

# Structural Investigation of Cell Wall Mannan Antigen Obtained from Pathogenic Yeast *Candida zeylanoides*

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## ABSTRACT

Antigenicity of pathogenic yeast *Candida zeylanoides* NBRC 10326 was found to have similar to that of *Candida guilliermondii*, but it was slightly different, *i.e.* NBRC 10326 strain cells react strongly with the factor antibodies 1, 4, and 9, and react relatively weakly with factor antibody 5. We have investigated the structural characteristic of cell wall mannan of this strain using a non-degradable method, nuclear magnetic resonance analysis. The results obtained from NMR analysis, the two-dimensional homonuclear Hartmann-Hahn, indicated that *C. zeylanoides* mannan contains  $\alpha$ -1,3-linked and  $\beta$ -1,2-linked oligomannosyl side chains corresponding to *C. guilliermondii* specific antigenic factor 9. In addition, it was found that the mannan contains  $\beta$ -1,2-linked oligomannosyl side chains attached to phosphate groups corresponding to antigenic factor 5, which were not found in *C. guilliermondii* mannan.

**Keywords:** *Candida zeylanoides*; *Candida guilliermondii*; Cell Wall Mannan; Antigenic Factor; Nuclear Magnetic Resonance

## 1. Introduction

Ten rabbit antibodies to antigenic factors of genus *Candida* (abbreviated as FAbs) was developed to identify clinical isolates from the patients with candidiasis by Fukazawa *et al.* [1] and Tsuchiya *et al.* [2,3]. These FAbs recognize the antigenic determinants in cell wall mannan of genus *Candida* [4]. We reported the chemical structure of cell wall mannans of several *Candida* species, including *C. albicans* [5], *C. tropicalis* [6] and *C. guilliermondii* [7]. Moreover, we revealed that the determinants of antigenic factors 5, 6, and 9 correspond to the oligomannosyl side chains containing  $\beta$ -1,2-linked mannose residue [7-9]. On the other hand, the determinants of *Candida* factors 1, 4, 13b, and 34 are  $\alpha$ -linked oligomannosyl side chains [10-12].

*Candida zeylanoides* is one of the opportunistic pathogen for candidiasis [13,14], and its cell wall mannan would be responsible for most of antigenic activity of the cell surface. The chemical structure of the antigenic mannan of this pathogen has not yet been determined. In the present study, we analyzed the purified mannan of *C. zeylanoides* NBRC 10326 strain by a non-degradable me-

thod, two-dimensional nuclear magnetic resonance.

## 2. Materials and Methods

### 2.1. Strains and Culture

*C. zeylanoides* NBRC 10326 strain (abbreviated as Z-strain), *C. guilliermondii* NBRC 10279 strain (abbreviated as G-strain), and *C. albicans* NBRC 1060 strain (serotype A) were obtained from the Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Japan. *C. albicans* NIH B-792 strain (serotype B) was gift from Dr. T. Shinoda, Meiji College of Pharmacy, Tokyo, Japan. The cells of these strains were cultivated in the yeast extract-Sabouraud's liquid medium [0.5% (w/v) yeast extract, 1% (w/v) peptone, and 2% (w/v) glucose] at 27°C for 72 hrs on a reciprocal shaker.

### 2.2. Slide Agglutination Reaction of the Cells of Z-Strain Cells with Factor Antibodies (FAbs)

Slide agglutination reaction was carried out as previously

described [15]. Factor antibodies of the genus *Candida* (abbreviated as FAbs) 1, 4, 5, 6, 8, 9, 11, 13, 13b, and 34 were prepared by Fukazawa *et al.* [1]. The agglutinations of *C. albicans* and *C. guilliermondii* cells are shown for comparative purposes.

### 2.3. Preparation of Mannan from Z-Strain

Mannans were extracted with hot water and precipitated by short-term treatment with Fehling solution [16]. The mannan obtained from the Z-strain was designated Fr. Z. The yield of Fr. Z was 10.5% of the dry cell weight.

### 2.4. Nuclear Magnetic Resonance (NMR) Analysis of Fr. Z

<sup>1</sup>H-nuclear magnetic resonance (<sup>1</sup>H-NMR) and two-dimensional homonuclear Hartmann-Hahn (2D-HOHAHA) spectrograph were recorded using D<sub>2</sub>O at 45°C with acetone as the internal standard (2.217 ppm) on a Jeol JNM-GSX 400 spectrometer. The percentage of the different kinds of oligomannosyl side chains in Fr. Z was calculated based on the peak dimensions of the corresponding H-1 signals in the <sup>1</sup>H-NMR spectrum [17].

### 2.5. Other Methods

Total carbohydrate was determined by the phenol-H<sub>2</sub>SO<sub>4</sub> method [18] using mannose as a standard. Total protein was determined by the Folin method of Lowry *et al.* [19] using bovine serum albumin as a standard. Total phosphate was determined using the Ames-Dubin method [20] using KH<sub>2</sub>PO<sub>4</sub> as the standard.

## 3. Results and Discussion

The results of the slide-agglutination assays of Z-strain cells with factor antibodies of the genus *Candida* are shown in **Table 1**. FAbs 1, 4, 5, and 9 are significantly positive to the Z-strain cells. Fab 1 is the antibody against common antigen of genus *Candida* that reacts with most of *Candida* species. The recognition of FAbs 4 and 5 suggests that Z-strain mannan contains the common antigen of two *Candida* species, *C. albicans* and *C. tropicalis*.

The recognition of FAbs 9 suggests that the determinant of specific antigen of *C. guilliermondii* is contained in Z-strain mannan. The lack of response to FAbs 6 and 13b suggests that the mannan does not contain the side chains corresponding to specific antigen of *C. albicans* serotype A and B. Therefore, the chemical structure of Z-strain mannan may be a crossbreed with that of the mannans of *C. guilliermondii* [7], *C. albicans* [5], and/or *C. tropicalis* [6].

Component analysis indicated that Fr. Z contained 94.4% total carbohydrate, 2.5% total protein, and 1.1% total phosphate. The phosphate group that was not previously observed in the mannan of *C. guilliermondii* [7] was detected in Fr. Z. This finding agreed with the positive reactivity of Z-strain cells against Fab 5 in the slide-agglutination (**Table 1**).

The chemical structure of Fr. Z was analyzed by means of 2D-HOHAHA (**Figure 1**). All H-1-H-2 cross-peaks were assigned according to published data [21-25] as shown in **Table 2**. The quartet signal corresponding peak 1 at 5.4 - 5.7 ppm indicates the presence of the phosphorylated oligomannosyl side chain and  $\beta$ -1,2-linked oligomannosyl unit. This result is intended to support the two facts that Fr. Z contains phosphate group and Z-strain cells react with Fab 5 (**Table 1**). The high intensity of H-1-H-2 cross-peak 6 indicates the presence of branched side chains containing  $\alpha$ -1,2-,  $\alpha$ -1,3- and  $\alpha$ -1,6-linked mannose residues. In addition, two H-1-H-2 cross-peaks 2 and 7 correspond to the internal and non-reducing terminal  $\alpha$ -1,3-linked mannose residues, respectively. The high intensity of H-1-H-2, H-1-H-4 and H-2-H-4 cross-peaks 11, 16 and 17 of the non-reducing terminal  $\alpha$ -1,6-linked mannose also indicated the presence of a 3,6-branched side chain. Both cross-peaks of two kinds of non-reducing terminal  $\alpha$ -1,3-linked mannose residues in a linear side chain and in a branched side chain appear in a similar range (cross-peak 7: H-1, 5.132 ppm; H-2, 4.064 ppm). The total peak dimension of two H1-signals at 5.363 ppm and at 5.132 ppm was not in agreement with that of the H-1 signal at 4.924 ppm corresponding to the non-reducing terminal  $\alpha$ -1,6-linked mannose. These results indicate that Fr. Z contains the small amount of

**Table 1.** Slide agglutination assay for whole cells of *C. zeylanoides*, *C. guilliermondii*, and *C. albicans* with anti-*Candida* factor antibodies, FAbs.

Strain	Agglutination <sup>a</sup> with FAb								
	1	4	5	6	8	9	11	13b	34
<i>C. zeylanoides</i> NBRC 10326	++	++	+	-	-	++	-	-	-
<i>C. guilliermondii</i> NBRC 10279 <sup>b</sup>	++	+	-	-	-	++	-	-	-
<i>C. albicans</i> NBRC 1060 (serotype A)	++	++	+	+	-	-	-	-	-
<i>C. albicans</i> NIH B-792 (serotype B)	++	++	+	-	-	-	-	+	-

<sup>a</sup>Agglutination was scored from high (++) to low (+) and no agglutination (-); <sup>b</sup>The agglutinations of *C. albicans* and *C. guilliermondii* cells are shown for comparative purposes.

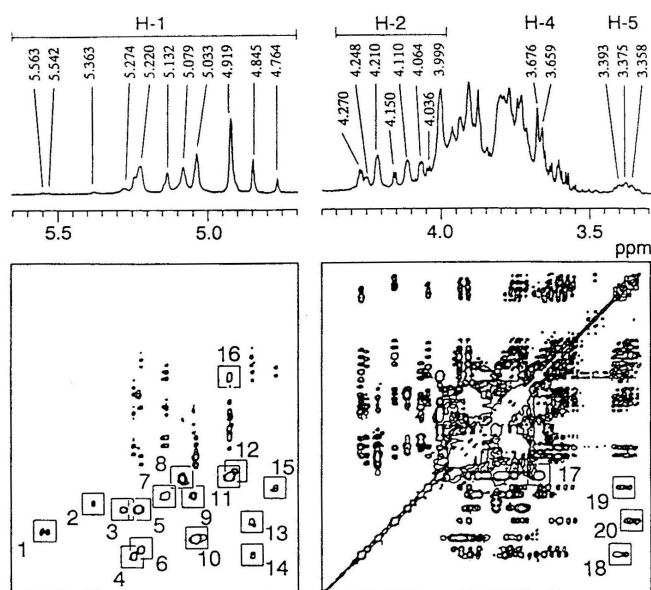


Figure 1. 2D-HOHAHA spectrum of Fr. Z. This was conducted at 45°C using acetone (2.217 ppm) as an internal standard.

Table 2. Assignment of chemical shifts of cross-peaks in 2D-HOHAHA map of Fr. Z.

Cross-peak	Chemical shift (ppm) <sup>a</sup>		Sugar residue <sup>b</sup>
	H-1	H-2	
1	5.563	-	$\beta$ 1-2Ma1-H <sub>2</sub> PO <sub>3</sub>
	5.542	-	
2	5.363	4.089	$\alpha$ 1-2Ma1-3
3	5.274	4.110	$\alpha$ -1-2Ma1-2
4	5.248	4.27	$\beta$ 1-2Ma1-3
5	5.220	4.105	( $\alpha$ 1-3M) $\alpha$ 1-2Ma1-2
			↑6 $\alpha$ 1
6	5.220	4.248	( $\beta$ 1-2M) $\beta$ 1-2Ma1-3
7	5.132	4.064	Ma1-3
8	5.079	4.01	$\alpha$ 1-6Ma1-6
			↑2 $\alpha$ 1
9	5.049	4.064	Ma1-2
10	5.037	4.21	$\alpha$ 1-3Ma1-2
11	4.919	3.999	Ma1-6
12	4.906	3.995	$\alpha$ 1-6Ma1-6
13	4.848	4.15	M $\beta$ 1-2 (M $\beta$ 1-2Ma1-3)
14	4.842	4.27	$\beta$ 1-2 M $\beta$ 1-2 (Ma1-3)
15	4.767	4.036	M $\beta$ 1-2 (Ma1-3)
16	4.919	3.668	Ma1-6
			(H-1) (H-4)
17	3.999	3.668	Ma1-6
			(H-2) (H-4)
18	4.842	3.393	$\beta$ 1-2M $\beta$ 1-2 (Ma1-3)
			(H-2) (H-5)
19	4.049	3.387	M $\beta$ 1-2 (Ma1-3)
			(H-2) (H-5)

<sup>a</sup>Measured using acetone (2.217 ppm) as a standard; <sup>b</sup>M denotes a mannose residue.

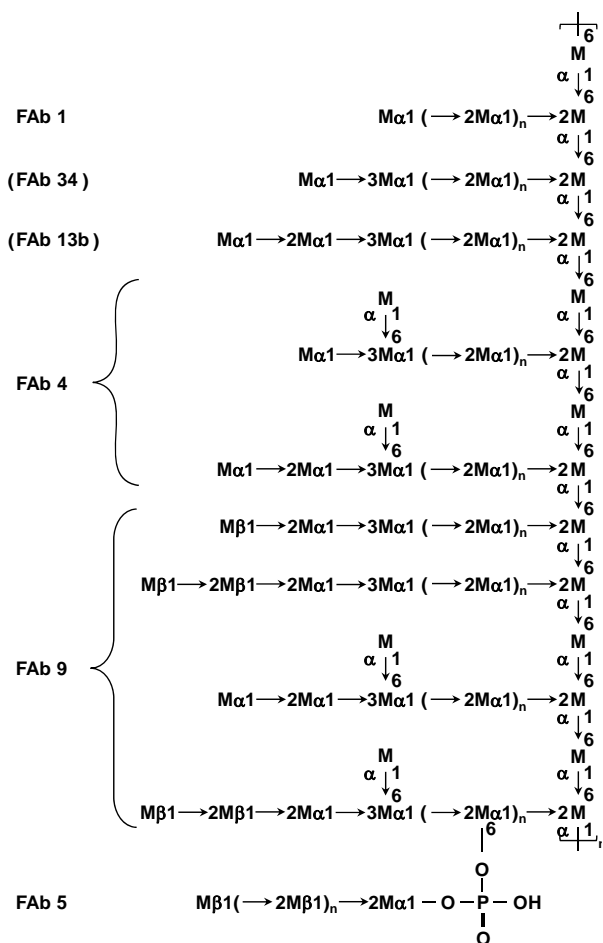
$\alpha$ -1,3-linkage-containing linear side chains. The appearance of H-1-H-2 cross-peaks 3 and 12 and H-2-H-5 cross-peak 15 indicates the presence of  $\beta$ -1,2- and  $\alpha$ -1,3-linked oligomannosyl residues. Unfortunately, it is difficult to distinguish several overlapping signals, H-1 and H-2 ranges of 5.25 - 5.30 ppm and 4.10 - 4.12 ppm, corresponding to the inner  $\alpha$ -1,2-linked mannose in the side chains.

As a result of the present study, the chemical structure of Fr. Z and the recognition sites of FAbs have been shown in **Figure 2**. The  $\alpha$ -1,2-linked oligomannosyl side chains and the 3,6-branched side chains corresponding to the determinants of antigenic factors 1 and 4 are presented in Fr. Z. Furthermore, Fr. Z possess the determinants of antigenic factors 5 and 9, which were previously observed in the mannans of *C. guilliermondii* [7] and the both serotypes of *C. albicans* [8]. However, Fr. F does not possess the determinant of antigenic factors 6, which was previously observed in the mannans of *C. albicans* serotype A [16,23]. Even though the cells of Z-strain do

not react with factor antibodies 13b and 34 (**Table 1**), the side chains corresponding to antigenic factors 13b and 34 were detected in Fr. Z by NMR analysis (**Figure 1** and **Table 2**). This phenomenon may be due to the steric hindrance in the mannan molecule, *i.e.*, it is estimated that a relatively short  $\alpha$ -linked oligomannosyl side chains are shielded by the long  $\beta$ -1,2-linkage-containing oligomannosyl side chains on the Z-strain cell surface.

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**Figure 2.** Structure of Fr. Z and the side chains corresponding to antigenic factors 1, 4, 5, and 9. M denotes a mannose residue. Side chain sequence is not specified. The repeat count "n" is not determined.

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