Antigenicity of Cell Wall Mannans of *Candida albicans* NIH A-207 Strain Cells Cultured in Galactose-Added Yeast Nitrogen Base Medium

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The cultivations of the *Candida albicans* NIH A-207 strain (A-strain) for 5 d at 27 and 37 °C in 500 mM galactose-added yeast nitrogen base medium (YNB-Gal) decreased the growth of blastoconidia and the pH in the cultures, with dry weights of 56 and 47% and with pHs of 2.41 and 2.47, compared with the dry weight of 100% and pH of 5.63 for a standard cultivation of 2 d at 27 °C in the yeast extract-added Sabouraud liquid medium (YSLM). The cells obtained by cultivations at 27 and 37 °C in the YNB-Gal clearly decreased the agglutination against serum factors 4, 5, and 6 in the commercially available kit 'Candida Check', especially at 37 °C, in contrast to those obtained by the standard cultivation. It was also revealed by ¹H-NMR analysis that both the mannans obtained from cultures at 27 and 37 °C in the YNB-Gal had drastically lost a phosphate group and a β-1,2-linked mannopyranose unit, and increased the non-reducing terminal α-1,3-linked mannopyranose unit, especially at 37 °C.

Key words *Candida albicans*; yeast nitrogen base (YNB)-galactose medium; mannan; antigenicity; structure

The adherence to host cells of *Candida albicans* is believed to play a critical role in the colonization of mucosal surfaces and subsequent infection. In 1981, Douglas *et al.* reported that yeast nitrogen base medium (Difco) containing 500 mM galactose (YNB-Gal) is useful for the studies of the adhesion to acrylic surfaces¹⁾ and buccal epithelial cells^{2,3)} of *C. albicans*, and recently, of the biofilm formations.⁴⁾ They and others⁵⁾ mentioned that the protein portion of the mannoprotein adhesin of the yeast is more important than the carbohydrate moiety. On the other hand, in separate experiments, several authors have shown that the cell-wall mannan parts are implicated to have at least some adhesive function to the host cells.^{6–10)} In 1989, we attempted the isolation of the adhesin from the *C. albicans* NIH A-207 strain (A-strain) using the YNB-Gal. At that time, we (Okawa and Giummelly, a private message) noticed that the cultures of the A-strain in the YNB-Gal had an acidic pH 2–3, and an drastic changing of the structure of the mannan in the supernatant. By using the pH information, we investigated the antigenicities and the mannan structures of *C. albicans* cells cultured at the acidic pH 2.0,^{11–13)} and then at the high temperatures of 37 and 40 °C,^{14,15)} in yeast extract-added Sabouraud liquid medium (YSLM). Until now there have been no reports about the structures of the cell wall mannans of the A-strain cells cultured in YNB-Gal. In this paper, we describe the changes in the antigenicity and the chemical structure of the mannans.

MATERIALS AND METHODS

Strain and Culture Conditions The *C. albicans* NIH A-207 strain (A-strain) was kindly supplied by Dr. T. Shinoda, Meiji College of Pharmacy, Tokyo, Japan. The cultivation and preparation of cells were conducted as previously described. The A strain was precultured in yeast extract-added Sabouraud liquid medium (YSLM) at 27 °C for 24 h on a rotary shaker (150 rpm).¹⁴⁾ The cells (2 × 10⁷) in 100 to 200 μl aliquots were then inoculated into each 200 ml of YSLM and of yeast nitrogen base medium containing

500 mM galactose (YNB-Gal)¹⁾ in 500 ml Erlenmeyer flasks, and the main cultivations were separately conducted at 27 and 37 °C for 2 and 5 d with shaking. After harvesting the cells by centrifugation, the pHs of the supernatants were measured and the precipitated cells were washed three times with saline by centrifugation. The morphologies of the washed cells were photomicroscopically evaluated. To evaluate the cell density at the end of each culture period, portions of the cultures were freeze-dried. Cells washed with saline were dehydrated with a large volume of acetone.

Slide Agglutination Reaction Slide agglutination assays were conducted as described elsewhere^{16,17)} with suspensions of the heat-killed cells which were harvested after cultivation.

Preparation of Mannans Mannans were prepared as previously described.¹⁸⁾ Briefly, this was done by a combination of extraction with hot water and short-term precipitation with Fehling solution. The mannan fractions obtained from the A strain cultured at 27 °C for 2 d in YSLM, at 27 °C for 5 d in YNB-Gal, and at 37 °C for 5 d in YNB-Gal were designated mannans A-27-M, Y-27-M, and Y-37-M, respectively.

¹H-NMR Analyses of the Mannans The ¹H-NMR spectra were recorded using a JEOL JNM-GSX 400 spectrometer. Each sample was dissolved in D₂O at a concentration of 1% (wt/vol), and the determination was conducted at 45 °C with acetone as the internal standard (2.217 ppm).

RESULTS AND DISCUSSION

A comparison of the pH values and cell growth rates after cultivation of the A-strain in YSLM and YNB-Gal at different temperatures was conducted (Table 1). Cultivations of the A-strain for 5 d at 27 and 37 °C in YNB-Gal decreased the growth of the blastoconidia and the pH in the cultures, with dry weights of 56 and 47% and with pHs of 2.41 and 2.47, compared with the dry weight of 100% and pH of 5.63 in a standard cultivation of 2 d at 27 °C in YSLM. The A-strain cells cultivated in YNB-Gal for 5 d at 27 and 37 °C grew in the budding yeast phase.^{1–3)} We already reported that the

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Table 1. Measurement of pH and Cell Growth in Cultures of A-Strain Cells Grown at Several Conditions

Medium	Growth temp. (°C)	Incubation period (d)	Cultures	
			pH	Cell growth (dry weight, g/l)
YSLM	27	2	5.63	4.94
YNB-Gal	27	5	2.41	2.79
YNB-Gal	37	5	2.47	2.33

Table 2. Slide Agglutination of A-Strain Cells Grown at Several Conditions with Polyclonal Anti-factor Sera

Medium	Growth temp. (°C)	Incubation period (d)	Factor serum			
			1	4	5	6
YSLM	27	2	+++	+++	+++	+++
YNB-Gal	27	5	+++	++	+	+
YNB-Gal	37	5	+++	+	±	±

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

A-strain cells decreased the growth rate by both the changes in pH, pH 5.6 to 2.0,¹¹⁾ and temperatures, 27 °C to 37 and 40 °C,¹⁴⁾ in YSLM. Based on the present results, we can state that the cell growth of the A-strain in the YNB-Gal was influenced by the severe conditions such as the low nutrition, the acidic pH, and the high temperature.

We then performed a series of agglutination tests between serum factors from a commercially available serum factor kit (Candida Check).^{16,17)} and cells from the A strain grown in YNB-Gal at 27 and 37 °C and in YSLM at 27 °C (Table 2). The cells obtained by cultivations at 27 and 37 °C in the YNB-Gal clearly decreased the agglutination against factor sera 4, 5, and 6, and retained the reactivity with factor serum 1, in contrast to those obtained by the standard cultivation in YSLM. These results show that the changes in antigenicity, namely, in the mannan structure, of the A-strain cells are caused by cultivation in YNB-Gal.

We next determined the chemical structure of the mannans by ¹H-NMR (Fig. 1). Both mannans (Y-27-M and Y-37-M) obtained from the A-strain cells cultured at 27 and 37 °C in the YNB-Gal had lost a phosphate group, the 1-*O*-phosphorylated mannose unit, 5.535 and 5.552 ppm, and a β-1,2-linked mannopyranose unit, 4.776, 4.843, and 4.854 ppm, especially Y-37-M, but increased the internal α-1,2-linked mannopyranose unit, 5.287—5.288 ppm, the internal α-1, 6-linked mannopyranose unit, 5.095 ppm, substituted by the α-1,2-linked one, and the internal α-1,6-linked mannopyranose unit, 4.910 ppm.¹³⁾ The non-reducing terminal α-1, 3-linked mannopyranose unit, 5.135 ppm, clearly appeared in Y-37-M instead of the α-1,2-linked mannopyranose unit, 5.145 ppm, substituted by the β-1,2-linked oligomannosyl unit in A-27-M. Y-37-M clearly lost the reactivity to factor sera 5 and 6, because these sera recognize the side chains containing the β-1,2-linked mannose residues.^{14,15,19,20)} The α-1,6-branched mannopyranose unit, 5.235 ppm, of *Candida* mannan functions as the determinant of the antigenic factor 4.¹⁹⁾ As all three mannans used in this experiment contain nearly the same amounts of the branched side chain, it seems that the differences of the reactivity with the factor serum 4

Fig. 1. ¹H-NMR Spectra of Mannans of A-Strain Cells Grown at Several Conditions
A, A-27-M; B, Y-27-M; C, Y-37-M.

depend on the remaining amounts of β-1,2-linked mannopyranose units in the mannans as discussed in the preceding paper.^{14,15)} Serum factor 1 has been shown to recognize the α-1,2-linked oligomannosyl side chains in mannan.^{13,15)} The three mannans have significantly the non-reducing terminal α-1,2-linked mannopyranose unit, 5.048—5.049 ppm, and the internal α-1,2-linked mannose unit, 5.287—5.288 ppm. This is the reason that the three mannans have the same reactivity with factor serum 1.

Based on these results, we can state that most of the structures of the mannans obtained from the A-strain cells cultivated in YNB-Gal consist of the α-mannosyl residues. This finding may influence the properties of *Candida* cells cultivated in YNB-Gal. It is also very similar to the antigenicities and the mannan structures of the *C. albicans* serotype A strain cells cultured at the acidic pH 2.0,^{11–13)} and at the high temperatures of 37 and 40 °C¹⁴⁾ in YSLM. Therefore, the present results seem to indicate that transferase activities participating in the introduction of mannosylphosphate and β-1,2-linked mannopyranose units underwent inactivation by culturing in YNB-Gal.²¹⁾ Detailed studies of these mannosyltransferases of *Candida* species is now in progress.

It is possible to mention that the mannan obtained from *C. albicans* cells by cultivation for 5 d in YNB-Gal changed the antigenicities and the mannan structures by changing not only the pH but also the temperature (Tables 1, 2, Fig. 1). We also have obtained the same structured mannan fractions as Y-37-M in the supernatant of the *C. albicans* cultures in YNB-Gal (unpublished data). It has been known that the growth in a high-galactose medium (YNB-Gal) promotes the synthesis of a mannoprotein adhesin, resulting in increased adhesion to the various animal cells.^{2,3,5)} The results of the present YNB-Gal study seem to be useful for determining the structure and the function of the mannoprotein adhesin.

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