FEBS Letters 345 (1994) 167–171

FEBS 14048

Temperature-dependent change of serological specificity of *Candida albicans* NIH A-207 cells cultured in yeast extract-added Sabouraud liquid medium: disappearance of surface antigenic factors 4, 5, and 6 at high temperature

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Received 7 March 1994; revised version received 11 April 1994

Abstract

The cells of *Candida albicans* NIH A-207 strain (A-strain) cultivated in YSLM at high temperatures (37 and 40°C) did not undergo agglutination with the factor sera 4, 5, and 6 in a commercially available factor serum kit, 'Candida Check', and formed a grape-like shape. The mannans isolated from the cells had lost their reactivity against the factor sera in ELISA. It was also revealed by ^{1}H NMR analysis that the mannans contained neither a phosphate group nor a β-1,2-linked mannopyranose unit, although these mannans increased the non-reducing terminal α-1,3-linked mannopyranose unit. The cells and the mannans prepared by cultivation at such high temperatures followed by 27°C in the same medium entirely recovered the reactivity with the factor sera.

Key words: Candida albicans; Cell-wall mannan; β-1,2 Linkage; Phosphodiester; Antigenic factor; High temperature; Aggregation

1. Introduction

The surface of the cell wall of *Candida albicans* participates in the role of initial contact with its host and subsequent host defense mechanisms [1,2]. Mannoprotein constitutes a large proportion of *C. albicans* cell wall components [3] and is proposed to be involved in the pathogenesis of candidiasis as immunosuppressive factor [4,5].

In the previous papers [6–11], structural analyses of the mannans of *C. albicans* NIH A-207 strain (A-strain) have been reported. In one of the papers it was reported that, upon morphological change from the yeast (Y)-form to mycelia (M)-form, the contents of β-1,2- and α-1,3-linked oligomannosyl residues in the mannan prepared from the M-form cells was depressed compared to that of the Y-form cells [7]. Recently, it was further revealed that the mannans isolated from *C. albicans* serotype A strain cells grown in YSLM at pH 2.0 contained neither the phosphate group nor the β-1,2-linked oligomannosyl residue, although these mannans retained non-reducing terminal α-1,3-linked mannose units [8,11]. It is known that the lesions infected with *C. albicans* are usually typified by the presence of yeast and hyphae (pseudo- and true hyphae) form cells [12]. From this fact, it is important to investigate the antigenic features of the Y-form cells grown under several different environmental conditions next to the M-form cells.

The antigenic patterns of the seven medically important *Candida* species have been demonstrated by Tsuchiya and co-workers [13,14]. A slide agglutination scheme was designed which uses factor sera [15], whereby it is possible to make a rapid and accurate identification of medically important *Candida* species [16], i.e. a kit of polyclonal factor sera, 'Candida Check'. In a recent study, we noticed that Y-form cells of the A-strain grown in YSLM at high temperatures (37 and 40°C) lost their reactivity with the factor sera 4, 5, and 6, in 'Candida Check'. It was suggested that some structural change might take place on the cell wall mannans, although the manual of 'Candida Check' documents the preferable cultivation conditions, either at 25°C for 48 h or at 37°C for 18 h on yeast extract-added Sabouraud agar plates in accordance with previous descriptions [13,16]. In this paper, we show the analyses of the relationship between changes in antigenicity and chemical structure of the mannans of the A-strain cells caused by cultivation in YSLM at such high temperatures.

2. Materials and methods

2.1. Culture conditions

The *Candida albicans* NIH A-207 strain (A-strain) was kindly supplied by Dr. T. Shinoda, Department of Microbiology, Meiji College of Pharmacy, Tokyo, Japan. The strain was precultivated in YSLM at 27°C for 24 h on a rotary shaker (150 rpm). The medium was prepared by autoclaving 200 ml of 0.5% (w/v) yeast extract, 1% (w/v) peptone,

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Abbreviations: YSLM, yeast extract-added Sabouraud liquid medium; ELISA, enzyme-linked immunosorbent assay; ^{1}H NMR, ^{1}H nuclear magnetic resonance.

SSDI 0014-5793(94)00434-W

and 4% (w/v) D-glucose in 500-ml cultivation flasks at 121°C for 20 min. Then the cells (2×10^7), 100–200 μl of the culture were inoculated to YSLM, and the main cultivations were separately conducted on a rotary shaker at 27, 37, and 40°C for 48 h on the shaker. After harvesting by centrifugation 1,500 × g, the cells were washed 3 times with saline by centrifugation, and the washed cells (2×10^7) were recultured in 200 ml of YSLM at 27°C for 48 h on the shaker, and the washed cells with saline were prepared in the same way. Morphologies of the washed cells were evaluated photomicroscopically. At the end of each culture, the number of strain cells was counted by means of a haemocytometer. Part of the cells were also freeze-dried to evaluate cell growth. The washed cells were dehydrated with large volumes of acetone.

2.2. Slide agglutination reaction

This was conducted as described elsewhere [17] using a suspension of heat-killed cells which were harvested after each cultivation of 48 h.

2.3. Preparation of the mannans

This was performed as described previously [18]. Briefly, this was done by a combination of hot-water extraction and fractional precipitation by short-term treatment with Fehling solution. The mannan fractions obtained from the A strain cultured at 27, 37, 37 followed by 27, 40, and 40 followed by 27°C, were designated as mannans A-27-M, A-37-M, A-37–27-M, A-40-M, and A-40–27-M, respectively.

2.4. NMR studies of the mannans

^{1}H NMR spectra were recorded by means of a JEOL JNM-GSX 400 spectrometer. Each sample was dissolved in D_2O in 1% (v/v), and the determination was conducted at 45°C, and acetone was used as the internal standard (2.217 ppm).

2.5. ELISA of mannans

An ELISA of mannans was conducted as described previously [19] using factor sera 1, 4, 5, and 6 of 'Candida Check' (lot no. R-260), a kit of rabbit polyclonal factor sera for serological identification of medically important *Candida* species purchased from Diatron (formerly Iatron, Tokyo, Japan).

2.6. Another method

Total phosphate was determined by the method of Ames and Dubin [20], using KH_2PO_4 (nakarai tesque, Kyoto, Japan) as a standard.

3. Results and discussion

A comparison of pH values and cell growth rates after cultivation of the A-strain in YSLM at different temperatures was conducted. The growth rate of Y-form cells decreased concomitantly with the elevation of the temperature, i.e. dry weight of the cells was 58% at 37°C, and 14% at 40°C compared with that (4.9 g/l) of 27°C. The growth of the cells at 37°C followed by 27°C and 40°C followed by 27°C was similar to that of 27°C. The pH values (5.5–5.7) in the media after cultivation at such high temperatures were almost the same as those at 27°C, 37°C followed by 27°C, and 40°C followed by

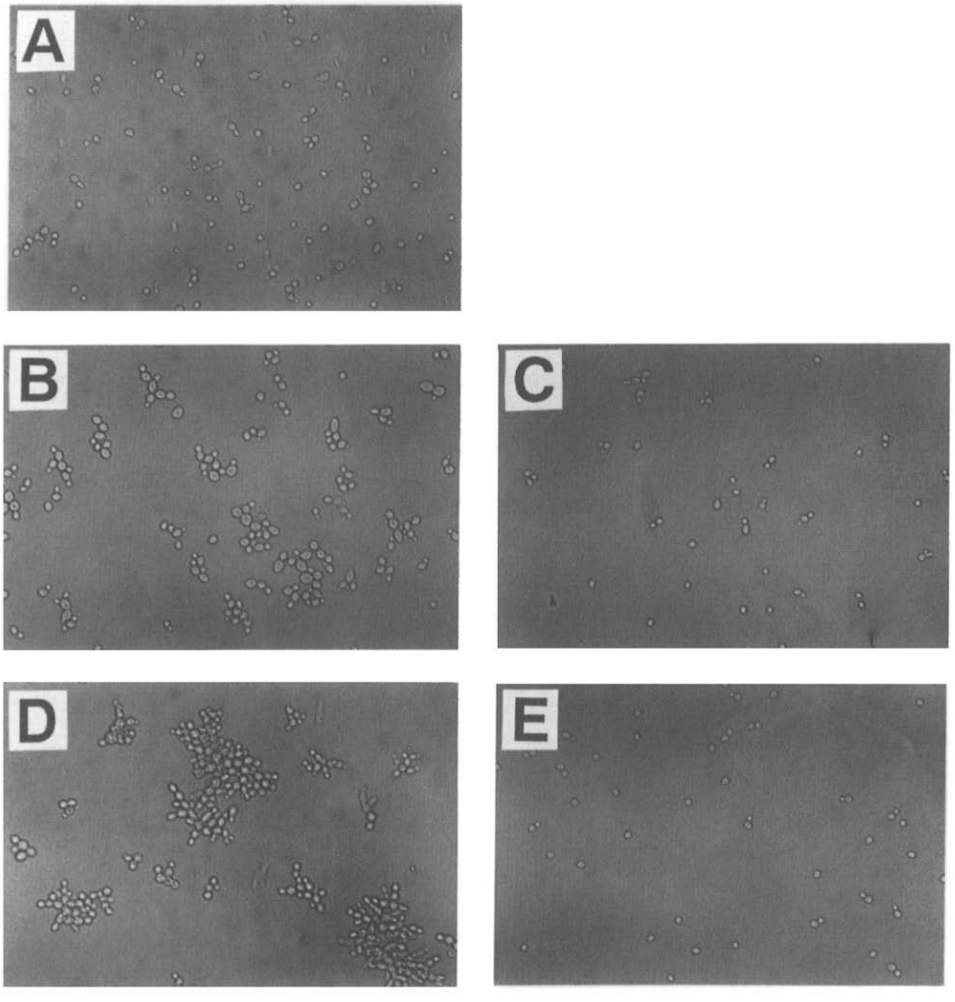

Fig. 1. A-strain cells grown in YSLM at several temperatures, ×200. A, 27°C; B, 37°C; C, 37–27°C; D, 40°C; E, 40–27°C.

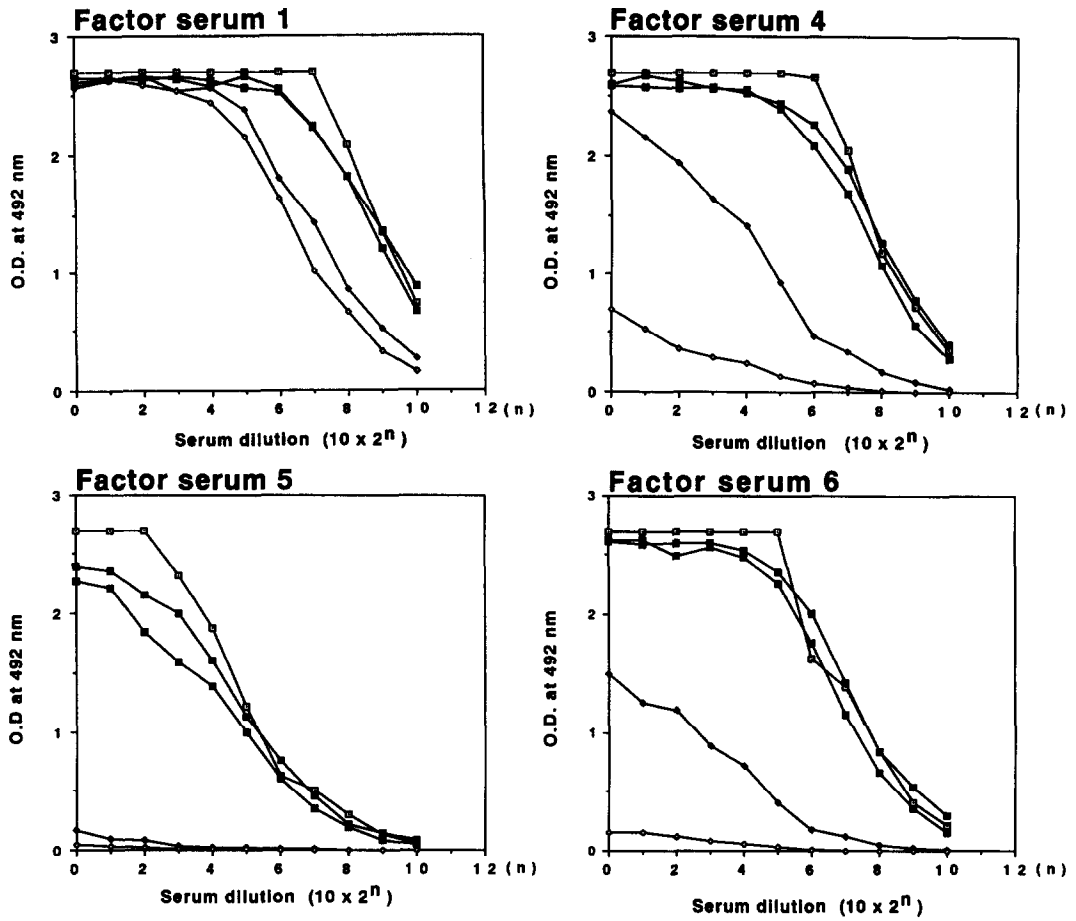

Fig. 2. ELISA of mannans of A-strain cells grown at several temperatures with factor sera of 'Candida Check'. ⊡, A-27-M; ◆, A-37-M; ⧈, A-37–27-M; ◇, A-40-M; ■, A-40–27-M.

27°C. Photomicroscopic observations of the cells cultured at both high temperatures, more especially at 40°C, revealed that these cells had the common feature of a grape-like shape by failure of mother cells to separate from daughters during cell division, chain formation (Fig. 1). This is comparable with our previous findings [8], demonstrating that A-strain cells grown in YSLM at pH 2.0 exhibited a remarkable flock-forming ability. It is obvious from the above results that the cultivation of the A-strain in YSLM under the non-suitable conditions, such as low-pH or the high temperatures, produces similar yeast-form cells with a grape-like shape. It is well known that the serotype A cells undergo agglutination by factor sera 1, 4, 5, and 6. Therefore, we attempted a series of agglutination tests between the five cell groups of the A-strain cultured at different temperatures and the factor sera kit, 'Candida Check'. The results summarized in Table 1 indicate that the cells cultivated at such high temperatures (37 and 40°C) did not undergo agglutination with the factor sera 5 nor 6, corresponding to the common epitope of *C. albicans* serotypes A and B cells and the serotype A-specific epitope, respectively, in addition to the factor serum 4, and retained the reactivity with factor serum 1. These results suggest that the antigenic factor 1 seems to be important as a diagnostic common antigen to widely detect pathogenic *Candida* species in situ, especially of that at the infected sites of patients with deep-seated candidiasis. When the cells cultured at high temperatures in YSLM were recultivated in the same medium at 27°C, the agglutination activities with the factor sera 4, 5, and 6 were entirely restored.

Table 1
Slide agglutination of A-strain cells grown in YSLM at different temperatures with polyclonal anti-factor sera

Culture temp. (°C)	Factor serum									
	1	4	5	6	8	9	11	13	13b	34
27	++	++	++	++	–	–	–	–	–	–
37	++	+	–	±	–	–	–	–	–	–
37-27	++	++	+	++	–	–	–	–	–	–
40	++	–	–	–	–	–	–	–	–	–
40-27	++	++	+	++	–	–	–	–	–	–

Agglutination was scored from high (++) to low (±), and no agglutination (–).

The results of ELISA of the mannans with factor sera is shown in Fig. 2. There are small differences in their reactivities against factor serum 1. On the other hand,

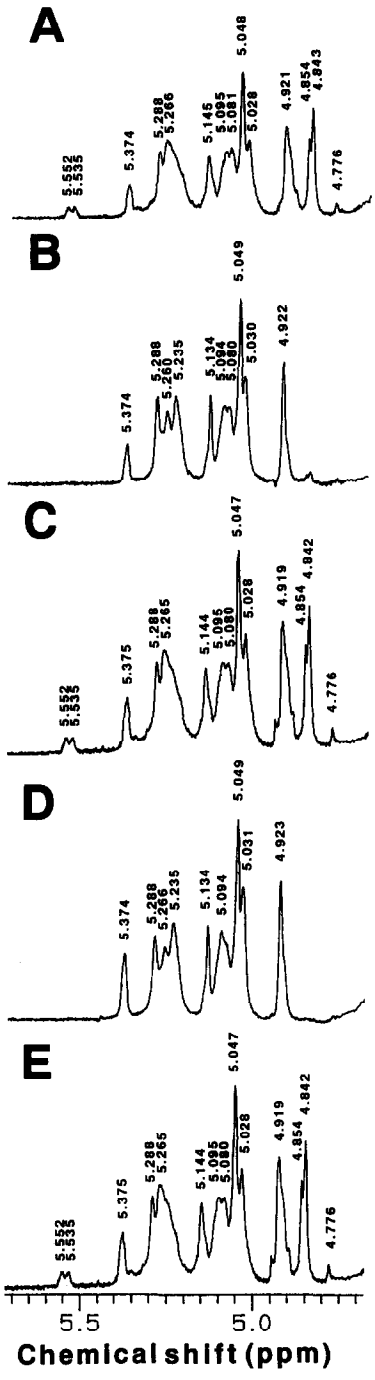

Fig. 3. ^{1}H NMR spectra of mannans of A-strain cells grown in YSLM at several temperatures. A, A-27-M; B, A-37-M; C, A-37–27-M; D, A-40-M; E, A-40–27-M.

the intensity of the reactivities of these mannans against factor sera 4, 5, and 6 significantly decreased in A-37-M and A-40-M, especially in the latter. A-37–27-M and A-40–27-M gave the same reaction patterns as that of A-27-M. A similar change was observed on the mannan of another *C. albicans* serotype A strain, J-1012, grown under the same conditions (data not shown). However, the cells of the *C. tropicalis* IFO 0199 strain, which possess antigenic factors 1, 4, 5, and 6 [21], did not exhibit a significant change in agglutinability (data not shown).

Fig. 3 shows ^{1}H NMR spectra of the five mannans, A-27-M, A-37-M, A-37–27-M, A-40-M, and A-40–27-M. Comparison of the NMR spectra of the five mannans resulted in an interpretation that lack of β-1,2-linked oligomannosyl residues and phosphate group in both mannans, A-37-M and A-40-M, especially of the latter one, is evident because the same chemical shifts corresponding to β-1,2-linked mannopyranose units [6–11,18], 4.776, 4.843, and 4.854 ppm, and to the 1-*O*-phosphorylated mannose unit [6–9,11,18], 5.535 and 5.552 ppm, disappeared concomitantly with the increase of the cultivation temperatures, in comparison with those of three other mannans. The results of chemical analysis of the phosphate content also substantiated these findings (Table 2). A-37–27-M and A-40–27-M gave exactly the same ^{1}H NMR patterns as A-27-M [6–9,11,22]. These results are very similar to those of the Y-form cells cultured at pH 2.0 [8,11]. The chemical shift of 5.134 ppm, which appeared clearly in A-37-M and A-40-M compared to the other three mannans, showed to contain considerable amounts of non-reducing terminal α-1,3-linked mannopyranose unit [10,23,24]. Similar changes, increase and expression of the non-reducing terminal α-1,3-linked mannopyranose unit with loss of the β-1,2-linked one, were also observed in the mannans of *C. albicans* and *C. tropicalis* strains by cultivation at low pH [11,25]. The chemical shift of 5.145 ppm in A-27-M, A-37–27-M, and A-40–27-M corresponds to the α-1,2-linked mannopyranose unit substituted by the β-1,2-linked oligomannosyl units in the acid-stable domain [7,9,10,18]. The chemical shift corresponding to the α-1,3-linked mannopyranose unit [6,7,9–11,18,24], 5.375 ppm, substituted by the α-1,2-linked one could be observed in all mannans (Fig. 3).

In recent studies, it was reported that the oligosaccharides containing both β-1,2 and α-1,2 linkages, Man*pβ*1–2Man*pα*1–2-Man*pα*1–2-Man*pα*1–2Man and Man*pβ*1–2Man*pβ*1–2Man*pα*1–2-Man*pα*1–2Man*pα*1–2Man, correspond to the specific epitopes of *C. albicans* serotype A [7,9,18], antigenic factor 6 [26], and that the consecutive β-1,2-linked oligomannosyl residues [27–29], which are attached to the phosphate groups, served as major common epitopes for antigenic factor 5, through-

Table 2
Total phosphate contents of mannan of A-strain cells grown in YSLM at different temperatures

Mannan	Total phosphate (%)[a]
A-27-M	1.49
A-37-M	0.31
A-37-27-M	0.86
A-40-M	< 0.10
A-40-27-M	1.12

[a] Determined by the Ames-Dubin method as $-PO_3H_2$.

out *C. albicans* serotype A and B strains [19]. Until now, it has been known that the structures of the factor 4 epitope are α-1,6- and α-1,2-linked mannopyranose units [30]. The present results suggest that factor serum 4 reacts with β-1,2-linked mannopyranose units as well as α-linked mannooligosaccharide side chains as reported in the preceeding paper [19]. The present results seem to indicate that transferase activities participating in the introduction of mannosyl phosphate and β-1,2-linked mannopyranose units underwent inactivation by elevation of temperature of the environment. It has been reported that the antigenic factors 1, 4, 5, and 6 were expressed to the same extent by each group of the parent *C. albicans* cells cultivated under different growth conditions [31]. On the other hand, Shimokawa et al. [32] reported in 1986 that *C. albicans* rough-colony phenotype mutant cells were defective in all antigenic factors 1, 4, 5, and 6, although any evidences for the chemical structures corresponding to these antigenic factors were not provided at that stage. It is further documented that growth of *Candida* is influenced by temperature accompanied by a change in expression of a variety of physiological characteristics, such as the virulence [33], adherence [34], morphology [35], antigenicity [35], and so on. As the normal temperature of living mammals is about 37°C, the present findings must be useful for the in situ detection of the antigens by serological diagnostic procedure in various infected sites and also in the consideration of the other physiological nature of *C. albicans* under various circumstances together with our previous findings [7,8,11]. Further studies should be carried out using cultivation media possessing closely identical compositions to those of blood plasma and body fluid of the host mammals.

Acknowledgements: We thank Dr. K. Hisamichi, the First Department of Medicinal Chemistry of this College for recording the NMR spectra.

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