypes with IF6 and B9E antibodies. When the reactivity of serotype A cells grown on BHI at different pH values with IF6 antiserum was compared, a statistically significant increase in reactivity was observed, the highest being in cells grown at pH 5.9 (Fig. 4a). However, no statistically significant differences in reactivity of serotype A cells with mAb B9E were observed (Fig. 4b). The reactivity of serotype B cells with IF6 antiserum increased in cells grown at pH values of 3.3 and 4.6 (Fig. 4a). However, a marked increase in reactivity of serotype B cells with mAb B9E along the increase in the pH of the culture medium was observed (Fig. 4b).

The decrease in expression of serotype A antigens at low pH was also confirmed by Western blotting. Both mAb B9E and factor 6 antibodies did not stain any component in serotype B yeast cells grown at pH 3.3 and 9.5. However, they reacted with components of very high molecular mass in serotype A yeast cells grown at pH 3.3 and with components of molecular masses > 60 kDa in cells grown at pH 9.5 (Fig. 5).

DISCUSSION

Antigenic expression in the *C. albicans* cell wall surface is a very dynamic process, which may reflect its ability to adapt to the host. Using polyclonal antibodies, antigenic

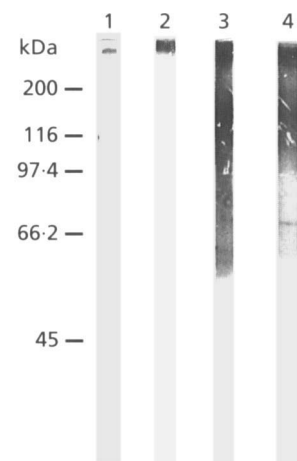


Fig. 5. Western blots of 13% (w/v) slab gels loaded with DTT extracts from serotype A yeast cells grown at pH 3.3 (lanes 1 and 2) or 9.5 (lanes 3 and 4), stained with mAb B9E (lanes 1 and 3) and IF6 antiserum (lanes 2 and 4). Molecular mass standards are given to the left of the gel.

variability has been shown to be influenced by environmental and nutritional factors, as well as by the origin of the strain (Poulain *et al.*, 1985). The use of mAbs has confirmed these observations. Thus, differences in expression of the H9 epitope, which has been shown to

correlate with serotype but it is not identical to serotype A antigen (Brawner & Cutler, 1989), between *C. albicans* strains isolated from immunocompromised patients and those isolated from immunocompetent individuals have been described (Brawner & Cutler, 1989). The growth of *C. albicans* in different synthetic media induced variability in H9 reactivity (Brawner & Cutler, 1984) but had no effect on serotype as determined by both Hasenclever's (Brawner & Cutler, 1989) and IF6 antisera (Brawner, 1991). However, the data presented in this study show that certain environmental factors, such as pH, may have an important effect on the expression of the IF6 serotype, since there was a decrease in expression of the epitopes in parallel with the decrease in the pH of the growth medium. Therefore, at pH 2.0, serotype A strains behave as serotype B. Similar results were observed with regard to the B9E epitope. The low reactivity of serotype A yeast cells grown at pH 2.0 with IF6 antiserum is in agreement with the data of Kobayashi *et al.* (1991), who observed that the cell wall mannans of serotype A strains cultured in Sabouraud dextrose broth supplemented with yeast extract at pH 2.0 did not react with IF6 antiserum since they contained neither a phosphate group nor a 1,2- β -linked mannopyranose unit. The low reactivity detected in the serotype A cells grown at pH 2.0 in our study may be related to the sensitive system used to measure the antibody reactivity, since as reported by Kobayashi *et al.* (1991), the cells grown at pH 2.0 were not reactive with IF6 antiserum by latex agglutination (data not shown). Conversely, we observed an increase in the expression of the epitope by serotype B yeast cells along with the increase of the pH of the culture medium and, therefore, at alkaline pH serotype B cells appeared as serotype A. Although only results from one serotype A and B (NCPF 3153 and NCPF 3156) strain have been shown, the influence of pH on the expression of antigens conferring serotype A specificity can be regarded as a general phenomenon, since similar results were observed with two other serotype A (UPV 93380, UPV 93703) and B (UPV 2000, UPV 1866) strains (data not shown).

The expression by serotype B strains of antigens commonly found in serotype A strains and the decrease in expression of serotype A antigens in serotype A strains grown at low pH is a transient phenomenon, since the strains show the reactivity expected for their serotype when they are subcultured at the standard conditions used for serotyping (Sabouraud dextrose agar, pH 5.8 ± 0.1 or glucose-yeast extract-peptone agar, pH 6.5 ± 0.1). These results may be related to the activity of the 1,2- β -mannosyltransferase, since as it has been recently proposed, the activity of this enzyme is suppressed or lowered at pH 2.0 (Kobayashi *et al.*, 1994) and, conversely, it could be enhanced at alkaline pH, especially in serotype B strains.

The influence of the pH on the expression of antigens responsible for *C. albicans* serotype A may explain the reactivity shown by serotype B cells *in vivo* with both IF6 and B9E antibodies. The pH of the renal tissue (6.53 in the normal rabbit renal tissue versus 6.26 for tissue infected with serotype A strain and 6.21 for tissue infected with

serotype B strain) and, in general, of other deep tissues should allow the expression of the antigen by both serotype B yeast cells and germ tubes and therefore, they will react with serotype A antisera or will induce an antibody response against antigen 6 (Poulain *et al.*, 1983). However, in other areas of the body such as the vagina, where the pH is lower, serotype B cells do not express serotype A antigens and, as shown in this study, they are negative by both IF6 and B9E staining. Despite the marked influence of the pH of the growth medium on the expression of serotype A antigens, other factors may also play a role. Thus, the growth in BHI seems to maintain the reactivity of serotype A yeast cells at pH 2.0 and to increase the reactivity with mAb B9E of serotype B yeast cells grown at pH 7.2. In addition to the differences in medium composition between Sabouraud dextrose broth and BHI, the modification of pH during the growth of *C. albicans* could have also played a role in the increase in reactivity observed in serotype B yeast cells, since final pH values of BHI medium were, in general, higher than those observed in Sabouraud dextrose broth.

Other mAbs with the ability to differentiate *C. albicans* serotypes have been described. Brawner & Cutler (1984) produced an agglutinating IgM mAb (H9) which reacted with a polysaccharide antigen expressed on *C. albicans* serotype A, *C. tropicalis* and *C. glabrata*. This mAb strongly agglutinated *C. albicans* serotype A strains but the epitope was also expressed in serotype B strains, since these strains were weakly agglutinated by the mAb (Brawner & Cutler, 1989). The H9 epitope is not the same as that reacting with the IF6 antiserum since only an agreement of 64% was reached with both antibodies when they were used to serotype *C. albicans* (Brawner, 1991). Miyakawa *et al.* (1986) produced an agglutinating IgM mAb (CA4-2) which reacted with *C. albicans* serotype A, *C. tropicalis* and *C. glabrata*, and although it did not completely correspond to IF6 reactivity, it could replace IF6 antisera for typing *C. albicans*. Reiss *et al.* (1986) produced an IgM mAb (CB6) in mice immunized with *C. tropicalis* cell walls which reacted with *C. albicans* serotype A and *C. tropicalis*. It binds to an epitope which is probably the factor 6 described by Tsuchiya *et al.* (1984) and an agreement of 95.8% was observed when the mAb was compared with IF6 antisera when serotyping *C. albicans* (Reiss *et al.*, 1986). Although the data for comparison are scarce, our mAb B9E seems to bind to an epitope different to that reacting with mAb CA4-2, since it reacts with *C. guilliermondii* and could be similar to mAb CB6. However, by indirect immunofluorescence, mAb B9E shows a different reactivity against some *Candida* species to that shown by IF6 antiserum and it can not replace the IF6 antiserum in competitive binding experiments (data not shown).

The epitope identified by the mAb B9E seems to be predominantly polysaccharide in nature, since it is resistant to heat, DTT and proteolytic enzymes but it is sensitive to oxidation with periodate, which is in agreement with the data obtained for the antigen giving the specificity for serotype A (Poulain *et al.*, 1985). *In vitro*, the epitope is expressed on both the serotype A yeast cell and

germ tube cell walls and, therefore, expression seems to be similar to that of antigen 6. However, the *in vitro* expression of B9E epitope in serotype B strains seems to be different to that of the antigen 6, since the former is seldom expressed on the germ tube cell wall and when it is, the reactivity of mAb B9E is restricted to a limited part. Preliminary experiments seem to relate this observation with adhesion to glass or plastic surfaces and, in fact, the *in vitro* expression of serotype A antigens by serotype B germ tubes is higher when the germ tubes grow adhering to a surface (data not shown). Miyakawa *et al.* (1992) have recently shown that serotype-A-specific determinants are largely involved in the mechanisms of adherence of *C. albicans* serotype A to human buccal epithelial cells and differences in adhesion between both serotypes have been described. Conversely, the expression of antigen 6 by serotype B germ tubes seems to be low but uniformly distributed on the whole cell wall surface, which is in agreement with the data of Poulain *et al.* (1983).

In conclusion, using two different antibodies directed against different epitopes present in the *C. albicans* cell wall, we have shown that the expression of antigens responsible for serotype A specificity is modulated by the pH of the medium where *C. albicans* is growing. According to Brawner (1991), it is not possible to make valid comparisons between studies which compare serotype prevalence unless the same methods are used to serotype the yeasts. The results presented in this study show that a precise use of the methods will be important also to avoid the influence of environmental factors on the expression of antigens conferring serotype specificity.

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